

# Interactions of the basolateral amygdala with the dorsal hippocampus and dorsomedial prefrontal cortex regulate drug context-induced reinstatement of cocaine-seeking in rats

Rita A. Fuchs, Jessica L. Eaddy, Zu-In Su and Guinevere H. Bell

Department of Psychology, University of North Carolina, CB#3270, Davie Hall, Chapel Hill, NC27599-3270, USA

**Keywords:** asymmetrical inactivation, baclofen, extinction, muscimol, self-administration

## Abstract

The basolateral amygdala (BLA), dorsomedial prefrontal cortex (dmPFC) and dorsal hippocampus (DH) are critical elements of the neurocircuitry of drug context-induced reinstatement of cocaine-seeking; however, little is known about functional interactions between these brain regions. The present study tested the hypothesis that serial information processing by the BLA and dmPFC mediates drug context-induced cocaine-seeking, whereas the BLA and DH independently control this behaviour. Rats were trained to self-administer cocaine in a distinct environment (cocaine-paired context) followed by extinction training in a different environment (extinction context). On the test days, rats received unilateral microinfusions of baclofen + muscimol or of vehicle into the BLA and either the contralateral or ipsilateral dmPFC or DH. Cocaine-seeking behaviour (i.e. nonreinforced presses on the cocaine-associated lever) was then assessed in the cocaine-paired and extinction contexts. Following vehicle pretreatment, exposure to the cocaine-paired context reinstated extinguished cocaine-seeking behaviour. BLA–dmPFC asymmetrical inactivation attenuated cocaine-seeking behaviour relative to vehicle treatment; however, this impairment equaled that produced by ipsilateral BLA–dmPFC inactivation. Furthermore, unilateral inactivation of the BLA or dmPFC did not alter this behaviour. BLA–DH asymmetrical inactivation selectively attenuated cocaine-seeking behaviour relative to vehicle treatment whereas ipsilateral or unilateral inactivation of the BLA and DH did not alter this behaviour. These findings indicate that the BLA and DH exhibit sequential information processing within the relapse circuitry. In contrast, interactions between the BLA and dmPFC are more complex and include parallel loops of information processing and/or necessary interhemispheric input from the dmPFC to the BLA, probably in addition to direct intrahemispheric interactions.

## Introduction

Drug-associated environmental contexts (e.g. drug-taking neighbourhood) are comprised of multimodal background stimuli, which constitute a setting in which conditioned stimulus–drug and/or response–drug associations can form. Consequently, these contexts come to signal response-contingent drug availability, elicit drug-seeking behaviour in laboratory animals (Alleweireldt *et al.*, 2001; Crombag & Shaham, 2002), and induce physiological arousal, craving and relapse in drug users (Ehrman *et al.*, 1992; Foltin & Haney, 2000).

The dorsomedial prefrontal cortex (dmPFC), basolateral amygdala (BLA), and dorsal hippocampus (DH) play critical roles in drug context-induced cocaine-seeking behaviour. Consistent with this, these brain regions exhibit context-dependent neuronal activation (Neisewander *et al.*, 2000; Ciccocioppo *et al.*, 2001). Glutamate, the neurotransmitter released by projection neurons of these brain regions, is necessary for context-induced drug-seeking behaviour (Baptista *et al.*, 2004; Bossert *et al.*, 2004; Bossert *et al.*, 2006). Furthermore, lesion or inactivation of these brain regions abolishes the ability of a cocaine-paired context to trigger cocaine-seeking behaviour (Fuchs *et al.*, 2005; Di Pietro *et al.*, 2006) or place preference (Tzschentke &

Schmidt, 1999; Ferbinteanu & McDonald, 2001; Fuchs *et al.*, 2002). While this evidence suggests that the functional integrity of the BLA, dmPFC and DH is necessary for drug context-induced reinstatement, it is unclear whether these brain regions interact with one another or independently control drug-seeking behaviour via discrete subcircuits.

To explore putative functionally significant interactions between these elements of the context-induced relapse circuitry, the present study assessed effects of BLA–dmPFC and BLA–DH functional disconnection, or asymmetrical inactivation (Gaffan *et al.*, 1993), on context-induced reinstatement of cocaine-seeking behaviour. To temporarily disrupt intrahemispheric communication between two brain regions, baclofen + muscimol (B/M), GABA agonists that suppress neural activity in cell bodies without affecting fibers of passage, were infused into the BLA in one hemisphere and into the dmPFC or DH in the contralateral hemisphere prior to a test of cocaine-seeking behaviour. Control groups received ipsilateral B/M infusions, which produce the same amount of neuronal suppression as contralateral infusions while sparing intrahemispheric interactions between the target brain regions in one hemisphere. Additional control groups received ipsilateral or contralateral vehicle infusions. We predicted that functionally significant intrahemispheric interaction would be evident as greater behavioural impairment following contralateral inactivation (disconnection) than following ipsilateral inactivation or vehicle treatment.

