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Reconditioning of heroin place preference requires the basolateral amygdala

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Abstract

To investigate the role of the basolateral amygdala (BLA) in the reacquisition of heroin seeking, we studied the effect of BLA inactivation after heroin re-exposure in the presence of drug-conditioned cues. We employed a heroin conditioned place preference task [Leri F, Rizos Z. Reconditioning of drug-related cues: a potential contributor to relapse after drug re-exposure. Pharmacol Biochem Behav 2005;80:621–30.], where after initial conditioning and subsequent extinction, rats received a single reconditioning session (explicit compartment–heroin re-pairing), followed by a test of heroin seeking 24 h later. Rats were infused with $GABA_A$/$GABA_B$ agonists (muscimol and baclofen, 0.03 and 0.3 nmol, respectively/0.3 μl) or vehicle, either 15 min or 6 h following the heroin reconditioning session. Animals that received vehicle infusions, whether they were given 15 min or 6 h following reconditioning, showed a significant preference for the heroin-paired compartment 24 h later. However, inactivation of the BLA 15 min post-reconditioning, but not 6 h following reconditioning, completely blocked the reacquisition of heroin seeking. These results suggest that the BLA plays an important role in a putative learning process initiated by drug re-exposure which may underlie the process of relapse.

Keywords: Basolateral amygdala; Extinction; Reconditioning; Reacquisition; Heroin; Relapse; Conditioned place preference; Rat

1. Introduction

Cognitive behavioral models conceptualized relapse as a process initiated by discrete instances of drug re-exposure following abstinence and rapidly culminating in a return to problematic drug use (Marlatt and Gordon, 1985). The learning-based aspect of this process can be investigated using an animal model of reacquisition of drug seeking behavior (Leri and Rizos, 2005). In this model, environmental cues present during drug exposure acquire conditioned incentive properties and thus the ability to elicit drug seeking behavior. Repeated exposure to drug-conditioned cues in the absence of drug effects extinguishes responding to these cues. Extinction, however, does not abolish their conditioned incentive value (Bouton, 2000), but rather promotes the acquisition of new learning (Rescorla, 2001) which competes with the original learning to control drug seeking behavior. It is possible, therefore, that drug re-exposure in the presence of drug-conditioned cues following extinction may induce rapid reacquisition of drug seeking behavior because new information are learned, and this facilitates the expression of the original learning. In these experiments, we investigated the role of the basolateral amygdala (BLA) in this putative learning process. The BLA plays a critical role in the acquisition of conditioned responses to stimuli with motivational value (Cardinal et al., 2002; LeDoux, 2000). For example, lesions to the BLA prevent the acquisition of a place preference induced by cocaine (Fuchs et al., 2002), block the ability of rats to encode action–outcome contingencies motivated by food (Balleine et al., 2003), and prevent the acquisition of cocaine seeking behavior assessed by a second-order schedule of reinforcement (Whitelaw et al., 1996).

It is believed that activity in the BLA during a period of time following a learning experience is crucial to the formation of memory for that experience (McGaugh, 2000), possibly because of modulation of memory consolidation in different memory systems of the brain (McGaugh, 2004). Memory consolidation refers to a process by which newly acquired associations are transformed from a labile state to a more stable and permanent neural representation (Block, 1970; McGaugh, 2000) through the expression of genes that modulate synaptic plasticity (Kandel, 2001). In support for the role of the BLA in

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memory consolidation, it has been found that post-conditioning inactivation of this region blocks the acquisition of amphetamine and food place preference (Hsu et al., 2002; Schroeder and Packard, 2000). Interestingly, the BLA is also involved in the acquisition of memory for extinction, as post-extinction infusions of glucose or a muscarinic receptor agonist in this region facilitate extinction of amphetamine place preference (Schroeder and Packard, 2003, 2004). Given this evidence, therefore, it is possible that the BLA may also be involved in the putative learning process underlying reacquisition of drug seeking behavior.

