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THE ROLE OF DIFFERENT SUBREGIONS OF THE BASOLATERAL AMYGDALA IN CUE-INDUCED REINSTATEMENT AND EXTINCTION OF FOOD-SEEKING BEHAVIOR

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Abtract—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 induced by cues associated with natural rewards is unclear. The present study assessed the effects of inactivation of different regions of the BLA in cue-induced reinstatement of food-seeking behavior and in the extinction of instrumental responding for food. In experiment 1, rats acquired a lever pressing response for food reward paired with a light/tone conditioned stimulus (CS). They were then subjected to extinction training, where both food and the CS were withheld. Reinstatement of extinguished responding was measured during response-contingent presentations of the CS alone. Following saline infusions into the caudal or rostral BLA, rats displayed a significant increase in lever pressing during reinstatement sessions. Inactivation of these subregions with bupivacaine did not attenuate responding for the CS in the absence of food delivery. In fact, inactivation of the caudal BLA potentiated responding relative to vehicle treatments. Analysis of within-session responding revealed that caudal BLA inactivation retarded extinction of lever pressing in response to the CS. In experiment 2, inactivation of the caudal BLA on the first or second day of extinction training significantly retarded the acquisition of extinction learning on the following day. These data indicate that that the caudal BLA may play a specific role in the extinction of appetitive conditioned responses, by monitoring changes in the reinforcing value of pavlovian conditioned stimuli linked to action–outcome associations once these associations have been formed. Moreover, these findings support a growing body of evidence indicating that separate neural circuits incorporating the BLA may play different roles in mediating reinstatement of reward-seeking behaviors induced by either drug or food related stimuli.

Key words: pavlovian conditioning, instrumental learning, drug addiction, relapse, rat.

Since the seminal study by de Wit and Stewart (1981), there has been a growing interest in the neural circuitries that mediate reinstatement of drug-seeking behavior induced by drug-related cues. Converging evidence suggests that the basolateral amygdala (BLA) is a key brain region that mediates this form of reinstatement of cocaine- or heroin-seeking behavior (Ciccocioppo et al., 2001; Kruzich and See, 2001; Fuchs and See, 2002; Kantak et al., 2002; McLaughlin and See, 2003). Excitotoxic lesions (Meil and See, 1997) or reversible inactivation (Grimm and See, 2000; Kantak et al., 2002; McLaughlin and See, 2003) of the BLA attenuates the ability of cocaine-paired stimuli to reinstate extinguished instrumental responding. Furthermore, the caudal and rostral subregions of the BLA may play dissociable roles in cue-induced reinstatement. Kantak et al. (2002) reported that lidocaine infusions into the more rostral regions of the BLA significantly attenuated reinstatement of drug-seeking behavior induced by a discriminative cocaine-associated cue. However, similar infusions into the caudal BLA had no such effect and actually caused a slight increase in lever pressing upon presentation of a drug-related conditioned stimulus (CS) alone, although this manipulation did block reinstatement induced by concurrent CS and cocaine prime delivery. Thus, the BLA plays an integral role in mediating reinstatement of instrumental responding by drug-associated cues, which may be viewed as a form of responding for conditioned reinforcement.

On the surface, the involvement of the BLA in reinstatement by drug-related cues is not surprising, considering the vast literature implicating this nucleus in the acquisition of stimulus–reward associations using natural reinforcers such as food (Robbins and Everitt, 1996; Baxter and Murray, 2002). For example, lesions of the BLA block the formation of a conditioned place preference for food (Everitt et al., 1991; McDonald and White, 1993). BLA lesions also impair the acquisition of a new instrumental response for conditioned reinforcement, where intact animals press a lever for presentation of a classically conditioned CS associated with food reward (Burns et al., 1993). However, the BLA also appears to guide instrumental action in response to changes in reward value after the original stimulus–reward associations have been acquired. BLA-lesioned rats show resistance to extinction when conditioned reinforcement is omitted in a discrimination learning task, and are unable to recognize changes in the motivational salience of conditioned reinforcers associated with food rewards in the absence of primary reinforcement (Burns et al., 1999; Lindgren et al., 2003). In a similar vein, BLA lesions impair performance on a differential outcome procedure, such that animals are insensitive to changes in the incentive value of instrumental outcome (Balleine et al., 2003; Corbit and Balleine, 2005). These findings indicate

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Abbreviations: BLA, basolateral amygdala; CS, conditioned stimulus; FR, fixed ratio; VR, variable ratio.

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that the BLA mediates the initial formation of stimulus–reward associations for natural reinforcers, and also plays a role in recognizing changes in the relative salience of conditioned reinforcers after the initial associations have been formed.