Correspondence: Dr Rita A. Fuchs, as above.

E-mail: rfuchs@unc.edu

Received 2 April 2007, revised 9 May 2007, accepted 31 May 2007

It has been hypothesized that inputs from various elements of the relapse circuitry, including the BLA and DH, converge at the level of the dmPFC before information can enter the nucleus accumbens (NAC), the input nucleus to the basal ganglia motor system that mediates the execution of cocaine-seeking behaviour (Kalivas & McFarland, 2003). Consistent with this hypothesis, we predicted that BLA–dmPFC asymmetrical inactivation would disrupt drug context-induced cocaine-seeking behaviour to a greater extent than ipsilateral inactivation or vehicle infusion. Furthermore, we predicted that BLA–DH asymmetrical inactivation would fail to impair cocaine-seeking behaviour more than ipsilateral inactivation. Remarkably, the results from the present study were not consistent with either of these predictions and have led us to re-evaluate our understanding of information processing within the relapse circuitry.

## Materials and methods

### Animals

Experimentally naïve male Sprague–Dawley rats (Charles River;  $n = 123$ ), weighing 250–275 g at the start of the experiment, were individually housed in a temperature- and humidity-controlled vivarium on a reversed light–dark cycle. Rats were maintained on 20–25 g of rat chow per day, with water available *ad libitum*. The housing and treatment of the rats followed the guidelines of the ‘Guide for the Care and Use of Laboratory Rats’ (Institute of Laboratory Animal Resources on Life Sciences, National Research Council, 1996), and the study protocol was approved by the Institutional Animal Care and Use Committee of the University of North Carolina at Chapel Hill.

### Food training

In order to expedite the acquisition of cocaine self-administration, rats were first trained to lever press on a fixed ratio (FR) 1 schedule of food reinforcement (45-mg pellets; Purina, Richmond, IN, USA) in standard sound-attenuated operant conditioning chambers (26 × 27 cm base × 27 cm high; Coulbourn Instruments, Allentown, PA, USA) during a 16-h overnight food-training session. The chambers were equipped with two retractable levers and a food pellet dispenser between the levers. During the session, each lever press on the right (active) lever resulted in delivery of a food pellet only. Lever presses on the left (inactive) lever had no programmed consequences. Following food training, food pellet dispensers were removed from the chambers.

### Surgery

Forty-eight hours after food training, rats were anaesthetized using ketamine hydrochloride and xylazine (66.6 and 1.3 mg/kg, respectively; *i.p.*). Chronic indwelling catheters were constructed using a bent steel cannula with a screw-type connector (Plastics One, Roanoke, VA, USA), Silastic tubing (10 cm long; *i.d.*, 0.64; *o.d.*, 1.19 mm; Dow Corning, Midland, MI, USA), Prolite monofilament mesh (Atrium Medical Corp., Hudson, NH, USA) and cranioplastic cement, as described previously (Fuchs *et al.*, 2006b). The end of the catheter was inserted into the right jugular vein and was secured to surrounding tissue with suture. The catheter ran subcutaneously and exited on the rat’s back, posterior to the shoulder blades.

Immediately after the catheter surgery, the rats were placed into a stereotaxic instrument (Stoelting, Wood Dale, IL, USA). They received stainless steel guide cannulae (26-gauge; Plastics One),

aimed at the right or left BLA and the right or left dmPFC (experiment 1) or DH (experiment 2) using standard stereotaxic procedures (BLA: AP, –2.7; ML, ±5.2; and DV, –6.8 mm; dmPFC: AP, +3.0; ML, ±0.6; and DV, –2.2; DH, cannulae angled rostrally by 15°: AP, –4.1; ML, ±2.1; and DV, –2.7 mm relative to bregma). Three small screws and cranioplastic cement secured the guide cannulae to the skull. Stylets (Plastics One) and Tygon caps were placed into the guide cannulae and catheter, respectively, to prevent occlusion. Rats were given 5 days for postoperative recovery before the start of the experiment.

To extend catheter patency, the catheters were flushed through once daily for 5 days following surgery with 0.1 mL of an antibiotic solution of cefazolin (100.0 mg/mL; Schein Pharmaceutical, Florham Park, NJ, USA) dissolved in heparinized saline (70 U/mL; Baxter Healthcare Corp., Deerfield, IL, USA). Thereafter, catheters were flushed with 0.1 mL heparinized saline (10 U/mL) prior to each self-administration session, and with 0.1 mL of the cefazolin solution and 0.1 mL of heparinized saline (70 U/mL) after each session. Catheter patency was periodically verified by infusing 0.1 mL of propofol (10 mg/mL, IV; Abbot Laboratories, North Chicago, IL, USA), an ultra-short-acting barbiturate which produces a rapid loss of muscle tone only when administered intravenously.

