ABSTRACT

Reinstatement of previously extinguished instrumental responding for drug-related cues has been used as an animal model for relapse of drug abuse, and is disrupted by inactivation of the basolateral amygdala (BLA). However, the role that the BLA plays in reinstatement for natural rewards is currently unknown. In Experiments 1A and 1B, rats with bilateral cannulae implanted into either the caudal or rostral BLA were trained to press a lever to receive delivery of food reward paired with a complex light/tone conditioned stimulus (CS). Following initial training, they underwent extinction of lever pressing in the absence of the CS. Reinstatement of extinguished lever pressing was measured during response-contingent presentations of the CS alone. Rats receiving saline infusions into the caudal or rostral BLA displayed a significant increase in lever pressing during reinstatement sessions relative to their last day of extinction training. Inactivation of these subregions with bupivacaine did not attenuate responding for the CS in the absence of food delivery, and in fact, caudal BLA inactivation potentiated responding relative to vehicle controls. Analysis of within-session responding revealed that caudal BLA inactivation retarded extinction of lever pressing in response to the CS. In a separate series of experiments, inactivation of the caudal BLA on day 1 or day 2 of extinction training significantly disrupted consolidation of extinction learning on the following day. These data suggest that neural circuits which underlie cue-induced reinstatement for drug-related stimuli are different from those which mediate responding for conditioned reinforcers associated with natural rewards. Moreover, they suggest that the caudal BLA may play a role in extinction of instrumental responding for conditioned reinforcement in the absence of primary reinforcement.
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LIST OF ABBREVIATIONS

cm: Centimeter
g: Gram
hr: Hour
kg: Kilogram
mA: MilliAmp
mg: Milligram
min: Minute
mm: Millimeter
s: Second
μL: Microlitre

BLA: Basolateral Amygdala
CeA: Central Amygdala
CPP: Conditioned Place Preference
CR: Conditioned Reinforcer
CS: Conditioned Stimulus
DA: Dopamine
FR-1: Fixed Ratio-1
FR-2: Fixed Ratio-2
OFC: Orbitofrontal Cortex
VI: Variable Interval
VR-5: Variable Ratio-5
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INTRODUCTION

Drug addiction is arguably one of the most devastating and debilitating impediments facing society today. However, the most difficult problem in treating addiction is not withdrawing addicts from drugs, but preventing relapse. Although pathological drug addiction emerges in only a small proportion (15-17%) of those using drugs, it is estimated that up to 90% of those individuals experience at least one relapse episode (Anthony et al., 1994). Various external and internal stimuli often lead to increased motivation towards drug-seeking and drug-taking behavior (Childress et al., 1993; Carter & Tiffany, 1999). Human clinical data and retrospective self-reports suggest that the urge to relapse is often triggered by exposure to the self-administered drug (Meyer & Mirin, 1979; de Wit, 1996), drug-associated cues (e.g., drug paraphernalia or locations where a drug was previously consumed) (Childress et al., 1993; Carter & Tiffany, 1999), and/or stress or negative affect due to social, emotional, or physical factors (Sinha, 2001). Moreover, exposure to cocaine-associated stimuli significantly contributes to maintenance of drug-taking (Gawin, 1991; O'Brien et al., 1998) and high rates of relapse in detoxified cocaine abusers (Jaffe, 2002).

A great deal of research on the cause and pharmacotherapeutic treatment of drug relapse has focused on animal models of drug relapse. In 1981, de Wit and Stewart reported that non-contingent priming injections of cocaine or re-exposure to cocaine-paired cues reinstated lever-pressing behavior following extinction of the drug-reinforced behavior. Based on these data and those from earlier studies (Stretch et al., 1971; Davis & Smith, 1976), de Wit and Stewart suggested that their "reinstatement model" could be used to study factors involved in relapse to drugs. Broadly defined,
reinstatement refers to the resumption of a previously reinforced behavior (i.e. drug-seeking) caused by exposure to drug or non-drug stimuli, following extinction (Stewart & de Wit, 1987). This model is intuitively appealing for scientists and clinicians because those precipitants reported to provoke relapse and craving in humans also reliably reinstate drug seeking in animals. In the laboratory, reinstatement of responding in the absence of drug delivery can be achieved either by; i) exposure to the drug (de Wit & Stewart, 1981), ii) exposure to stressors (Shaham et al., 2000), or of direct relevance to the present study, iii) response-contingent presentation of a conditioned stimulus (CS) paired with drug delivery cues (cue-induced reinstatement) (Meil & See, 1997).

However, for the purpose of this paper, only research on cue-induced reinstatement will be discussed.

Converging evidence suggests that the basolateral amygdala (BLA) functions as a key component of the neural circuitry underlying cue-induced reinstatement of cocaine or heroin seeking (Ciccocioppo et al., 2001; Kruzich & See, 2001; Fuchs & See, 2002; Kantak et al., 2002, McLaughlin & See, 2003). For instance, excitotoxic lesions (Meil & See, 1997) or reversible inactivation (Grimm & See, 2000; McLaughlin & See, 2003) of the BLA profoundly attenuates the ability of cocaine-paired stimuli to reinstate extinguished lever responding. Furthermore, intra-BLA injections of the dopamine (DA) D₁-receptor antagonist SCH23390 blocks reinstatement for discrete cues associated with cocaine presentation (See, Kruzich, & Grimm, 2001), while intra-BLA infusion of amphetamine (Ledford et al., 2003) potentiates the expression of conditioned-cued reinstatement of cocaine-seeking behavior.
Recent studies have demonstrated that inactivation of the caudal and rostral subregions of the BLA produce dissociable effects on cognitive performance (Kantak et al., 2001). In addition, lidocaine-induced inactivation of the rostral BLA significantly attenuates reinstatement of drug-seeking behavior induced by cocaine-associated cues, while caudal BLA inactivation has no such effect and actually causes a slight increase in lever pressing for such cues (Kantak et al., 2002). These findings suggest that the rostral and caudal BLA are functionally heterogeneous with regard to processing associative aspects of cue-induced reinstatement. Neuroanatomical studies also lend support to this theory. Projection neurons from these regions appear to innervate distinct compartments of the nucleus accumbens core and shell (Groenewegen et al., 1990), which in turn, have dissociable control over reward-relevant behavior (Parkinson et al., 1999; Ito et al., 2000).

Reinstatement of lever pressing in response to CS associated with a drug may be viewed as a form of responding for conditioned reinforcement. The neural basis of this type of learning has been well delineated (Robbins & Everitt, 1996). Excitotoxic lesions of the BLA have been shown to reduce the control over instrumental behavior exerted by conditioned reinforcers such as cues associated with food (Burns, Robbins, & Everitt, 1993), sexually receptive females (Everitt, Cador, & Robbins, 1989), and cocaine (Whitelaw et al., 1996).

The BLA also appears to guide instrumental action in response to changes in reward value, such that BLA-lesioned rats show a significantly enhanced resistance to extinction when conditioned reinforcement (previously paired with sucrose) is omitted in a discrimination learning task (Burns, Everitt, & Robbins, 1999). Specifically, BLA-
lesioned animals are unable to recognize changes in motivational salience exerted by conditioned reinforcers associated with natural rewards in the absence of primary reinforcement (Burns et al., 1999). Inactivation of the BLA also alters behavior on tasks where the magnitude of an expected reward changes (Salinas, Packard, & McGaugh, 1993). Similarly, BLA lesions impair performance on a differential outcome procedure, such that animals are insensitive to changes in the incentive value of instrumental outcome (Balleine, Killcross, & Dickinson, 2003; Corbit & Balleine, 2005).