In these experiments, we examined the role of the BLA in the reacquisition of an extinguished heroin conditioned place preference (CPP). In this paradigm, rats are initially trained to associate an environment with heroin administration (i.e., conditioning). Subsequently, the resulting heroin CPP is extinguished by pairing vehicle administration with both the previous vehicle- and heroin-paired compartments. Rats are then reconditioned by re-pairing heroin with the heroin-paired compartment on a single occasion. Finally, a test of CPP is given 24 h after reconditioning, in drug-free conditions. In Experiment 1, the BLA was reversibly inactivated 15 min following heroin reconditioning via intracranial microinjections of a combination of $GABA_A$/$GABA_B$ agonists (McFarland and Kalivas, 2001). In order to verify the time course of BLA involvement in reacquisition, in Experiment 2 the BLA infusions were administered 6 h (Schafe and LeDoux, 2000) following heroin reconditioning.

2. Methods

2.1. Subjects

Subjects were adult male Sprague–Dawley rats (Charles River, Quebec) weighing 225–250 g at the beginning of the experiments. Rats were singly housed and maintained on a reverse light/dark cycle (8:00 am lights off; 8:00 pm lights on) with free access to food and water except during testing, which occurred during their dark cycle. All experiments were approved by the Animal Care Committee of the University of Guelph and were carried out in accordance with the recommendations of the Canadian Council on Animal Care.

2.2. Surgery and histology

Following 6 days of acclimation to the facility, animals were anesthetized with pentobarbital (15 mg/kg Somnotol, IP), morphine (5 mg/kg, SC) and diazepam (1 mg/kg, SC) and implanted with bilateral guide cannulas (20 guage, cut to 18 mm, Plastics One) aimed at the BLA. The coordinates relative to bregma were: A/P −2.3, M/L ±4.8, and D/V +3.3 from the interaural line (Paxinos and Watson, 2005). The guide cannulas were attached to the skull via stainless steel screws and dental acrylic. Obdurators (22 gauge, Plastics One) were cut to the same length as the cannulas, and were inserted to prevent occlusions. Animals were allowed 5 days to recover before the beginning of conditioning.

For the histology, rats received an overdose of pentobarbital (65 mg/kg Somnotol, IP) and were then perfused intracardially using 0.9% saline followed by 4% paraformaldehyde. After the brains were removed, they were placed in a 30% sucrose and paraformaldehyde solution for at least 48 h before slicing. Coronal sections were taken on a cryostat at a thickness of 25 μm throughout the BLA, and were stained with cresyl violet to verify cannula placements.

2.3. Apparatus

Six custom made (University of Guelph) place conditioning boxes were used in these experiments. The boxes were located in the middle of a laboratory room. Each place conditioning box was made of dark gray PVC, and comprised of three compartments: two large ($30 \times 40 \times 26$ cm) and one smaller, middle ($23 \times 30 \times 26$ cm) compartment. Removable inserts, with or without small arch-way openings (10×10 cm), formed the center compartment. The two large compartments differed primarily in visual cues; one large compartment was dark gray while the other had a white wall and a 10 cm white stripe painted along the top of the other walls. In addition, there were cues that provided spatial information external to the compartments, such as posters on walls, benches, and lights. In this apparatus, rats do not display a significant spontaneous preference for any of the compartments (i.e., the apparatus is balanced). The entire apparatus was covered by black wire mesh to allow video tracking of the rats during testing. The tracking software employed was EthoVision (v.3, Noldus Information Technology, The Netherlands).

2.4. Drugs

Diacetylmorphine hydrochloride (heroin) was obtained from Almat Pharmachem (Concord, Ontario, Canada), dissolved in 0.9% physiological saline, and injected subcutaneously at a volume of 1.0 ml/kg. Vehicle (0.9% physiological saline) was injected at the same volume and by the same route. Muscimol hydrobromide and baclofen hydrochloride were obtained from Sigma-Aldrich (St. Louis, MO, USA), and dissolved in sterile distilled water.

2.5. Behavioral procedures

2.5.1. Handling and habituation

Animals were handled for 10 min on 2 occasions before habituation to the apparatus. During this session, the inserts with openings were used, the rats had free access to the 3 compartments for 20 min, and spontaneous preference for each large compartment was measured.