### Self-administration

Self-administration training was conducted during 2-h sessions on a minimum of 10 consecutive days during the rats’ dark cycle. Rats were trained to press a lever according to an FR 1 schedule of cocaine reinforcement (cocaine hydrochloride; 0.10 mg in a 0.05-mL infusion; National Institute on Drug Abuse, Research Triangle Park, NC, USA) with a 20-s time-out period. The catheters were connected to liquid swivels (Instech, Plymouth Meeting, PA, USA) via polyethylene 20 tubing that was encased in steel spring leashes (Plastics One). The swivels were suspended above the operant conditioning chamber and were connected to infusion pumps (Coulbourn). Data collection and reinforcer delivery were controlled using Graphic State Notation software version 2.102 (Coulbourn).

Self-administration training was conducted in operant conditioning chambers that contained one of two distinctly different sets of visual, auditory, olfactory and tactile contextual stimuli in addition to the active (right) and inactive (left) levers. Context 1 contained a continuous red houselight on the wall opposite the active lever, beeping pure tone (80 dB, 1 kHz; 2 s on, 2 s off), pine odour cue (4.5 × 2 cm; Car Freshener Corp., Waterton, NY, USA) and wire mesh floor (26 × 27 cm). Context 2 contained a blinking white stimulus light above the inactive lever (2 s on, 2 s off), continuous pure tone (75 dB, 2.5 kHz), vanilla odour cue (4.5 × 2 cm; Sopus Products, Moorpark, CA, USA), a slanted ceramic tile wall and bar floor (19 × 27 cm). Rats had no exposure to the self-administration context prior to self-administration training. The contextual stimuli were presented throughout each session independent of responding, as in our previous study (Fuchs *et al.*, 2005). Assignment of rats to cocaine self-administration training in Context 1 vs. Context 2 was random. Active lever presses resulted in a 2-s activation of the infusion pump only. After each infusion, responses on the active lever had no consequences during the 20-s time-out period. During the sessions, responses on the inactive lever had no programmed consequences but were recorded. Daily self-administration training sessions were continued until a rat reached the acquisition criterion (*i.e.* ≥ 10 infusions self-administered per session on a minimum of 10 training days).

### Extinction

Rats underwent 2-h extinction sessions on at least seven consecutive days, during which responses on either lever had no programmed consequences. Extinction sessions were conducted in Context 2 for rats that had previously self-administered cocaine in Context 1, and vice versa. Rats had no exposure to the extinction context prior to extinction training. On extinction day 2, the rats were adapted to the intracranial infusion procedure prior to placement in the chamber. During the adaptation procedure, stainless steel injection cannulae (33-gauge; Plastics One) were bilaterally inserted into the rat's guide cannulae to a depth of 1 mm (dmPFC, DH) or 2 mm (BLA) below the tip of the guide cannulae. Rats were held by the experimenter for 4 min while the injection cannulae were left in place but fluid was not infused through the infusion cannulae. Extinction training was terminated when the rats reached the criterion for extinction (i.e. minimum of 7 days of extinction training with  $\leq 25$  responses per session on the last two consecutive days) with a minimum of 7 days of extinction training.

### Intracranial microinfusions

For intracranial microinfusions, the injection cannulae were connected to 10- $\mu$ L Hamilton syringes (Hamilton Co., Reno, NV, USA) that were mounted on an infusion pump (KD Scientific, Holliston, MA, USA). A combination of baclofen hydrobromide and muscimol (B/M; 1.0 and 0.1 mM, respectively; Sigma-Aldrich) or phosphate-buffered saline vehicle (VEH; pH = 7.0 for both) were infused at volumes of 0.5  $\mu$ L over 2 min, and the injection cannulae were left in place for 1 min prior to and after the infusion. Muscimol, at doses of 1000 ng in 1  $\mu$ L and 20 ng in 1  $\mu$ L inhibits glucose utilization in a 1.6-mm radius (Martin, 1991) and electrophysiological activity in a 1-mm radius (Arikan *et al.*, 2002), respectively. These estimates probably include tissue that exhibits hypoactivity due to reduced synaptic input from inactivated neurons. In the present study, muscimol was administered at 20 ng in 0.5  $\mu$ L; the area of neural inactivation was thus probably smaller due to reduced infusion volume. Similar information about the spread of baclofen hydrobromide is not available, but its spread is probably limited by low lipophilicity (Leisen *et al.*, 2003). To minimize unintended spread, we used a dose of B/M that we had used in the past to demonstrate functional differentiation between the nucleus accumbens core and shell and between the dorsolateral caudate–putamen and overlying somatosensory cortex (Fuchs *et al.*, 2004; Fuchs *et al.*, 2006a). However, given that B/M was not traced directly in the brain, the possibility cannot be ruled out that brain regions adjacent to the target sites, either alone or together with the target brain regions, may have mediated the observed behavioural effects.

