Neuroscience 154 (2008) 877–884

OPPOSING ROLES FOR THE NUCLEUS ACCUMBENS CORE AND SHELL IN CUE-INDUCED REINSTATEMENT OF FOOD-SEEKING BEHAVIOR

S. B. FLORESCO,* R. J. McLAUGHLIN AND D. M. HALUK

Department of Psychology and Brain Research Centre, University of British Columbia, 2136 West Mall, Vancouver, BC, Canada V6T 1Z4

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 differentially affected by inactivation of the core and shell subregions of the nucleus accumbens (NAc). To compare the roles of these subregions in reinstatement induced by cues associated with natural and drug rewards, the present study assessed the effects of inactivation of the NAc core and shell on cue-induced reinstatement of food-seeking behavior. Rats acquired a lever pressing response for food reward paired with a light/tone conditioned stimulus (CS). They were then subjected to extinction, where both food and the CS were withheld. Reinstatement of responding was measured during response-contingent presentations of the CS. Following saline infusions into the NAc core or shell, rats displayed a significant increase in lever pressing during reinstatement sessions. Inactivation of the core, induced by infusion of GABA agonists muscimol and baclofen, attenuated responding for the CS, but did not affect pavlovian approach toward the food receptacle. In contrast, inactivation of the shell had the opposite effect, potentiating responding relative to vehicle treatments. These data suggest that the NAc core and shell play opposing, yet complementary roles in mediating the influence that food-associated conditioned stimuli exert over behavior. The core enables reward-related stimuli to bias the direction and vigor of instrumental responding. In contrast, the shell facilitates alterations in behavior in response to changes in the incentive value of conditioned stimuli. The fact that the NAc core appears to play a similar role in cue-induced reinstatement induced by both natural and drug rewards suggests that this region of the ventral striatum may be a final common pathway through which both drug- and food-associated stimuli may influence the direction and magnitude of ongoing behavior.

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

Exposure to drug-associated stimuli is a major factor that contributes to relapse of drug seeking and taking in human addicts. Cue-induced reinstatement paradigms have served as a useful tool in delineating the neural circuits that mediate the ability of drug-associated cues to induce relapse of drug-seeking behavior in experimental animals. Increases in previously extinguished instrumental responding induced by presentation of drug-related stimuli are mediated by interconnected neural networks which include the basolateral amygdala (BLA), the medial prefrontal cortex (PFC) and the nucleus accumbens (NAc) core (Kantak et al., 2002; McLaughlin and See, 2003; Fuchs et al., 2004). These findings are in keeping with imaging studies in human addicts, where presentation of drug-related stimuli activates these same brain regions and increases subjective ratings of drug craving (Childress et al., 1999; Kilts et al., 2004).

Cue-induced reinstatement tests share similarities with tests of pavlovian-to-instrumental (PIT) transfer, where a classically conditioned stimulus (CS) can invigorate instrumental responding. It is important to note that there are key differences in the cortico-limbic-striatal circuits that mediate instrumental responding induced by drug related stimuli, compared with cues associated with natural reinforcers (e.g. food). For example, the BLA has been shown repeatedly to mediate cue-induced reinstatement for cocaine-related stimuli (Kantak et al., 2002; McLaughlin and See, 2003). However, using a similar protocol, we have observed that inactivations of different subregions of the BLA either do not affect, or actually potentiate reinstatement of responding induced by food-related cues (McLaughlin and Floresco, 2007). Thus, to obtain a comprehensive understanding of how the brain processes drug-related cues that may facilitate relapse, it is critical to understand the similarities and differences in neural circuits that mediate the conditioned reinforcing properties of cues associated with either natural or drug rewards.

As noted above, the NAc plays a critical role in mediating the ability of drug-related cues to induce cravings in humans, and in reinstatement of responding for cocaine-associated stimuli in animals. The role of different subregions of the NAc in mediating the ability of food-related conditioned stimuli to influence instrumental behavior has been studied in some detail, although there are some discrepancies as to which subregions are involved in the phenomenon. Thus, lesions of either the NAc core (Hall et al., 2001; de Borchgrave et al., 2002) or shell (Corbit et al., 2001) made prior to training have been reported to disrupt the ability of pavlovian cues to invigorate instrumental responding. Moreover, the precise roles of these subregions in mediating reinstatement of previously extinguished responding induced by food-related cues after pavlovian and instrumental contingencies have been acquired remains unclear. To explore these issues further, we

*Corresponding author. Tel: +1-604-827-5313; fax: +1-604-822-6923.
E-mail address: floresco@psych.ubc.ca (S. B. Floresco).
Abbreviations: AP, anteroposterior; BLA, basolateral amygdale; CS, conditioned stimulus; DV, dorsoventral; ML, mediolateral; NAc, nucleus accumbens; PFC, prefrontal cortex; PIT, pavlovian-to-instrumental transfer; VR, variable ratio.