2.5.2. Conditioning

Place conditioning commenced the day following habituation. For this phase, the compartments were separated with solid inserts. Each day rats received two 30-min conditioning sessions, one in the morning and one in the afternoon (morning session: between 9:00 am and 12:00 pm; afternoon session:

between 2:00 and 5:00 pm), with 0.3 mg/kg heroin (dissolved in 0.9% physiological saline, SC) or vehicle (Leri and Rizos, 2005). The heroin-paired compartment was counterbalanced across rats. The time of heroin session (am or pm) was also counterbalanced across rats and, for each rat, across days of conditioning. Animals received a total of 4 heroin and 4 vehicle injections over 4 days of conditioning.

2.5.3. Test I

The day following conditioning, the solid inserts were replaced by those with openings, and a preference test was given to measure the effect of place conditioning. For this test, the rats were placed in the middle compartment and given 20 min of free access to all compartments. No injections were given on this day.

2.5.4. Extinction

After Test I, rats were left undisturbed in the colony room for one day before the beginning of extinction. Extinction was similar to conditioning in that it was carried out over four days, with two 30-min conditioning sessions each day. However, during extinction, rats received vehicle injections before confinement in both compartments. We have already demonstrated that this extinction procedure blocks the expression of heroin CPP (Leri and Rizos, 2005). Specifically, animals primed with a vehicle injection did not display a CPP (Experiment 1), and animals reconditioned with vehicle did not display a CPP when tested 24 (Experiment 2) or 96 h following (Experiment 3) reconditioning.

2.5.5. Reconditioning

Following the last day of extinction, rats received a single day of reconditioning during which they received one session with heroin (0.3 mg/kg) and the other with vehicle, in the compartments that were previously paired with heroin and vehicle, respectively. On this day, all animals received the vehicle session first, followed 2 h later by the heroin reconditioning session.

2.5.6. Microinjections

For Experiments 1 and 2, rats received bilateral intracranial infusions (0.3 μl infused over 1 min) in their home cage, either 15 min or 6 h following the reconditioning session with heroin, respectively. Injectors extended 2 mm past the tip of the cannulas, and were left in place for 1 min following the infusions. In Experiment 1, 13 rats received infusions of vehicle and 39 rats received infusions of the muscimol (0.03 nmol)/baclofen (0.3 nmol) mixture (McFarland and Kalivas, 2001). In Experiment 2, 8 rats received vehicle and 16 rats received the mixture at the same dosage.

2.5.7. Test II

The day following reconditioning and the intracranial infusions, the solid inserts were replaced by those with openings, and rats received a second preference test (i.e., Test II) that lasted 20 min. No injections were given on this day.

2.6. Data analyses

Relative preferences for the two large compartments during habituation and Test I were evaluated using separate paired *t*-tests. Preference during Test II was evaluated using 2-way mixed design ANOVAs with Muscimol/Baclofen Group as the between factor and Compartment (vehicle-paired vs heroin-paired) as the within factor (Leri and Rizos, 2005). In case of a significant interaction or a significant main effect, multiple-comparisons were performed using the Fisher's LSD method in order to identify individual mean differences ($\alpha = 0.05$). The specific values of negative findings are not reported. All statistical analyses were performed using Statistica (version 1.5 for Windows, StatSoft Inc).

3. Results

3.1. Histology

The injection sites for Experiment 1 are shown in Fig. 1. Thirteen rats received vehicle infusions localized within the BLA (i.e., VEH group). Of the rats that received muscimol/baclofen ($n = 39$), 25 had infusion sites within the BLA (M+B group), while 14 had infusion sites outside the BLA. The behavioral data from these latter animals were used to generate