### Reinstatement testing

Three test days were conducted using an ABA test design. During the test sessions, lever presses were recorded on the previously active and inactive levers but had no programmed consequences.

#### Test day 1

To examine potential nonspecific effects of B/M on operant responding, rats received unilateral microinfusions of B/M or VEH into the BLA and into the contralateral or ipsilateral dmPFC (experiment 1) or DH (experiment 2). Immediately after receiving the microinfusions, rats were placed into the extinction context for a

2-h test session. Assignment to B/M vs. VEH pretreatment within each surgery group was counterbalanced based on previous cocaine intake. Test day 1 was scheduled for extinction day 4, when lever pressing was expected to be only partially extinguished, in order to maximize the sensitivity for detecting a potential B/M-induced decrease in responding.

#### Test day 2

To examine the effects of B/M on the ability of the cocaine-paired context to elicit cocaine-seeking behaviour, rats received unilateral intracranial microinfusions of B/M or VEH into the BLA and into the contralateral or ipsilateral dmPFC (experiment 1) or DH (experiment 2) on the day after they reached the extinction criterion (see above), using the infusion procedure described above. Assignment to B/M vs. VEH pretreatment was the same as for test 1. Immediately after receiving the microinfusions, rats were placed into the cocaine-paired context for a 2-h test session. During the test session, rats were connected to the infusion apparatus in order to allow for similar interaction with the spatial and tactile elements of the context (e.g. slanted tile) as during cocaine self-administration training. However, fluids were not infused through the catheter as a consequence of lever pressing.

#### Test day 3

To determine whether repeated administration of B/M would have a nonspecific effect on operant responding relative to acute administration (i.e. to detect a potential carry-over effect), the procedure for test 3 was identical to that for test 1. Rats received unilateral microinfusions of B/M or VEH into the BLA and into the contralateral or ipsilateral dmPFC (experiment 1) or DH (experiment 2), using the infusion procedure described above. Immediately after receiving the microinfusions, rats were placed into the extinction context for a 2-h test session. Assignment to B/M vs. VEH pretreatment was the same as for tests 1 and 2. Test day 3 occurred after rats underwent additional daily extinction sessions following test day 2, until they reached the extinction criterion again (i.e.  $\leq 25$  responses per session on two consecutive days).

### Locomotor activity test

To further assess possible nonspecific effects of intracranial B/M microinfusions on general activity, we examined the effects of intracranial administration of B/M or VEH on locomotion in a novel context. Forty-eight to seventy-two hours following the last test session, rats received unilateral microinfusions of B/M or VEH into the BLA and into the contralateral or ipsilateral dmPFC or DH using the infusion procedures described above. Immediately thereafter, horizontal locomotor activity was measured in novel Plexiglas chambers (42  $\times$  20 cm base  $\times$  20 cm high). The chambers were equipped with an array of eight photodetectors and corresponding light sources that emitted photobeams 4.5 cm apart, 6 cm above the chamber floor. A computerized activity system (San Diego Instruments, San Diego, CA, USA) recorded the number of times photobeams were broken by a rat moving in the chamber during a 2-h test session.

### Food-reinforced operant behaviour

As a follow up to experiment 2, the effects of BLA–DH asymmetrical inactivation on food-reinforced lever pressing behaviour were examined in a separate group of experimentally naïve rats in order to further assess whether this manipulation produced motor impairment

selective to operant behaviour. Using procedures identical to those employed in experiment 2, rats first underwent a single food training session and then received guide cannula implants into the right or left BLA and the contralateral DH. After postsurgical recovery, rats were trained to press a lever according to an FR 1 schedule of food reinforcement (45 mg; Purina) with a 20-s time out period. Training was conducted in Context 1 or 2 during daily 2-h sessions on nine consecutive days. Rats were adapted to the intracranial infusion procedure immediately prior to session 4. After responding stabilized (< 10% variability in active lever responding on two consecutive days), two test days were conducted using a within-subjects design, with two training days intervening. On the test days, rats received unilateral microinfusions of B/M or VEH into the BLA and into the contralateral DH immediately prior to placement in the operant conditioning chamber. Active lever presses resulted in food reinforcement whereas inactive lever presses were recorded but had no programmed consequences. The order of B/M and VEH microinfusions was counterbalanced across the two test days based on baseline active lever responding.