With respect to the cue-induced reinstatement model, it is notable that the presentation of drug-related stimuli induces an increase in previously extinguished instrumental responding that no longer results in drug delivery. Thus, rats also learn about changes in the relevance of the drug-related CS and the instrumental response. These tests may be viewed as a form of extinction, where animals learn that the CS associated with the response no longer predicts delivery of primary reinforcement. As noted above, lesions of the BLA retard the extinction of conditioned responding induced by stimuli paired with food reward, where animals are slower to cease responding when the CS and/or instrumental response is no longer paired with food delivery (Burns et al., 1999; Lindgren et al., 2003). Yet, lesions or inactivation of the BLA exerts the opposite effect during reinstatement tests for drug-related cues, as these manipulations block the increase in previously-extinguished responding induced by drug-related cues (Kruzich and See, 2001; Kantak et al., 2002; McLaughlin and See, 2003). Hence, the BLA may be differentially involved in these two types of conditioned responding, whereby it facilitates responding for a CS previously associated with drug reward but mediates extinction of responding for a CS associated with food reward.

In order to explore these issues further, the present study was designed to investigate the role of the BLA in reinstatement of previously extinguished responding induced by stimuli associated with a natural reinforcer (i.e.; food-seeking behavior, experiment 1). We used an experimental protocol patterned after that used by McLaughlin and See (2003), who observed that inactivation of the BLA blocked cue-induced reinstatement for cocaine-related stimuli. By using a similar protocol, we would be able to compare the role of the BLA in reinstatement of responding for food and drug related stimuli. We also undertook a second study (experiment 2) that assessed the effects of BLA inactivation on extinction of instrumental responding, to further characterize the role of this nucleus in mediating this type of learning.

EXPERIMENTAL PROCEDURES

Experiment 1

Animals. Male Long-Evans rats (Charles River Laboratories, Montreal, QC, Canada) weighing 275–350 g were used. Upon initial arrival into the colony, rats were group housed in plastic cages in a temperature-controlled colony room on a 12-h light/dark schedule, with *ad libitum* access to water for the duration of the experiment. All testing was in accordance of the Canadian Council of Animal Care and the Animal Care Committee of the University of British Columbia. All efforts were made to minimize the number of animals used and their suffering.

Surgery. Rats were anesthetized with 100 mg/kg of ketamine hydrochloride and 7 mg/kg xylazine, and implanted with bilateral 23 gauge stainless-steel guide cannulae. In our initial experiment (experiment 1A), animals were implanted with one pair of cannulae into the more caudal regions of the BLA (flat skull AP$=-3.1$ mm from bregma, ML$=\pm5.0$ mm from midline, DV$=-6.3$ mm from dura). In a subsequent experiment (experiment 1B), a second group of animals was implanted with identical cannulae in the rostral BLA (AP$=-2.1$ mm, ML$=\pm5.0$ mm, DV$=-5.9$ mm) (Paxinos and Watson, 1998).

Four steel screws and dental acrylic were used to permanently affix the guide cannulae to the skull. 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. All rats were given 1 week of recovery before behavioral testing began. During this recovery period, animals were food restricted to 85% of their free-feeding weight, and maintained on food restriction for the entire duration of the experiment.

Apparatus. Eight operant chambers (30.5×24×21 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, USA) 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. Each chamber was illuminated by a single 100-mA house light located in the top-center of the wall opposite the levers. Four infrared photobeams were mounted on the sides of each chamber, and another photobeam was located in the food receptacle. Locomotor activity was indexed by the number of photobeam breaks that occurred during a session. Similarly, approaches toward the food cup (nosepokes) were assessed using the number of beam breaks of the photobeam located in the food receptacle. All experimental data were recorded by an IBM personal computer connected to the chambers via an interface.

Lever pressing and extinction training. We patterned our training and testing protocols as closely as possible to that used by McLaughlin and See (2003), taking into account differences in the manner animals respond for food versus cocaine reinforcement. Approximately 1 week following surgery, rats were introduced to the testing apparatus. The first 2 days consisted of 30-min familiarization sessions where reward pellets (Bioserv) were dispensed on a variable-interval 60 schedule of reinforcement with no CS paired with food presentation. On the following day, rats received the first of seven, 20 min lever pressing training sessions. These were shorter than the 3 h sessions used by McLaughlin and See (2003) to ensure that rats did not become sated during these training sessions, as rats display higher rates of lever pressing for food versus cocaine reward. Here, both levers were introduced with one of the levers designated the active and the other the inactive lever (the side counterbalanced across animals). Before the animal was placed in the chamber, two to three pellets were placed in the food cup and crushed on the active lever to facilitate the learning of the instrumental response. On the first day of lever press training, the reinforcement schedule was set to a fixed-ratio-1 (FR-1) where presentation of the food reward was contingent upon one press on the active lever. Delivery of a pellet was always paired and preceded by a 5 s light–tone CS, which entailed illumination of the stimulus light above the active lever and presentation of an 80 dB, 3 kHz tone. This was followed by a 20-s time-out period, where pressing the active lever did not result in food/CS delivery. Pressing the inactive lever had no programmed consequences. On the second day, the schedule was increased to an FR-2. A variable-ratio-5 (VR-5) schedule was implemented on days 3 through 7, ensuring that rats were re-