Thus, the BLA is essential for recognizing changes in the salience of natural conditioned reinforcers in the absence of primary reinforcement. In BLA-lesioned animals, this deficit is manifested behaviorally by increased lever pressing within an extinction session where the outcome value of conditioned reinforcement is suddenly diminished. However, the BLA is also critically involved in conditioned-cued reinstatement of cocaine-seeking. In this paradigm, inactivation of the BLA causes a decrease in lever pressing for a cue previously associated with drug reward, despite the absence of primary reinforcement. Hence, the BLA may be differentially involved in two similar types of conditioned responding. The BLA facilitates responding for a CS previously associated with drug reward while mediating the extinction of responding for a CS associated with food reward.

The BLA may be differentially involved in these types of conditioned responding because of variations between experimental paradigms. Alternatively, chronic drug exposure is known to cause long-lasting molecular, cellular, and neurochemical adaptations in the brain that underlie different facets of addiction, including prolonged relapse vulnerability after cessation of drug use (Nestler, 2001; Kalivas, 2004). Over the
course of training, repeated psychostimulant exposure may induce neuroadaptations in
the BLA and its reciprocal connections (most notably the prefrontal cortex). This in turn,
may alter the way these brain regions contribute to this form of associative learning. For
example, addiction theories based on sensitization of incentive salience propose that
"drugs sensitize brain regions that are involved in incentive salience, fractionating
natural reward by intensifying 'wanting' disproportionately, leading to compulsive drug
taking" (Kelley & Berridge, 2002, p. 3309). Thus, when psychostimulant-sensitized
animals are tested for cue-induced reinstatement, the BLA may be improperly
processing changes in the salience of the cue, causing resistance to extinction and
enhanced responding despite the absence of primary drug reinforcement.

Taken together, the goal of Experiment 1 was to assess the role of the caudal
BLA (Experiment 1A) and rostral BLA (Experiment 1B) in cue-induced reinstatement of
food-seeking behaviour. The protocol was patterned after that used by McLaughlin and
See (2003), with the exception that food was used instead of cocaine as the primary
reinforcer. Thus, results from the two studies could be compared directly. If food-
seeking behavior is unaffected or potentiated by inactivation, then the BLA may be
contributing differently to drug and food seeking, perhaps because drug-sensitized
animals responding for a drug-related cue are unable to accurately perceive changes in
its predictive value. Alternatively, if BLA inactivation attenuates reinstatement for food-
related cues in a manner similar to drug-related cues, the BLA may be critically involved
in cue-induced reinstatement irrespective of the primary reinforcer used.

Over the duration of a reinstatement test session, instrumental responding for
reward-related cues undergoes extinction. The BLA is critically involved in mediating
extinction of responding for an appetitive Pavlovian CS (Lindgren et al., 2003), or when conditioned reinforcement is omitted in a discrimination learning task (Burns et al., 1999), but less is known about the neural substrates mediating extinction of instrumental responding for cues that are no longer predictive of reward. Thus, the goal of Experiment 2 was to further characterize the role of the caudal BLA in the extinction of instrumental responding across extinction sessions on the day following the last lever-press training session (when rats received both food and CS). The caudal BLA was transiently inactivated on either day 1 (Experiment 2A) or day 2 (Experiment 2B) of extinction training to determine whether this brain region plays a role in acquisition and/or retrieval of extinction in this instrumental learning paradigm.

EXPERIMENT 1

Methods

Subjects

Male Long-Evans rats (Charles River Laboratories, Montreal, Canada) weighing 275-350g at the beginning of the study were used. Rats were individually housed in plastic cages in a temperature-controlled (21 ± 1°C) colony room on a 12hr:12hr light-dark schedule. Immediately following surgery, all rats were restricted to 85% of their free-feeding weight, with ad-libitum access to water for the duration of the experiment. All experimental testing on animals was within accordance of the Canadian Council of Animal Care and the Animal Care Committee of the University of British Columbia.

Surgery

Approximately one week following arrival, rats were anesthetized (IP) with 7 mg/kg xylazine and 100 mg/kg ketamine hydrochloride and placed into a stereotaxic
instrument (Kopf Instruments). The connective tissue overlying the skull was removed and stainless steel, 23-gauge guide cannulae were bilaterally implanted into either the caudal BLA (anterior-posterior [AP]: -3.1 mm from bregma, medial-lateral [ML]: ± 5.0 mm from midline, dorsal-ventral [DV]: -6.3 mm from dura) or the rostral BLA (AP: -2.1 mm, ML: ± 5.0 mm, DV: -5.9 mm) according to coordinates derived from the neuroanatomical atlas of Paxinos and Watson (1998). Four steel screws and dental acrylic were used to permanently affix the guide cannulae to the skull. To prevent occlusion, stainless steel stylets (30-gauge) were inserted into the guide cannulae until the time of infusion. Immediately following surgery, antibiotic ointment was applied to the skull and surrounding incision to aid in healing. All rats were given one week of recovery before behavioral testing began. During this recovery period, animals were food restricted to 85% of their free-feeding weight.

**Apparatus**

Eight operant chambers (30.5 x 24 x 21 cm; Med-Associates, St. Albans, Vt., USA) enclosed in sound-attenuating boxes were used. Boxes were equipped with a fan to provide ventilation and to mask extraneous noise. Each chamber was fitted with two retractable levers, one located on each side of a central food receptacle where food reinforcement (45 mg; Bioserv, Frenchtown, NJ) was delivered by a pellet dispenser. Two identical 100-mA stimulus lights, 2.5 cm in diameter, were located above each lever. Auditory stimuli were delivered via a speaker connected to a programmable audio generator (ANL-926, Med-Associates) located in the top-left corner of the wall opposite the levers. Four infrared photobeams were mounted on the sides of each chamber to detect locomotor activity. Each chamber was illuminated by a single 100-mA house light.
located in the top-center of the wall opposite the levers. All experimental data were recorded by a IBM® personal computer connected to the chambers via an interface (Med-Associates).

**Lever-Press and Extinction Training**

Approximately one week following surgery, rats began the first of 9 days of lever-press training. The first two days consisted of a 30-min habituation session whereby sugar pellets were presented on a variable-interval (VI) 60 schedule of reinforcement without any CS paired with food presentation. The levers were introduced on day 3 (active and inactive) and the reinforcement schedule was switched to fixed-ratio-1 (FR-1) where presentation of the food reward paired with a 5-s light-tone conditioned stimulus (CS) was contingent upon one press on the active lever. This was followed by a 20-s time-out period. Pressing the inactive lever had no programmed consequences. Before the session started, 2-3 sugar pellets were placed in the food cup and crushed on the active lever to ensure animals quickly learned to press the active lever for food. On day 4, the schedule progressed to FR-2. A variable-ratio-5 (VR-5) schedule was implemented on days 5 through 9 ensuring that rats were responding reliably on the active lever by the end of training. This schedule of partial reinforcement is known to elicit more robust responding than fixed-ratio schedules when reinforcement is withheld (Mowrer & Jones, 1945).

Following the last day of VR-5 training, rats underwent a 20-min extinction session where neither food nor light-tone CS were presented after responding on either lever. Extinction training sessions continued on subsequent days until a criterion of
<10% of their baseline responding on the VR-5 schedule was reached (~30 presses), which typically took 3-6 days.