### Histological and data analysis

Rats were fully anesthetized with pentobarbital (Sigma, 100mg/kg, i.p.) and perfused with phosphate buffered saline and 10% formaldehyde solution. The brains were dissected out and stored in 10% formaldehyde solution until sectioning. Brains were sectioned in the coronal plane at a thickness of 75  $\mu$ m. Cannula placements were determined on cresyl violet-stained brain sections based on the rat brain atlas (Paxinos & Watson, 1997). Cocaine-reinforced active lever presses and cocaine intake in experiments 1 and 2 were analysed separately using one-way ANOVA. Non-reinforced active and inactive lever presses on the test days in experiments 1 and 2 were analysed separately using  $2 \times 2 \times 3$  mixed-factorial ANOVA with treatment (VEH, B/M) and surgery type (contralateral, ipsilateral) as between-subjects factors, and test day (1, 2, 3) as the within-subject factor. To test for a floor effect, nonreinforced active lever presses in the extinction context were further analysed using separate  $2 \times 2 \times 2$  mixed-factorial ANOVA with treatment and surgery type as between-subjects factors, and extinction test day (1, 3) as the within-subject factor. Locomotor activity counts in experiments 1 and 2 were analysed separately using  $2 \times 2$  ANOVA with treatment and surgery type as between-subjects factors. Non-reinforced active and inactive lever presses on the test days in unilateral controls were analysed separately using  $4 \times 3$  mixed-factorial ANOVA with group (BLA B/M, dmPFC B/M, DH B/M, VEH control) as the between-subjects factor and test day as the within-subject factor. Locomotor activity counts in unilateral controls were analysed using a one-way ANOVA with group as the between-subjects factor. Food-reinforced active lever presses and food pellets obtained following B/M vs. VEH treatment were analysed separately using within-subjects *t*-tests. Significant ANOVA main and interaction effects were followed up by *post hoc* comparisons (Tukey's LSD test) when appropriate. The hemispheric laterality of significant effects was investigated separately using planned *t*-tests because the variables BLA hemisphere (right, left) and dmPFC or DH hemisphere (right, left) are not orthogonal.

## Results

### Histology

A schematic diagram illustrating the distribution of injection cannula placements in the brains of rats is included in Fig. 1, and photomicrographs of representative cannula tracts are shown in

Fig. 2. The target regions were defined as the anterior cingulate and prelimbic regions of the medial prefrontal cortex (dmPFC), the lateral and basolateral nuclei of the amygdala (BLA) and the dorsal hippocampus proper (DH). The most ventral point of each injection cannula track was located within the target brain region for the following number of rats per group (Fig. 1, top): right BLA–left dmPFC VEH,  $n = 5$ ; left BLA–right dmPFC VEH,  $n = 6$ ; right BLA–left dmPFC B/M,  $n = 5$ ; left BLA–right dmPFC B/M,  $n = 5$ ; right BLA–right dmPFC VEH,  $n = 5$ ; left BLA–left dmPFC VEH,  $n = 6$ ; right BLA–right dmPFC B/M,  $n = 5$ ; left BLA–left dmPFC B/M,  $n = 5$ ; right BLA–left DH VEH,  $n = 5$ ; left BLA–right DH VEH,  $n = 5$ ; right BLA–left DH B/M,  $n = 7$ ; left BLA–right DH B/M,  $n = 6$ ; right BLA–right DH VEH,  $n = 6$ ; left BLA–left DH VEH,  $n = 5$ ; right BLA–right DH B/M,  $n = 5$ ; left BLA–left DH B/M,  $n = 5$ . The data of one rat from the right BLA–right DH VEH group was omitted from all analyses because his active lever pressing behaviour on test day 2 was 3.2 SD above the group mean (final group size reported above). In the within-subjects food control experiment, the most ventral point of each injection cannula track was located within the target brain region for the following number of rats per group: right BLA–left DH food,  $n = 5$ ; left BLA–right DH food,  $n = 4$ ; data not shown). Microscopic examination of the brain sections did not reveal differences between the B/M- and VEH-treated groups in cell loss or gliosis at the infusion site.