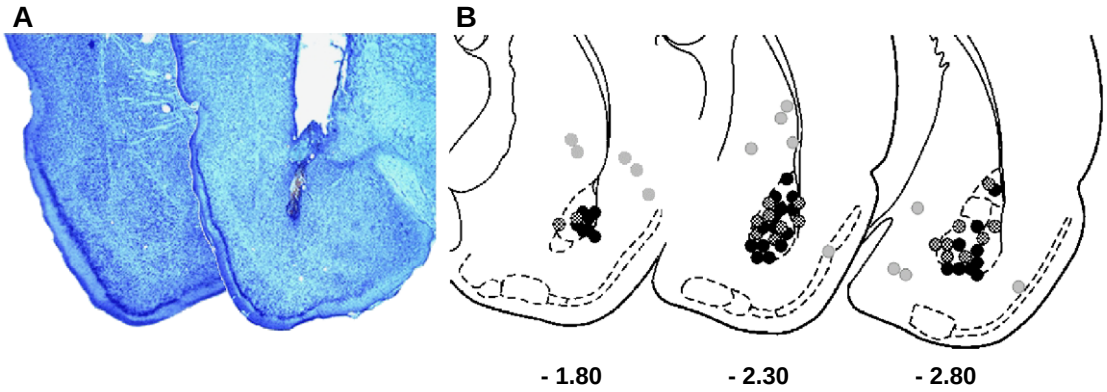

Fig. 1. Panel A: Coronal section of a normal brain and one with a representative cannula track aimed at the BLA. Panel B: Traces of cannula placements for Experiment 1 (black dots = VEH and M+B groups; gray dots = M+B not BLA group) and Experiment 2 (hatched pattern dots).

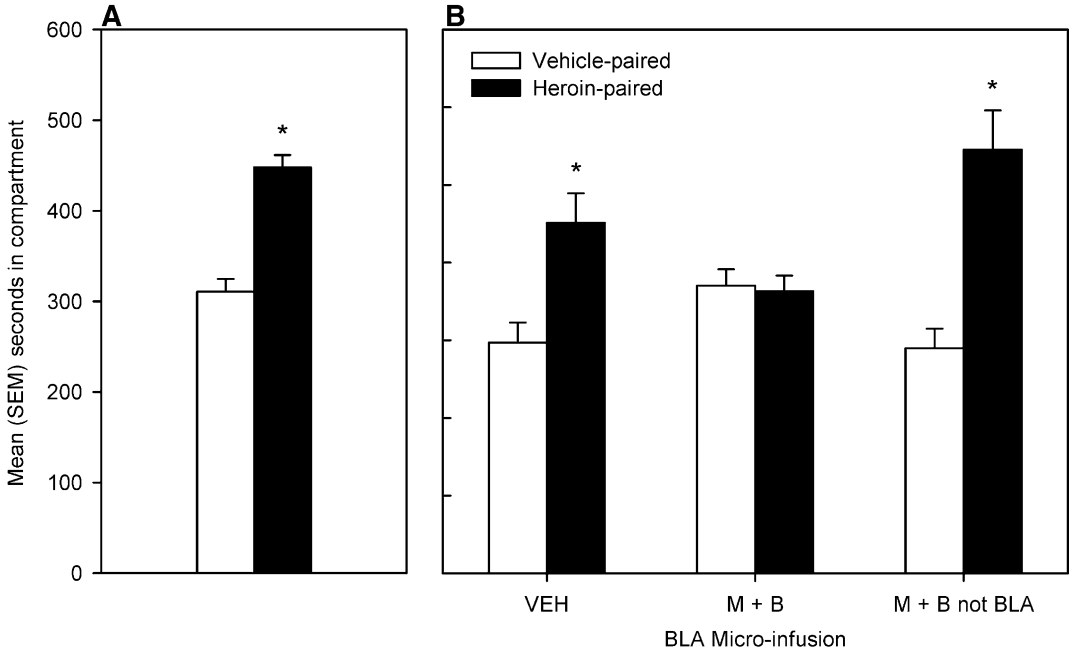

Fig. 2. Mean (SEM) seconds spent in vehicle- and heroin-paired compartments on: Panel A — Test I in animals conditioned with 0.3 mg/kg heroin ($n=52$); Panel B — Test II in animals reconditioned with 0.3 mg/kg heroin and micro-infused 15 min later with vehicle (VEH, $n=13$) or muscimol and baclofen (M+B, $n=25$). The data for the M+B not BLA ($n=14$) group was combined following histological analyses to control for muscimol/baclofen injections in areas surrounding the BLA. The asterisk indicates a significant difference between vehicle- and heroin-paired compartments.