**Cue-Induced Reinstatement and Microinfusion Procedure**

Once criterion was reached, rats underwent a 20-min reinstatement test session, where pressing the active lever elicited the presentation of the light-tone CS in the absence of food reinforcement. Just prior to reinstatement testing, separate groups of animals received bilateral infusions into either the caudal BLA (Experiment 1A) or rostral BLA (Experiment 1B) through 30-gauge injection cannulae extending 0.8 mm below the tip of the guide cannulae. The injection cannulae were connected to gastight Hamilton syringes (10 μL; Hamilton Co., Reno, Nev., USA) positioned in an infusion pump. Saline or the local anesthetic bupivacaine hydrochloride (0.75%; Abbott Laboratories, Saint Laurent, Quebec, Canada) was infused at a rate of 0.5 μL per 72 s by a microsyringe pump (Sage Instruments Model 341). Injection cannulae were left in place for an additional 1 min to allow for diffusion. Each rat remained in its home cage for a further 5 min prior to the reinstatement test session.

A within-subjects design was implemented, whereby half of the rats received saline before the first reinstatement test and bupivacaine before the second test, while the other half received the reverse order. The order of infusions was counterbalanced based on the average number of active and inactive lever presses on the last days of both VR-5 and extinction training. Test sessions were separated by at least 2 days of extinction to re-establish baseline responding.

**Histology**
After completion of behavioral testing, all rats were euthanized in a carbon dioxide chamber. Brains were removed and fixed in a 4% formalin solution. The brains were frozen and sliced in 50 μm sections before being mounted and stained with cresyl violet. Placements were verified with reference to the atlas of Paxinos and Watson (1998). Rats with cannulae placements that were not in the caudal or rostral portions of the BLA were excluded from data analysis.

**Statistical Analysis**

Responding on both active and inactive levers during reinstatement and extinction test sessions were analyzed using repeated measures analysis of variance (ANOVA), where appropriate. All significant main effects and interactions were further analyzed using Tukey’s pairwise tests.

**Results**

**Histology**

Schematic diagrams of cannulae tip placements for caudal and rostral BLA (Experiment 1A and 1B, respectively) are presented in Figure 1A. Placements were verified with reference to the neuroanatomical atlas of Paxinos and Watson (1998). All animals with placements ventral to the amygdaloid complex or encroaching the central nucleus of the amygdala (CeA) were excluded from analyses. Following histological analysis, a total of 15 caudal BLA and 9 rostral BLA rats were included in the analysis.

**Experiment 1A: Cue-Induced Reinstatement (Caudal BLA)**

By the end of VR-5 training, all animals were responding robustly on the active lever for food reinforcement (overall mean = 604.87 ± 219.87) and animals required an average of 5 days to achieve extinction criterion before the beginning of reinstatement
testing (overall mean = 26.30 ± 9.31). Both groups were evenly matched for active lever responding on the last day of VR-5 training and extinction days immediately prior to an infusion. Inactive lever presses were consistently low throughout lever-press training and extinction and no significant between- or within-group differences were found (all F's < 0.665, n.s.).

All animals received both a bupivacaine and saline counter-balanced infusion on separate reinstatement test days. Thus, it was of interest to first determine whether a treatment order effect could have compromised the use of a within-subjects design. Active lever pressing was compared between groups receiving a bupivacaine infusion immediately prior to the first versus second reinstatement session. There was not a significant effect of order [t (7) = 2.223, n.s.], indicating that animals receiving bupivacaine prior to the second reinstatement session responded similarly to those receiving bupivacaine on the first test session and vice versa. This validated the collapse of these groups and the use of one-way ANOVAs for further analyses.

Figure 2 displays active and inactive lever presses for the last day of VR-5 training, extinction sessions immediately prior to reinstatement testing, reinstatement following saline infusions, and reinstatement following bupivacaine infusions into the caudal BLA. One-way ANOVAs were used to examine active and inactive lever pressing as well as nosepoking (the number of head entries into the food receptacle), across both extinction days prior to reinstatement, vehicle reinstatement, and bupivacaine reinstatement test days. This analysis revealed a significant effect of day on active lever pressing [F (3,39) = 9.841, p < .001] but no effect on inactive lever pressing or nosepoking (both F's < 2.012, n.s.). Tukey's pairwise comparisons
confirmed that when animals received saline infusions prior to reinstatement testing, they responded significantly more on the active lever relative to the previous day of extinction (p < .05). Infusions of bupivacaine prior to reinstatement testing also resulted in higher active lever pressing rates relative to the previous day of extinction (p < .05), suggesting that inactivation of the caudal BLA does not attenuate cue-induced reinstatement of food-seeking behavior. In fact, comparison of active lever-pressing on saline versus bupivacaine test days revealed that animals actually emitted significantly more lever presses on days in which the caudal BLA was inactivated (p < .05). This result was particularly surprising because inactivation of the BLA disrupts reinstatement of responding for drug-related cues (McLaughlin & See, 2003).

To further dissect this finding, within-session active lever pressing was then examined for each of the two reinstatement test sessions, which were broken down into five 4-min epochs (Fig. 3). A 2x5 ANOVA with treatment (bupivacaine vs. saline) and epoch as two within-subjects factors revealed an overall effect for treatment [F (1,14) = 4.745, p < .05] and epoch [F (4,56) = 8.325, p < .001], but no treatment x epoch interaction [F (4,56) = 1.461, n.s.]. Although subjects in both treatment conditions achieve comparable rates of lever pressing by the end of the reinstatement session, Figure 3 illustrates a resistance to extinction especially during the first two epochs in animals that received bupivacaine infusions prior to testing. This effect warranted further investigation into the effects of caudal BLA inactivation on extinction of instrumental responding, thus we explored this finding in Experiment 2.

To ensure that any effects seen on cue-induced reinstatement with bupivacaine were not due to changes in locomotor ability, a paired samples t-test was performed
comparing locomotor ability on both reinstatement test days. As expected, there were no significant differences between bupivacaine and saline treatments \( t(14) = 0.812, \text{n.s.} \).

**Experiment 1B: Cue-Induced Reinstatement (Rostral BLA)**

As with Experiment 1A, all animals were responding robustly on the active lever for food reinforcement by the final day of VR-5 training (overall mean = 354.89 ± 167.85) and animals required an average of 5 days to achieve extinction criterion before the beginning of reinstatement testing (overall mean = 18.44 ± 5.68). Both groups were evenly matched for active lever responding on the last day of VR-5 training and extinction days immediately prior to an infusion. Inactive lever presses were consistently low throughout lever-press training and extinction, and no significant between- or within-group differences were found (all F's < 1.095, n.s.).

Analysis of active lever pressing revealed that there was not a significant effect of order \( t(4) = 2.011, \text{n.s.} \), suggesting that animals receiving bupivacaine prior to the second reinstatement session responded similarly to those receiving bupivacaine on the first test session.

Figure 4 displays active and inactive lever presses for the last day of VR-5 training, extinction sessions immediately prior to reinstatement testing, reinstatement following saline infusions, and reinstatement following bupivacaine infusions into the rostral BLA. As in Experiment 1A, one-way ANOVAs were used to examine active and inactive lever pressing and nosepoking behaviour, across four days of interest (both extinction days prior to reinstatement, vehicle reinstatement, and bupivacaine reinstatement). This analysis revealed a significant effect for day \( F(3,18) = 4.198, p < \).
.05] on active lever pressing, but not on inactive lever pressing or the number of nosepokes [both F's (3,18) < 1.095, n.s.].