Data from rats with one correct placement in the BLA, dmPFC or DH, coupled with an incorrect placement in an area just dorsal relative to a target brain region, were grouped to form additional control groups ('unilateral inactivation control'; Fig. 1, bottom). We have shown previously that bilateral inactivation of brain regions adjacent to the BLA, dmPFC or DH (i.e. barrel fields of the somatosensory cortex, ventral prefrontal cortex and trunk region of the somatosensory cortex) failed to alter context-induced cocaine-seeking behaviour (Fuchs *et al.*, 2005). These groups were thus included in order to (i) assess whether the sum of impairments produced by separate unilateral inactivations of two target brain regions approximated the impairments seen after ipsilateral inactivation of the same target brain regions, and (ii) provide information about the anatomical selectivity of our manipulations. A subset of rats with dorsal cannula misplacement was used because unintended spread of B/M was expected to be disproportional in the dorsal direction (Baker *et al.*, 1996; Neisewander *et al.*, 1998). Furthermore, at least in the case of the BLA and dmPFC, regions that (based on existing literature) could have potentially altered cocaine-seeking behaviour are indeed located in the dorsal direction relative to the target brain regions (i.e., central nucleus, caudate–putamen, motor cortex; Everitt *et al.*, 1991; Kruzich & See, 2001; Fuchs *et al.*, 2006a). In the present study, misplaced injector cannula tracks were located on the border of the anterior cingulate cortex, motor cortex and meninges (just dorsal to the dmPFC), in the trunk region of the somatosensory cortex (just dorsal to the DH), and in the central amygdaloid nucleus or adjacent caudate–putamen (just dorsal to the BLA). Sample sizes for these groups were as follows: unilateral BLA B/M,  $n = 5$ ; unilateral dmPFC B/M,  $n = 16$ ; unilateral DH B/M,  $n = 8$ .

### Experiment 1. Effects of BLA–dmPFC asymmetrical inactivation on context-induced cocaine-seeking

#### Self-administration and extinction

Rats exhibited stable responding on the active lever during the last three self-administration days with a within-subject variability of