a group controlling for the effect of muscimol/baclofen infusions in the areas surrounding the BLA (i.e., M+B not BLA group). The injection sites for Experiment 2 are also shown in Fig. 1. Three rats from the vehicle group (VEH 6 h) were removed from the analysis because of marked gliosis in the BLA. Three rats were removed from the M+B 6 h group because of inaccurate localization and their behavioral data were not used.

3.2. Experiment 1 — BLA inactivation 15 min following heroin reconditioning

Rats showed no significant spontaneous preference during habituation but, after conditioning (i.e., Test I) they displayed a significant CPP for the heroin-paired compartment (Fig. 2A; $[t(51)=-6.35,\ p<0.001]$). Rats were then tested 24 h after heroin reconditioning and BLA infusions (Test II, Fig. 2B), in

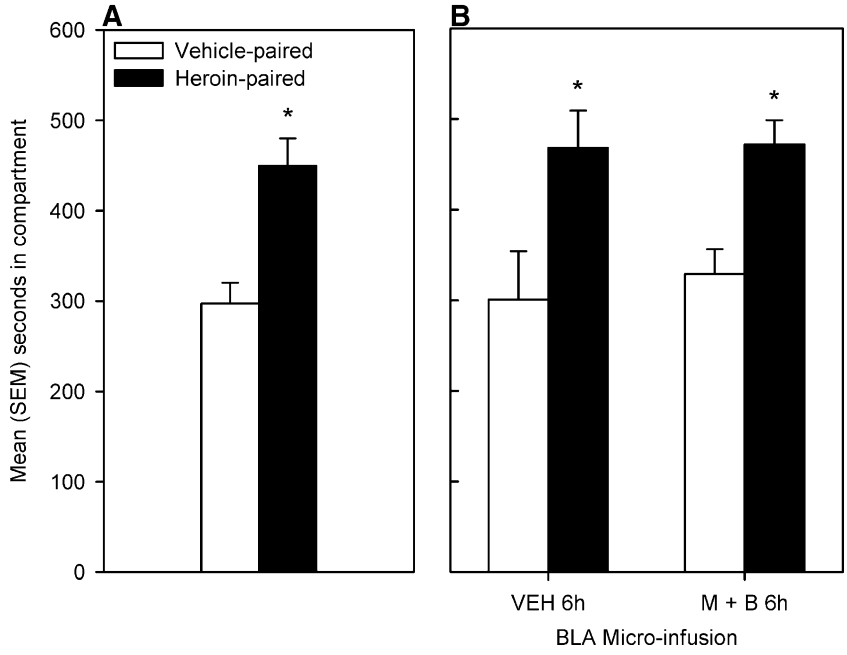

Fig. 3. Mean (SEM) seconds spent in vehicle- and heroin-paired compartments on: Panel A — Test I in animals conditioned with 0.3 mg/kg heroin ($n=18$); Panel B — Test II in animals reconditioned with 0.3 mg/kg heroin and micro-infused 6 h later with vehicle (VEH 6 h, $n=5$) or muscimol and baclofen (M+B 6 h, $n=13$). The asterisk indicates a significant difference between vehicle- and heroin-paired compartments.

heroin-free conditions. There was a significant Muscimol/Baclofen Group by Compartment interaction [$F(2,49)=10.69$, $p<0.001$] and a significant main effect of Compartment [$F(1,49)=27.67$, $p<0.001$]. Multiple comparisons identified significant heroin CPP in the rats that received vehicle infusions ($p<0.01$), and in the rats that received the muscimol/baclofen mixture in the areas surrounding the BLA ($p<0.001$). In contrast, rats infused with muscimol/baclofen in the BLA did not show a significant heroin CPP.