Tukey's pairwise follow-up analyses confirmed that infusions of saline prior to reinstatement testing resulted in significantly more responses on the active lever relative to the previous day of extinction (p < .05). Inactivation of the rostral BLA prior to reinstatement testing also exhibited higher active lever pressing rates relative to the previous day of extinction (p < .05), suggesting that inactivation of the rostral BLA does not attenuate cue-induced reinstatement of food-seeking behavior. Lastly, active lever-pressing on vehicle test days did not significantly differ from pressing on bupivacaine test days. This finding, along with results from Experiment 1A, suggest a functional dissociation exists between the rostral and caudal BLA on cue-induced reinstatement for natural rewards, such that inactivation of the caudal BLA potentiates responding for food-related cues in the absence of primary reinforcement, while inactivation of the rostral BLA has no effect.

A paired samples t-test was performed comparing locomotor ability on both reinstatement test days and no significant differences were found between bupivacaine- and saline-infused subjects [t (7) = 1.355, n.s.). Thus, inactivation of the rostral BLA does not alter locomotor activity.

**EXPERIMENT 2**

Over the duration of a reinstatement test session, instrumental responding for reward-related cues eventually undergoes extinction, during which the animal learns that presentation of the cue is no longer a salient predictor of the reward. The results from Experiment 1 indicate that caudal-BLA-inactivated animals show a noticeable
resistance to extinction within the reinstatement test session, especially during the first two epochs. Hence, the caudal BLA facilitates reinstatement of responding for conditioned reinforcers associated with a drug reward, but potentially mediates the extinction of responding for a CS associated with food reward once these associations have been established.

To date, most of what is known about the neural basis of extinction learning is based on fear conditioning paradigms. For instance, many studies have implicated the BLA in both the acquisition and extinction of Pavlovian conditioned fear (Pare, Quirk, & LeDoux, 2004; Phelps et al., 2004). Although much less is known about the neural substrates that mediate extinction of appetitive-conditioned responses, recent studies also point to critical role for the BLA in this type of learning (Burns et al., 1999; Lindgren, Gallagher, & Holland, 2003). Inactivation of the BLA leads to enhanced responding following extinction of an appetitive Pavlovian CS (Lindgren et al., 2003), while BLA-lesioned rats show an enhanced resistance to extinction when conditioned reinforcement is omitted in a discrimination learning task (Burns et al., 1999).

With that said, the goal of Experiment 2 was to further characterize the role of the caudal BLA in the extinction of instrumental responding across extinction sessions on the day following the final day of lever-press training (when rats received both food and CS). The caudal BLA was transiently inactivated on either day 1 (Experiment 2A) or day 2 (Experiment 2B) of extinction training to determine whether the caudal BLA plays a role in acquisition, consolidation, and/or retrieval of extinction in this instrumental learning paradigm.

**Methods**
Subjects, Surgery, and Histology

Male Long-Evans rats (Charles River Laboratories, Montreal, Canada) weighing 275-350g at the beginning of the study were used. All surgical and histological procedures were performed as described in Experiment 1.

Lever-Press and Extinction Training

Separate groups of rats were trained to press the active lever for a sugar pellet reward paired with a light-tone CS using the training protocol described above in Experiment 1. Following the final day of VR-5 training, rats underwent daily 20-min extinction sessions as described above, where food reward and light-tone CS were withheld, irrespective of lever pressing activity. Separate groups of animals were matched to experimental and control conditions with regard to the average number of active and inactive lever presses emitted on the last day of VR-5 training. Rats were given microinfusions of either saline or bupivacaine hydrochloride (0.75%) into the caudal BLA on extinction day 1 (Experiment 2A) or day 2 (Experiment 2B), using the microinfusion procedure described in Experiment 1. Each rat remained in its home cage for a further 5 min prior to the extinction test session.

Extinction sessions continued on subsequent days without any further infusions, until subjects reached <10% of baseline responding on the VR-5 schedule.

Statistical Analysis

Experiment 2 utilized a between-subjects design to directly compare extinction learning across days for bupivacaine- and saline-treated animals. Responding on both active and inactive levers during extinction sessions were analyzed using repeated measures ANOVA, where appropriate. Active lever pressing on extinction days was
transformed to a percentage of baseline VR-5 responding to further compensate for any
differences between groups on baseline levels of responding. All significant main effects
and interactions were further analyzed using Tukey's pairwise tests.

Results

Histology

Schematic diagrams of cannulae implanted into the caudal BLA for Experiments
2A and 2B are presented in Figure 1B. Cannulae tip placements were verified with
reference to the neuroanatomical atlas of Paxinos and Watson (1998). All animals with
placements ventral to the amygdaloid complex or encroaching the central nucleus of the
amygdala (CeA) were excluded from analyses. Following histology, a total of n=18 and
n=19 subjects were included in the analysis for Experiment 2A and 2B, respectively.
The final number of animals per treatment condition for Experiment 2A was: n=9
(bupivacaine) and n=9 (saline). The number of animals per treatment condition for
Experiment 2B was: n=10 (bupivacaine) and n=9 (saline).

Experiment 2A: Extinction Day 1 Inactivation

By the end of VR-5 training, all animals were responding robustly on the active
lever for food reinforcement and subjects were assigned to treatment or control groups
based on the number of active lever presses emitted on the last day of VR-5 training
(bupivacaine: mean = 462.67 ± 231.56; saline: mean = 458.33 ± 195.05).

Active lever pressing on the three extinction days was examined as a percentage
of responding on the last day of VR-5 training. In order to assess the effects of caudal
BLA inactivation (day 1) on active lever pressing over these three days of interest, a 2x3
between-within ANOVA was conducted, with treatment (bupivacaine vs. saline) and day
(extinction days 1 through 3) as between and within factors, respectively (see Fig. 5). This analysis revealed a significant effect for day \[F (2,32) = 22.081, p < .0001\], no effect for treatment \[F (1,16) = 0.170, \text{n.s.}\], and a significant treatment x day interaction \[F (2,32) = 5.494, p < .01\]. One-way ANOVAs on separate bupivacaine- and saline-treated groups again revealed a significant effect of day for bupivacaine-treated \[F (2,16) = 5.869, p < .05\] and saline-treated subjects \[F (2,16) = 80.224, p < .0001\]. Tukey's post-hoc analyses for bupivacaine-treated subjects revealed no significant change in this measure from day 1 to day 2, but a significant reduction in this index from day 2 to day 3 \(p < .05\). As for saline-treated animals, active lever pressing decreased significantly from day 1 to day 2 \(p < .05\) and day 2 to day 3 \(p < .05\).

Tukey's post-hoc comparisons of active lever pressing relative to the last day of VR-5 training between bupivacaine- and saline-treated subjects on each day of extinction revealed that bupivacaine-treated subjects pressed somewhat more on day 1 compared to controls. On day 2, bupivacaine-treated subjects pressed significantly more than saline-treated subjects \(p < .05\), while active lever pressing was nearly identical across groups on day 3. Thus, bupivacaine-treated animals may have experienced a minor deficit in the acquisition of extinction learning, or alternatively, any extinction learning on day 1 may not have been properly consolidated in these animals, resulting in increased lever pressing on day 2, following treatment.

In order to assess the effects of caudal BLA inactivation on inactive lever pressing over these three days of interest, another 2x3 between-within ANOVA was conducted, with treatment (bupivacaine vs. saline) and day (extinction days 1 through 3) as between and within factors, respectively. This analysis revealed no significant effect
of treatment or day on inactive lever pressing, and no significant interaction (all F's < 2.36, n.s.).