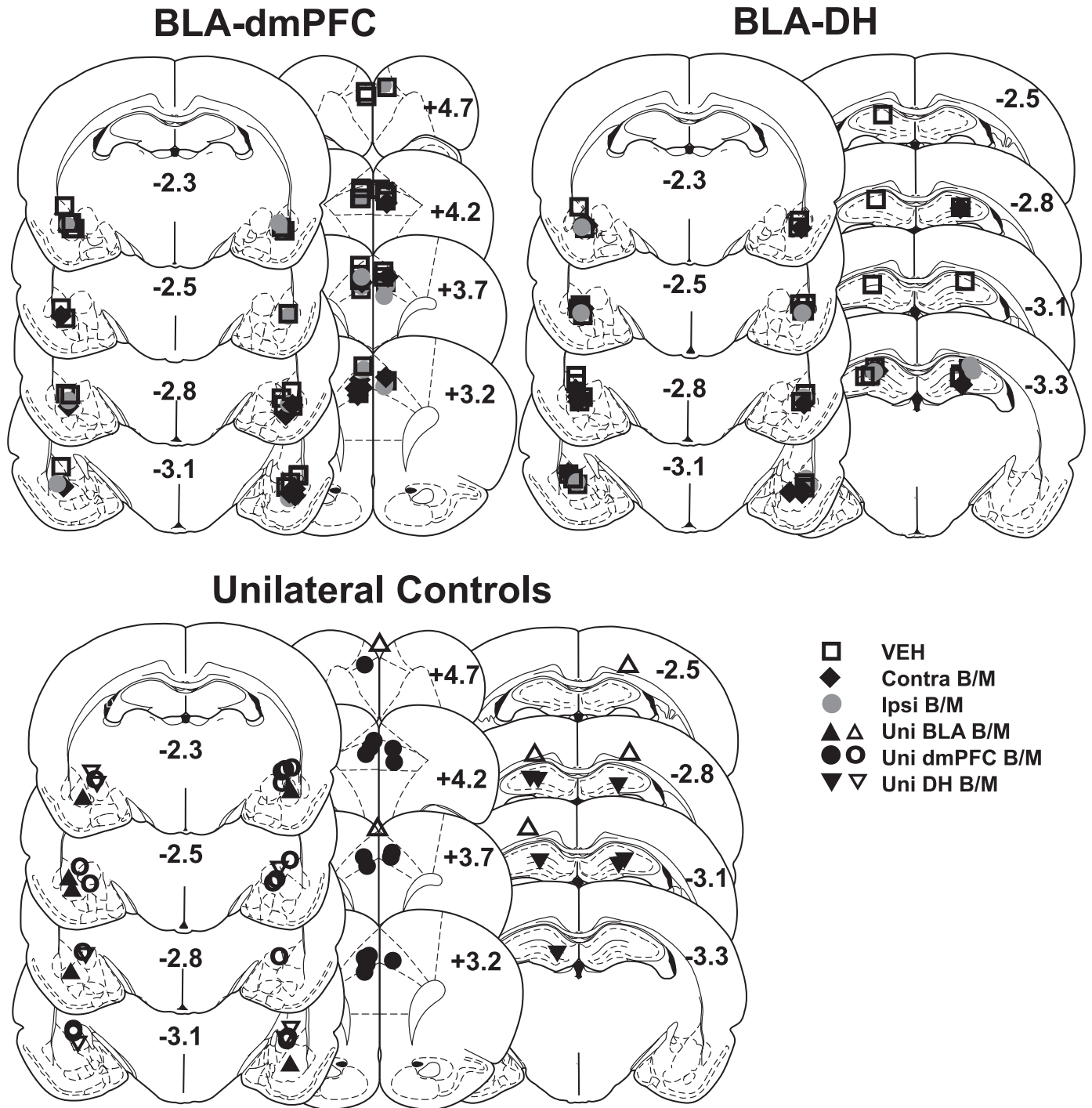


FIG. 1. Microinfusion cannula placement as verified on cresyl violet-stained sections. The symbols represent the most ventral point of the infusion cannula tract for each rat on coronal sections based on the atlas of Paxinos & Watson (1997). (Top) Schematics of cannula placements in rats that received ipsilateral or contralateral infusions of vehicle (VEH, open square) or baclofen/muscimol (B/M, diamond; filled grey circle, respectively) into the target brain regions in experiments 1 and 2. (Bottom) Schematics of cannula placements in B/M-treated rats with one correct (filled symbols) and one incorrect (open symbols) placement. Data from these rats were analysed separately (unilateral control groups). Numbers indicate the distance from bregma in mm.

<10% in daily cocaine intake. Collapsed across groups, the mean  $\pm$  SEM daily active lever responding and cocaine intake were  $107.10 \pm 18.93$  lever presses and  $37.72 \pm 3.64$  infusions ( $\sim 12.57 \pm 1.21$  mg/kg per session), respectively. There was no pre-existing difference in active lever responding ( $F_{7,33} = 0.73$ ,  $P = 0.64$ ) or cocaine intake ( $F_{7,33} = 0.45$ ,  $P = 0.86$ ) between the eight groups that subsequently received VEH or B/M into the right

or left BLA and the contralateral or ipsilateral dmPFC. Responding declined upon removal of cocaine reinforcement on extinction day 1 ( $50.12 \pm 8.98$  lever presses per session). The microinfusion adaptation procedure failed to alter responding on extinction day 2 (data not shown). Subsequently, responding gradually extinguished to criterion ( $\leq 25$  responses per day on the last two consecutive days with a minimum of seven extinction days) in all groups prior to