0306-4522/08$32.00+0.00
doi:10.1016/j.neuroscience.2008.04.004

examined the role of the core and shell regions of the NAc in reinstatement of previously extinguished responding induced by stimuli associated with a natural reinforcer (i.e.; food-seeking behavior). Our experimental protocol was similar to that used by Fuchs et al. (2004), who observed that inactivation of the NAc core, but not shell abolished reinstatement induced by cocaine-related stimuli.

EXPERIMENTAL PROCEDURES

Animals and surgery

Male Long-Evans rats (Charles River Laboratories, Montreal, Canada) weighing 275–350 g were used. Rats were acclimatized to the colony for 1 week prior to surgery. All surgical procedures and testing were conducted in accordance with international guidelines and of the Canadian Council of Animal Care and all efforts were made to minimize the suffering and number of animals used. Two groups of rats were anesthetized with 100 mg/kg of ketamine and 7 mg/kg xylazine, and implanted with bilateral 23 gauge stainless-steel guide cannulae into either the NAc core (flat skull: anteroposterior (AP)=+1.6 mm from bregma, mediolateral (ML)=±1.8 mm from midline, dorsoventral (DV)=−6.8 mm from dura) or NAc shell (flat skull: AP=+1.3 mm, ML=±1.0 mm DV=−6.2 mm) (Paxinos and Watson, 1998). Four steel screws and dental acrylic affixed 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 (Med-Associates, St. Albans, VT, USA) enclosed in sound-attenuating boxes were used. Chambers were fitted with two retractable levers, one located on each side of a central food receptacle where food reward pellets (45 mg; Bioserv, Frenchtown, NJ, USA) were delivered by a dispenser. Two identical 100-mA stimulus lights were located above each lever. Auditory stimuli were delivered via a speaker connected to a programmable audio generator located in the top-left corner of the wall opposite the levers. Each chamber was illuminated by a 100-mA house light located on the wall opposite the levers. Four infrared photobeams were mounted on the sides of each chamber, with another photobeam 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 (nose pokes) 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 used a training protocol identical to that we have described previously (McLaughlin and Floresco, 2007), that was patterned as closely as possible to that used by McLaughlin and See (2003) and Fuchs et al. (2004), 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 were dispensed on a variable-interval 60 s schedule, 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 Fuchs et al. (2004) 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 inserted, with one lever 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 where food was delivered after one active lever press. Food delivery was always preceded by a 5 s light-tone CS (illumination of the stimulus light above the active lever and presentation of an 80 dB, 3 kHz tone), followed by a 20-s time-out period, where lever presses did not result in food/CS delivery. Pressing the inactive lever had no programmed consequences. On the second day, the schedule was increased to a fixed ratio-2. A variable-ratio-5 (VR-5) schedule was implemented on days 3 through 7, ensuring that rats were responding reliably on the active lever by the end of training.

Following the last day of VR-5 training, rats underwent daily 20-min extinction sessions where neither food nor the light-tone CS was presented after responding on either lever. Extinction sessions continued on subsequent days until they reached an extinction criterion. This was achieved when a rat responded on the active lever with fewer than 10% of presses relative to the last day of training on the VR-5 schedule compared with its own and the group's mean number of presses (~30 presses). Thus, if on the last day of lever press training for food, an individual rat emitted 400 presses, criterion performance was achieved when it made less than 40 presses during an extinction session. Rats typically took 4–7 days to reach this criterion.

Cue-induced reinstatement and microinfusion procedure

On the day after extinction criterion was reached, a rat was subjected to the first of two 20-min reinstatement test sessions. Here, pressing the active lever elicited the presentation of the light-tone CS in the absence of food reinforcement on a VR-5 schedule, although the first CS was presented after one press. Prior to a reinstatement session, rats in each respective group received bilateral infusions into the NAc core or shell. Inactivation of NAc subregions was achieved by infusion of a drug solution containing the GABA A agonist muscimol (Sigma Aldrich Canada, Oakville, Ontario, Canada) and the GABA B agonist baclofen (Sigma Aldrich). Each drug was mixed separately at a concentration of 500 ng/μl, and then combined in equal volumes so that the final concentration of each compound in solution was 250 ng/μl. A volume of 0.3 μl was infused, so that the final dose of both drugs was 75 ng/side. Previous studies have shown that infusion of these compounds at this volume induces dissociable effects on behavior when administered into the NAc core or shell (McFarland and Kalivas, 2001; Floresco et al., 2006).

Infusions of GABA agonists or saline were administered via 30 gauge injection cannulae that protruded 0.8 mm past the end of the guide cannulae, at a rate of 0.3 μl/46 s by a microsyringe pump. 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 used, whereby half of the rats received saline before the first reinstatement test and muscimol/baclofen before the second test, while the other half received the reverse order. Assignment to a particular group (saline or muscimol/baclofen first) was based on the average number of active and inactive lever presses on the last days of both VR-5 and extinction training, so that rats in both order conditions displayed similar levels of responding. Following the first reinstatement test session, rats received at least two more days of extinction training