It was also of interest to examine nosepoking behavior across extinction days for bupivacaine- and saline-treated groups to determine whether this manipulation also affected extinction of Pavlovian approach to the food receptacle (Fig. 6). A 2x3 between-within ANOVA was conducted on the number of nosepokes in a manner similar to the previous three analyses. There was no effect for day or treatment (both F's < 1.264, n.s.), however, a significant treatment x day interaction was present [F (2,32) = 6.832, p < .01].

One-way ANOVAs were performed on bupivacaine and saline groups independently to determine the nature of the interaction. The change in the number of nosepokes across days was not statistically significant for bupivacaine-treated subjects [F (2,16) = 2.209, n.s.], while nosepoking in saline-treated subjects decreased significantly across days [F (2,16) = 8.915, p < .01]. Post-hoc analyses for saline-treated subjects revealed a significant decrease in nosepoking from day 1 to day 2 (p < .05) and day 2 to day 3 (p < .05), which suggests that the number of nosepokes emitted also followed a typical extinction pattern across days in control animals.

Post-hoc comparisons revealed that although infusions of bupivacaine depressed nosepoking on day 1, this was not statistically different from saline-treated subjects. However, bupivacaine-treated subjects nosepoked significantly more than controls on day 2 (p < .05) and day 3 (p < .05). Thus, Pavlovian approach to the food receptacle was somewhat reduced in bupivacaine-treated animals on the day of inactivation, but
extinction of this behavior was significantly retarded on the two days following inactivation (as Fig. 6 illustrates).

A paired samples t-test was performed comparing locomotor ability on day 1 of extinction training (infusion day), and no significant differences were found between bupivacaine- and saline-infused subjects \[t (16) = 0.628, \text{n.s.}\]. Thus, infusion of bupivacaine into the caudal BLA did not lead to any impairment in locomotor ability.

**Experiment 2B: Extinction Day 2 Inactivation**

By the end of VR-5 training, all animals were responding robustly on the active lever for food reinforcement and subjects were assigned to inactivation and control groups based on the number of active lever presses emitted on the last day of VR-5 training (bupivacaine: mean = 561.20 ± 152.64; saline: mean = 640.56 ± 249.73) and first day of extinction (bupivacaine: mean = 342.70 ± 143.10; saline: mean = 337.67 ± 141.39).

As in Experiment 2A, active lever pressing was examined as a percentage of responding on the last day of VR-5 training. To determine the effects of caudal BLA inactivation (day 2) on active lever pressing, a 2x3 between-within ANOVA was conducted, with treatment (bupivacaine vs. saline) and day (extinction days 2 through 4) as between and within factors, respectively. This analysis revealed a significant effect for treatment \[F (1,17) = 4.973, p < .05\], but no effect for day, and no treatment x day interaction (both F's < 2.923, n.s.).

Post-hoc analyses revealed that bupivacaine-treated subjects pressed significantly more (relative to VR-5 baseline responding) than saline-treated subjects on the days following treatment (\(p < .05\)). Thus, infusions of bupivacaine again induced a
moderate disruption of extinction learning even when administered on the second day (see Fig. 7).

With regard to inactive lever pressing, there was a significant effect for day \( [F (2,34) = 4.467, p < .05] \), no effect for treatment, and no treatment x day interaction (both \( F's < 2.235, \text{n.s.} \)), indicating that animals in both groups showed changes in responding on the inactive lever that were not due to the treatment administered on day 2.

Finally, nosepoking behavior was examined across extinction days for bupivacaine- and saline-treated groups (Fig. 8). A 2x3 between-within ANOVA was conducted on the number of nosepokes using the same factors as above. There was no effect for day or treatment (both \( F's < 2.062, \text{n.s.} \)), however, a treatment x day interaction approached significance \( [F (2,34) = 2.602, p = .089] \). As can be seen from Figure 8, rats receiving bupivacaine on day 2 displayed an increase in nosepoking on day 3, similar to Experiment 2A.

One-way repeated measures analyses within each treatment group revealed no significant differences in nosepoking behavior across days for saline- or bupivacaine-treated animals (both \( F's < 2.387, \text{n.s.} \)). Post-hoc comparisons of nosepoking between bupivacaine- and saline-treated subjects on each day of extinction revealed a significant difference between groups on day 3 \( (p < .05) \), but not on day 2 (treatment day) or day 4. Thus, inactivation of the caudal BLA did not significantly alter nosepoking on the treatment day, but led to a robust increase in nosepoking on the day following the infusion, which again parallels the findings from Experiment 2A.

A paired samples t-test was performed comparing locomotor ability on day 2 of extinction training (infusion day), and no significant differences were found between
bupivacaine- and saline-infused subjects (t (17) = 0.928, n.s.). Thus, local infusion of bupivacaine into the caudal BLA did not significantly alter locomotor ability.

DISCUSSION

The primary objective of the present study was to determine the role of the BLA in reinstatement for food-related cues, with the intent to directly compare these results with previous research using drug-related cues. In Experiment 1, we have demonstrated that inactivation of the rostral BLA did not disrupt responding for a CS associated with food reward, while caudal BLA inactivation in fact enhanced responding for the CS relative to saline controls. Furthermore, subjects given bupivacaine infusions prior to testing displayed a pronounced resistance to extinction within the reinstatement session, suggesting a potential role for the caudal BLA in the extinction learning of an instrumental response, specifically when the CS no longer predicts the availability of food reward.

In Experiment 2, we inactivated the caudal BLA on day 1 or day 2 of extinction training to further dissect the aforementioned finding. Results indicated that bupivacaine-treated subjects pressed and nosepoked significantly more than saline-treated subjects on the day following treatment (day 2 or day 3, respectively) and exhibited remarkably similar response rates to the day prior to treatment, regardless of whether inactivation occurred on the first or second day. Hence, the caudal BLA appears to critically mediate the acquisition and/or consolidation (but not retrieval) of extinction learning when the previously reinforced instrumental response no longer produces the expected reward.
As a whole, this series of experiments provides further evidence for the involvement of the BLA in recognizing changes in the motivational significance of natural conditioned reinforcers when primary reinforcement is absent (i.e., when a CS or previously conditioned response becomes irrelevant as illustrated in Experiments 1 and 2, respectively).

**Experiment 1: Cue-Induced Reinstatement of Food-Seeking Behavior**

In Experiment 1, animals with temporary lesions to the caudal or rostral BLA were tested for their ability to respond for food-related cues in the absence of primary reinforcement. All subjects were able to acquire this new response (i.e., responding for conditioned reinforcement only) during the reinstatement test session, regardless of treatment condition. Moreover, inactivation of the caudal BLA prior to reinstatement testing actually potentiated responding for the conditioned reinforcer previously associated with food presentation. Superficially, these results do not appear to harmonize with the large body of research suggesting that the BLA is critically involved in the acquisition of stimulus-response associations in a variety of Pavlovian and instrumental learning paradigms (Cador, Robbins, & Everitt, 1989; Everitt, Cador, & Robbins, 1989; Hatfield et al., 1996; Whitelaw et al., 1996; Everitt et al., 1999). Specifically, BLA lesions impair acquisition of various conditioned behaviors that depend on the formation of stimulus-reward associations such as conditioned place preference (CPP) (White & McDonald, 1993), acquisition of a new response with conditioned reinforcement (Burns, Robbins, & Everitt, 1993), second-order conditioning (Hatfield et al., 1996), and instrumental learning (Baldwin et al., 2000).
Although there are variations in protocols between these forms of stimulus-response learning, the involvement of the BLA appears to be conserved. In CPP, rats are given paired presentations of a food reward with one particular context, and no food with a different context (this can also be tested in a radial arm maze; see White & McDonald, 1993). After repeated trials, animals are given a choice between these two contexts with no reward present in either context. Intact animals spend more time in the context that had been initially paired with food reward. Animals with BLA lesions show marked deficits in forming this association and consequently spend less time in that context (Everitt et al., 1991; Hiroi & White, 1991; McDonald & White, 1993; White & McDonald, 1993).