3.3. Experiment 2 — BLA inactivation 6 h following heroin reconditioning

Rats showed no significant spontaneous preference during habituation but, after conditioning (i.e., Test I) they displayed a significant CPP for the heroin-paired compartment (Fig. 3A; [$t(17)=-3.47$, $p<0.01$]). Fig. 3B shows the preference of the groups on Test II. The ANOVA revealed a significant main effect of Compartment [$F(1,16)=11.24$, $p<0.01$], which indicates a significant preference for the heroin-paired compartment in both groups (Fig. 3B).

4. Discussion

Recently, we have reported a method to study reacquisition of heroin seeking behavior using the conditioned place preference procedure (Leri and Rizos, 2005). After original conditioning and extinction, rats received a single session of reconditioning (one vehicle and one heroin pairing) and were tested for CPP 24 h later. In this previous study it was found that animals injected with vehicle (i.e., 0 mg/kg heroin) during reconditioning displayed no preference, indicating that the extinction protocol was effective in blocking the expression of heroin CPP. In contrast, animals that received heroin (0.3, 1 and 3 mg/kg) displayed a dose-dependent place preference, indicating that a single reconditioning session can be sufficient to induce reacquisition of extinguished CPP.

The current studies show that the BLA, but not its surrounding areas, is critical for the reacquisition of heroin CPP. In fact, it was found that intra-BLA infusions of $GABA_{A/B}$ agonists administered immediately after reconditioning blocked the reacquisition of heroin CPP. The observation that intra-BLA infusions administered 6 h following reconditioning had no significant effect supports the conclusion that the BLA is required for the consolidation of information learned during reconditioning.

Our findings are in accordance with the suggestion that the BLA plays a central role in drug relapse. It is known that cocaine self-administration induces alterations in glutamate neurotransmission in the BLA which may underlie relapse vulnerability after periods of abstinence (Lu et al., 2005). It is also known that the BLA modulates drug seeking behavior precipitated by drug-conditioned stimuli. In fact, BLA inactivation/lesions abolish the ability of a drug-conditioned context, or discrete drug-conditioned cues, to reinstate extinguished self-administration behavior (Meil and See, 1997; Fuchs et al., 2005; Fuchs and See, 2002). The results reported in the present investigation further suggest that the BLA is involved in the rapid reacquisition of drug seeking behavior underlying the process of relapse. Such a finding is important because interference with noradrenaline activity in the BLA is known to block the consolidation of emotional memories (McGaugh, 2004). Therefore, it may be of interest to explore whether noradrenergic antagonists can be effective in reducing the escalation of drug seeking induced by discrete instances of drug re-exposure following abstinence.

There is extensive evidence demonstrating the importance of the BLA in the acquisition (Everitt et al., 1991; Hiroi and White, 1991; Hsu et al., 2002; Schroeder and Packard, 2000; Fuchs et al., 2002) as well as the extinction (Fuchs et al., 2002; Schroeder and Packard, 2003, 2004) of CPP. Because it is known that extinction is a distinct learning process, and not merely the degradation of the original CS–US association (Bouton, 2002), our finding that the BLA is involved in the reacquisition of CPP suggests that this may also be a separate learning process involving the establishment of additional associative strength between the neural representations of the CS and the US.

The results of fear conditioning studies, however, suggest an alternative interpretation. In fact, while the BLA is involved in acquisition of CS–US associations (Maren and Quirk, 2004), the ventro-medial prefrontal (vmPFC) cortex is involved in the long-term retention of extinction of these associations (Santini et al., 2004; Milad and Quirk, 2002). In light of reciprocal anatomical connections and functional interactions between these regions (Berretta et al., 2005), it is possible that reconditioning may not further strengthen the CS–US association but may rather interfere with a vmPFC-mediated inhibition of responding to the extinguished CS. Although Rescorla recently showed that retraining does not weaken the strength of extinction (Rescorla, 2001), the possibility remains that drug re-exposure precipitates rapid reemergence of drug seeking behavior because of compromised response inhibition to drug-conditioned cues.

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