These findings from CPP studies are also complemented by studies using conditioned reinforcement. In a similar protocol to that used in Experiment 1, Burns et al. (1993) trained animals to associate the onset of the house light and the sound of the liquid dispenser (CS) with the delivery of a sucrose reward. Animals were then trained to push the appropriate magazine panel door to gain access to the sucrose on a 30-s random interval schedule. Rats were then tested for acquisition of lever pressing for CS presentation in the absence of primary food reinforcement. Quinolinate-induced lesions of the BLA significantly impaired approach to a CS predictive of sucrose reinforcement and the acquisition of lever responding for the CS, confirming that BLA lesions reduce the control over behavior exerted by conditioned reinforcement.

Similarly, the BLA is also involved in the acquisition of an instrumental response, in which an animal learns a new motor response in order to obtain a positive outcome such as food, water, or drug reinforcement. Through interactions with its environment,
animals gain information about the consequences of their actions and use that information to behaviorally modify the current environment to produce more favorable conditions (Kelley, 2004). In this paradigm, animals are placed in an operant chamber and trained to associate the presentation of a reward with a neutral CS. Once this association has been made, a lever is introduced. Through natural foraging and exploration of the lever, the animal eventually presses the lever which produces delivery of the reward. The animal ultimately learns that pressing the lever results in a favorable outcome and this action-outcome contingency becomes strengthened over a number of trials. Infusions of N-methyl-D-aspartate (NMDA) receptor antagonists into the BLA impair acquisition of this instrumental response in these animals (Baldwin et al., 2000). In addition, renewal of extinguished instrumental responding for a sucrose reward is associated with an up-regulation of c-Fos protein in the BLA (Hamlin, Blatchford, & McNally, 2006). Together, these results suggest that the BLA critically mediates the acquisition of both Pavlovian and instrumental associations.

As a whole, these findings clearly demonstrate that the acquisition of stimulus-reward associations is BLA-mediated. Yet, inactivation of the caudal BLA in the present study actually potentiated responding for the conditioned reinforcer previously associated with food, which seemingly conflicts with the body of research cited above. Nevertheless, a number of studies suggest that the BLA is also involved in recognizing changes in the motivational significance of stimulus-reward associations. The reinforcer value of these associations requires rapid updating which is then used to form more accurate object representations within the environment. Rapidly updated stimulus–value
associations that support goal-directed action and instrumental learning are mediated by the BLA.

In a study by Salinas et al. (1993), rats were trained to run a straight alley for either 1 or 10 food pellets. After 10 days of training, half the animals in the 10 pellet reward group were shifted to a one pellet reward and received intra-BLA infusions of lidocaine immediately following shifted trials. Shifted training continued for 2 more days and response latencies were compared for control and BLA-inactivated animals. Shifted animals that received intra-BLA lidocaine infusions exhibited significantly lower latencies compared to the shifted control group on the second day of shifted training (Salinas, Packard, & McGaugh, 1993). This suggests that post-training inactivation of the BLA alters behavior on tasks where the magnitude of an expected reward changes.

A number of studies employing reward reduction in behavioral contrast paradigms have reported that amygdala lesions block the response to reductions in reward magnitude (Henke, 1972; Henke, Allen, & Davison, 1972; Becker et al., 1984; Salinas, Parent, & McGaugh, 1996). Similarly, BLA lesions impair performance on a differential outcome procedure, such that animals are insensitive to changes in the incentive value of instrumental outcome (Balleine, Killcross, & Dickinson, 2003; Corbit & Balleine, 2005). In addition, when CS-potentiated feeding is tested in sated rats with BLA lesions, presentation of the CS does not stimulate feeding (Holland, Hatfield, & Gallagher, 2001; Holland, Petrovitch, & Gallagher, 2002). It is assumed that the CS has failed to acquire reinforcing properties by virtue of its association with food (Baxter & Murray, 2002). BLA-lesioned animals are also unable to recognize changes in motivational salience exerted by conditioned reinforcers associated with natural rewards.
in the absence of primary reinforcement (Burns et al., 1999). Thus, in addition to
mediating the acquisition of stimulus-reward associations, the BLA is essential for
recognizing changes in the salience of natural CRs in the absence of primary
reinforcement. In BLA-lesioned animals, this deficit may be manifested behaviorally by
increased lever pressing within an extinction session where the outcome value of
conditioned reinforcement is suddenly diminished.

In addition, recording studies from BLA neurons also point to a critical role for the
BLA in coding the value of expected outcomes (Schoenbaum, Chiba, & Gallagher,
1998; Schoenbaum, Chiba, & Gallagher, 1999; Schoenbaum et al., 2003). The BLA
shares reciprocal connections with the orbitofrontal cortex (OFC) (Carmichael & Price,
1995) and there is evidence that the BLA and OFC work cooperatively to encode
expected learning outcomes in an instrumental learning task (Schoenbaum, Chiba, &
Gallagher, 1998). Moreover, Schoenbaum et al. (2003) performed selective BLA lesions
in one group of animals and sham lesions in another and then recorded from single
OFC neurons during performance on a go-no-go olfactory discrimination task. The
experimental design allowed for analysis of neural activity after a response had been
made, but before delivery of the reinforcer, as well as during odor sampling and
reinforcer delivery. They found that BLA lesions abolished acquisition of cue-specific
responses in a subpopulation of outcome-expectant OFC neurons (Schoenbaum et al.,
2003), thus providing evidence that BLA lesions have substantial effects on neural
representations of predictive value in the OFC. It is clear that these two brain regions do
interact during learning and that, without input from the BLA, predictive value coding in
the OFC becomes somewhat more impoverished and less adaptive (O'Doherty, 2003).
The results from the present caudal BLA inactivation study appear to converge quite nicely with this body of research, especially when responding within the reinstatement test session is taken into account (see Fig. 3). When the caudal BLA is inactivated prior to reinstatement testing, analysis of within-session responding reveals an enhanced resistance to extinction, especially during the first half of the session. These animals initially respond as if the CS is still a salient predictor of the food reward, and consequently take longer to acquire the "CS=no food" association, resulting in the potentiation of responding relative to saline controls described above. Therefore, the caudal BLA appears to be required for extinction of responding within a reinstatement session when the cue is no longer a salient predictor of an upcoming food reward. Furthermore, this adds to the body of evidence suggesting that the BLA is involved in recognizing changes in the motivational significance of stimulus-reward associations.

Although this may explain why inactivation of the caudal BLA potentiates cue-induced food seeking, it does not clarify the discrepancy between our results and the established literature on reinstatement for drug-related cues. The BLA functions as a key component of the neural circuitry underlying cue-induced reinstatement of cocaine or heroin seeking (Ciccocioppo et al., 2001; Kruzich & See, 2001; Fuchs & See, 2002; Kantak et al., 2002, McLaughlin & See, 2003). For instance, excitotoxic lesions (Meil & See, 1997) or reversible inactivation (Grimm & See, 2000; McLaughlin & See, 2003) of the BLA profoundly attenuates the ability of cocaine-paired stimuli to reinstate extinguished lever responding.

In addition, inactivation of the rostral BLA significantly attenuates this form of reinstatement, while caudal BLA inactivation slightly enhances this form of responding
Conversely, our results indicate that transient inactivation of the rostral BLA does not alter cue-induced reinstatement for food-related cues. It is becoming increasingly apparent that the rostral and caudal BLA are functionally heterogeneous with regard to processing associative aspects of cue-induced reinstatement, and our findings provide further evidence of a functional dissociation between these two subregions. Additionally, the rostral and caudal BLA appear to have differing roles based on the type of reinforcer used, which could have implications for studying the function of these two subregions in reward-related behavior.

Considering the established role of the BLA in recognizing changes in reward salience, it seems peculiar that inactivation of the BLA would attenuate responding for drug-related cues, especially if these animals are unable to re-evaluate the salience of the cue-reward association during the reinstatement session. One possible explanation for this discrepancy comes from research on the neuromodulatory effects of psychostimulants such as amphetamine and cocaine. Repeatedly exposing animals to these psychostimulants induces neuroadaptations in the mesocorticolimbic DA system which in turn, alters the way these brain regions contribute to this type of associative learning (Kelley & Berridge, 2002). Therefore, the differences between our findings and other reinstatement research may be explained by their use of chronic cocaine exposure during training, which may have resulted in neuromodulation within the BLA and its reciprocal connections. For example, addiction theories based on sensitization of incentive salience propose that drugs sensitize brain regions such as the BLA that are involved in incentive salience, fractionating natural reward by intensifying 'wanting' disproportionately, leading to compulsive drug taking (Kelley & Berridge, 2002). Thus,
when psychostimulant-sensitized animals are tested for cue-induced reinstatement, the BLA may be improperly processing changes in the salience of the cue, causing resistance to extinction and enhanced responding despite the absence of primary drug reinforcement. Therefore, in order to understand the brain mechanisms involved in cue-induced drug relapse, we must first acquire a better understanding of how critically involved brain regions (such as the BLA) contribute to reward learning in general.

Lastly, it should be noted that drug reinforcers are delivered differently than food reinforcers. Drug reinforcement involves passive administration of intravenous drug reward following an instrumental response, while food reinforcement engages a sequence of movements (i.e., Pavlovian approach) that lead to active consumption of the food reward. In this sense, instrumental responding for a drug reward may be more akin to responding for intracranial self-stimulation, which directly activates similar brain circuits triggered by drugs of abuse. By determining the latency between the response and the perceived effects of the drug, the route of administration partially determines several drug effects, including those that allow a substance to act as a reinforcer (Sanchis-Segua & Spanagel, 2006). Therefore, this discrepancy in amygdalar involvement with respect to responding for drug- vs. food-related cues may be a consequence of reinforcer administration differences. Nevertheless, there are currently no viable means of mimicking drug reinforcement by passively administering a food reward without somewhat compromising the integrity of the reinforcer.

**Experiment 2: Extinction of Instrumental Responding**

Experiment 1 demonstrated a noticeable resistance to extinction within the reinstatement test session when the caudal BLA was inactivated, signifying that this
region of the BLA mediates the extinction of responding for a CS associated with food reward once these associations have been established. Based on this finding, the aim of Experiment 2 was to further characterize the role of the caudal BLA in the extinction of instrumental responding across extinction sessions. When the caudal BLA was inactivated on the first day of extinction learning following VR5 training, bupivacaine-treated subjects pressed somewhat more on day 1 compared to controls. On day 2, bupivacaine-treated subjects pressed significantly more than control subjects, while lever pressing was nearly identical for the two groups on day 3. Figure 5 reveals that bupivacaine-treated subjects also did not show any decrease in active lever pressing from day 1 to day 2. Collectively, these data suggest that bupivacaine-treated animals may have experienced a deficit in the acquisition of extinction learning. Alternatively, any extinction learning on day 1 may not have been properly consolidated in these animals, resulting in similar lever pressing rates on the following day.

In Experiment 2B, the caudal BLA was inactivated on the second day of extinction learning and bupivacaine-treated subjects pressed significantly more than control animals on the day following treatment. Thus, infusions of bupivacaine again induced a moderate disruption of extinction learning even when administered on the second day. Figure 7 shows that on day 2, active lever pressing did not increase to day 1-like levels in bupivacaine-treated subjects, suggesting that any retrieval of extinction-related learning from day 1 is not affected by inactivation of the caudal BLA. However, these animals show a deficit in the acquisition of extinction learning on day 2. Furthermore, any extinction learning on day 2 may not have been properly consolidated, resulting in increased lever pressing on day 3. Together with the findings from
Experiment 2A, these results suggest that the caudal BLA may mediate the acquisition and/or consolidation (but not retrieval) of this form of extinction learning.

Although much less is known about the neural substrates that mediate extinction of appetitive-conditioned responding, accumulating research points to a critical role for the BLA in this type of learning (Burns et al., 1999; Balleine, Killcross, & Dickinson, 2003; Lindgren, Gallagher, & Holland, 2003). In monkeys, aspiration removals of the amygdala were reported to retard extinction of instrumental responding (Weiskrantz, 1956). BLA-lesioned rats show a significantly enhanced resistance to extinction when conditioned reinforcement (previously paired with sucrose) is omitted in a discrimination learning task (Burns, Everitt, & Robbins, 1999). Also, BLA lesions lead to a reduction in the sensitivity of instrumental performance to post-training changes in outcome devaluation (Balleine et al., 2003). Rats with amygdala lesions also behave more impulsively in a temporal discounting task, choosing fewer large, delayed rewards and more small, immediate rewards relative to controls (Winstanley, et al. 2004).

Of direct relevance to the present study, Lindgren et al. (2003) examined the role of BLA in the extinction of instrumental responding for a Pavlovian CS previously associated with food. During initial training, rats learned to associate the presentation of food with a visual CS. Following training, rats received either BLA- or sham-lesions and underwent extinction training where the visual CS was presented in the absence of food. When the BLA was lesioned, extinction training did not reduce responding for the visual CS, nor did it reduce the ability of the CS to reinforce second-order conditioning of an auditory stimulus. Hence, the BLA appears to be critical for both acquisition (Hatfield et al., 1996; Setlow et al., 2002) and extinction (Lindgren et al., 2003), but not
maintenance of a previously established value (Setlow et al., 2002; Lindgren et al., 2003). Together, these results demonstrate that animals with impaired amygdala functioning are more impulsive and unable to adapt to changes in the incentive value of a reinforcer. This ultimately causes a resistance to extinction when the CS or primary reinforcer is omitted or devalued, as in Experiment 2.

Lastly, it should be noted that in the present study, inactivation of the caudal BLA on day 1 or day 2 of extinction training led to a similar deficit in nosepoking to that seen for instrumental responding in the absence of reinforcement (see Fig. 6 and 8). It is particularly important to consider the effects of BLA inactivation on nosepoking and its relation to other events, because this behavior may represent the role of Pavlovian influences on instrumental responding. In other words, nosepoking is often considered a measure of the associative strength between the food reward and the familiar sound of food pellet delivery (Andrzejewski, Spencer, & Kelley, 2005). Since both Pavlovian approach to the food cup and instrumental lever responding were slower to extinguish in caudal-BLA-inactivated animals, it can be postulated that extinction of these behaviors is equally sensitive to manipulations of the caudal BLA, regardless of their associative nature (e.g., Pavlovian vs. instrumental).

The results from Experiment 2 suggest that caudal-BLA-inactivated animals may have experienced a deficit in the acquisition of extinction learning. On the other hand, the data can also be interpreted as a deficit in consolidation, since any extinction learning on the inactivation day may not have been properly consolidated, resulting in similar lever pressing rates on the following day. Using this paradigm, it is often difficult to differentiate between a deficit in the acquisition of an extinguished instrumental
response and consolidation of extinction memories. In most cases, acquisition is cumulative in that it does not occur over one trial, often taking a number of days. Given the role of the BLA in the extinction of appetitive- and fear-conditioned responding (Falls et al., 1992; Lee & Kim, 1998; Lu et al., 2001; Lindgren et al., 2003), one could logically assume that acquisition of this extinction response is being affected by caudal BLA inactivation. In opposition, the memory consolidation hypothesis (McGaugh, 2000; Dudai, 2004), claims that newly formed memories are initially present in a "labile" state, but the memory can become consolidated and resistant to disruption over time. The BLA is known to mediate the consolidation of appetitive associations (Vazdarjanova & McGaugh, 1999) and is involved in the consolidation of extinction learning in conditioned fear (Akirav, Raizel & Maroun, 2006) and place preference (Schroeder & Packard, 2002) paradigms. We postulate that extinction memories formed in an instrumental learning paradigm on day 1 (Experiment 2A) and day 2 (Experiment 2B) undergo consolidation which may be disrupted by caudal BLA inactivation on either of these days.

Concluding Remarks

In conclusion, the caudal and rostral BLA appear to be differentially involved in cue-induced reinstatement depending on the nature of the CS involved (natural vs. artificial). The caudal BLA facilitates reinstatement of responding for conditioned reinforcers associated with a drug reward, but mediates the extinction of responding for a CS associated with food reward, which is consistent with previous literature concerning conditioned responding for natural rewards. Furthermore, we have demonstrated that the caudal BLA is also critically involved in the acquisition and/or
consolidation (but not retrieval) of extinction memory when the previously reinforced instrumental response no longer produces the expected reward. As a whole, these experiments provide further evidence for the involvement of the BLA in recognizing changes in the motivational significance of natural conditioned reinforcers when primary reinforcement is absent (i.e., when a CS or previously conditioned response becomes irrelevant as illustrated in Experiments 1 and 2, respectively). Taken together, these studies have significantly contributed to our understanding of the similarities and differences in the neural circuitries that regulate instrumental learning for natural and drug rewards. In turn, this knowledge may facilitate the development of new animal models for drug-seeking behavior, which can potentially be applied to the phenomenon of drug relapse in humans.
Figure 1. Schematic of coronal sections of the rat brain showing placement of cannula tips for all rats receiving infusions into the caudal or rostral BLA for Experiments 1 and 2. Brain sections correspond to the atlas of Paxinos and Watson (1998).
Figure 2. Active (hatched bars) and inactive (black bars) lever responses on the last day of VR-5 training, extinction sessions prior to saline [EXT (SAL)] and bupivacaine [EXT (BUPI)] reinstatement testing, and cue-induced reinstatement sessions following bilateral bupivacaine (BUPI) or saline (SAL) infusion into the caudal BLA. Active lever pressing on reinstatement sessions following saline and bupivacaine infusions was significantly higher relative to prior extinction sessions ( * p < .05). Furthermore, active lever presses on the reinstatement session following bupivacaine inactivation were significantly higher than responses following saline infusion († p < .05).
Figure 3. Within-session active lever pressing across four 5-min epochs on reinstatement sessions immediately following bilateral infusions of bupivacaine (black circles) or saline (white squares) into the caudal BLA. Although subjects in both treatment conditions achieve comparable rates of lever pressing by the end of each reinstatement session, bupivacaine-treated subjects exhibit a resistance to extinction especially during the first two epochs.
Figure 4. Active (hatched bars) and inactive (black bars) lever responses on the last day of VR-5 training, extinction sessions prior to saline [EXT (SAL)] and bupivacaine [EXT (BUPI)] reinstatement testing, and cue-induced reinstatement sessions following bilateral bupivacaine (BUPI) or saline (SAL) infusion into the rostral BLA. Active lever pressing on reinstatement sessions following saline and bupivacaine infusions was significantly higher relative to prior extinction sessions ( * p < .05).
Figure 5. Panel A shows active lever pressing on the last day of VR5 training for treatment and control groups. Panel B illustrates the effect of caudal BLA inactivation (day 1) on active lever pressing (as a percentage of responding on the last day of VR-5 training) over the first three days of extinction training. Saline-treated animals showed a significant decrease in active lever pressing (relative to the last day of VR-5 training) from day 1 to day 2 (* p < .05) and day 2 to day 3 (* p < .05). As for bupivacaine-treated subjects, active lever pressing did not change from day 1 to day 2 but decreased significantly from day 2 to day 3 (* p < .05). Bupivacaine-treated subjects also pressed significantly more (relative to VR-5 baseline responding) than saline-treated subjects on the day following treatment (day 2) († p < .05).
Figure 6. The effect of caudal BLA inactivation (day 1) on nosepoking behavior over the first three days of extinction training. Saline-treated animals showed a significant decrease in the number of nosepokes from day 1 to day 2, and day 2 to day 3 (* p < .05), suggesting typical extinction learning occurred across days. Bupivacaine-treated subjects showed no differences in nosepoking across days, but nosepoked significantly more than saline-treated subjects on the two days following treatment (days 2 and 3) († p < .05).
Figure 7. Panel A shows the number of active lever presses for treatment and control groups on the last day of VR5 training and extinction day 1. Panel B illustrates the effect of caudal BLA inactivation (day 2) on active lever pressing (as a percentage of responding on the last day of VR5 training) over the first 4 days of extinction training. Saline-treated animals pressed significantly less than bupivacaine-treated subjects across days 2 through 4 (* p < .05).
Figure 8. The effect of caudal BLA inactivation (day 2) on nosepoking behavior over the first 4 days of extinction training. Both saline- and bupivacaine-treated animals showed no significant difference in nosepoking across days. However, bupivacaine-treated subjects nosepoked significantly more than saline-treated subjects on the day following treatment (day 3) (* p < .05).
REFERENCES


Appendix

ANIMAL CARE CERTIFICATE

Application Number: A06-0300

Investigator or Course Director: Stan Floresco

Department: Psychology, Department of

Animals: Rats Long Evans 160
         Rats Sprague Dawley 80

Start Date: July 1, 2006

Approval Date: July 7, 2006

Funding Sources:

Funding Agency: Canadian Institutes of Health Research
Funding Title: Functional interactions between basolateral amygdala and mesocortical dopamine inputs to the medial prefrontal cortex: electrophysiological and behavioral analyses

Funding Agency: Canadian Institutes of Health Research
Funding Title: Alternations in amygdala-prefrontal cortex circuitry by repeated psychostimulants: Electrophysiological and behavioural analyses

Unfunded title: n/a

The Animal Care Committee has examined and approved the use of animals for the above experimental project.
This certificate is valid for one year from the above start or approval date (whichever is later) provided there is no change in the experimental procedures. Annual review is required by the CCAC and some granting agencies.

A copy of this certificate must be displayed in your animal facility.

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