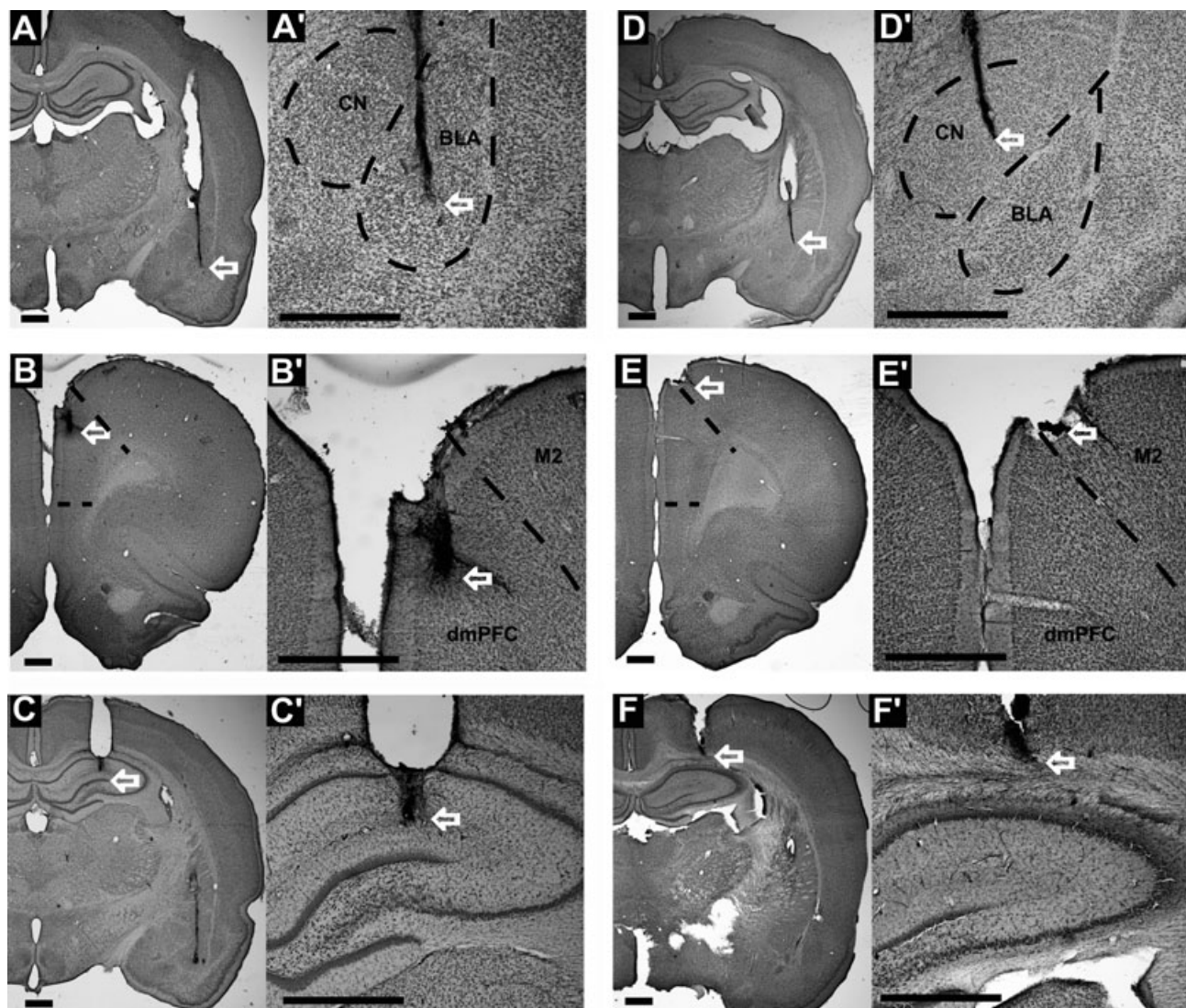


FIG. 2. Histological photomicrographs depicting representative cannula tracts in cresyl violet-stained brain sections from B/M-treated subjects. Cannulae were aimed at three target brain regions: (A and A') the BLA, (B and B') dmPFC, and (C and C') DH. Note that section C was collected from an ipsilaterally cannulated subject. In the unilateral control groups, due to cannula misplacement, cannula tracts were located dorsal relative to the target brain regions: (D and D') in the central nucleus of the amygdala (CN; 67% of cases) or adjacent caudate-putamen overlying the BLA (33% of cases; not shown); (E, E') on the border of the anterior cingulate cortex, motor cortex and meninges overlying the dmPFC; or (F and F') in the somatosensory cortex overlying the DH. The arrows identify the most ventral point of the infusion cannula tract. The scale bars represent 1 mm.

reinstatement testing (test 2). Mean active lever responding on the day preceding test 2 was  $8.61 \pm 1.13$  lever presses per session.

#### Cocaine-seeking behaviour

Independent of surgery type, B/M treatment selectively attenuated responding on the active lever in the cocaine-paired context (Fig. 3A). In the VEH control groups, but not in the B/M-treated groups, exposure to the cocaine-paired context produced a significant increase in responding on the active lever on test day 2 relative to exposure to the extinction context on test day 1 (test day  $\times$  treatment interaction effect,  $F_{2,74} = 12.20$ ,  $P = 0.0001$ ; Tukey's test,  $P < 0.01$ ; test day main effect,  $F_{2,74} = 51.343$ ,  $P = 0.0001$ ). Active lever responding on test day 2 exceeded responding on test day 3 both in the VEH control (Tukey's test,  $P < 0.01$ ) and B/M-treated groups (Tukey's test,  $P < 0.05$ ). Pretreatment with B/M into the BLA and dmPFC significantly attenuated reinstatement of respond-

ing in the cocaine-paired context (Tukey's test,  $P < 0.01$ ) but failed to alter responding in the extinction context relative to VEH. Remarkably, this effect was independent of whether the infusions were contralateral or ipsilateral (no surgery type  $\times$  treatment  $\times$  test day interaction:  $F_{2,74} = 0.16$ ,  $P = 0.85$ ), and was also independent of the particular hemisphere in which B/M was administered into the BLA ( $t_{17} = 0.46$ ,  $P = 0.64$ ) or the dmPFC ( $t_{17} = 1.316$ ,  $P = 0.21$ ). Furthermore, a separate ANOVA indicated that responding in the extinction context significantly decreased from test day 1 to test day 3 regardless of treatment or surgery type (test day main effect only,  $F_{1,37} = 17.4$ ,  $P = 0.0001$ ). It is thus unlikely that nonspecific effects of B/M in the extinction context on test day 1 were masked by a floor effect.

All groups exhibited an increase in inactive lever responding upon exposure to the cocaine-paired context (Fig. 3C) relative to exposure to the extinction context (test day main effect,

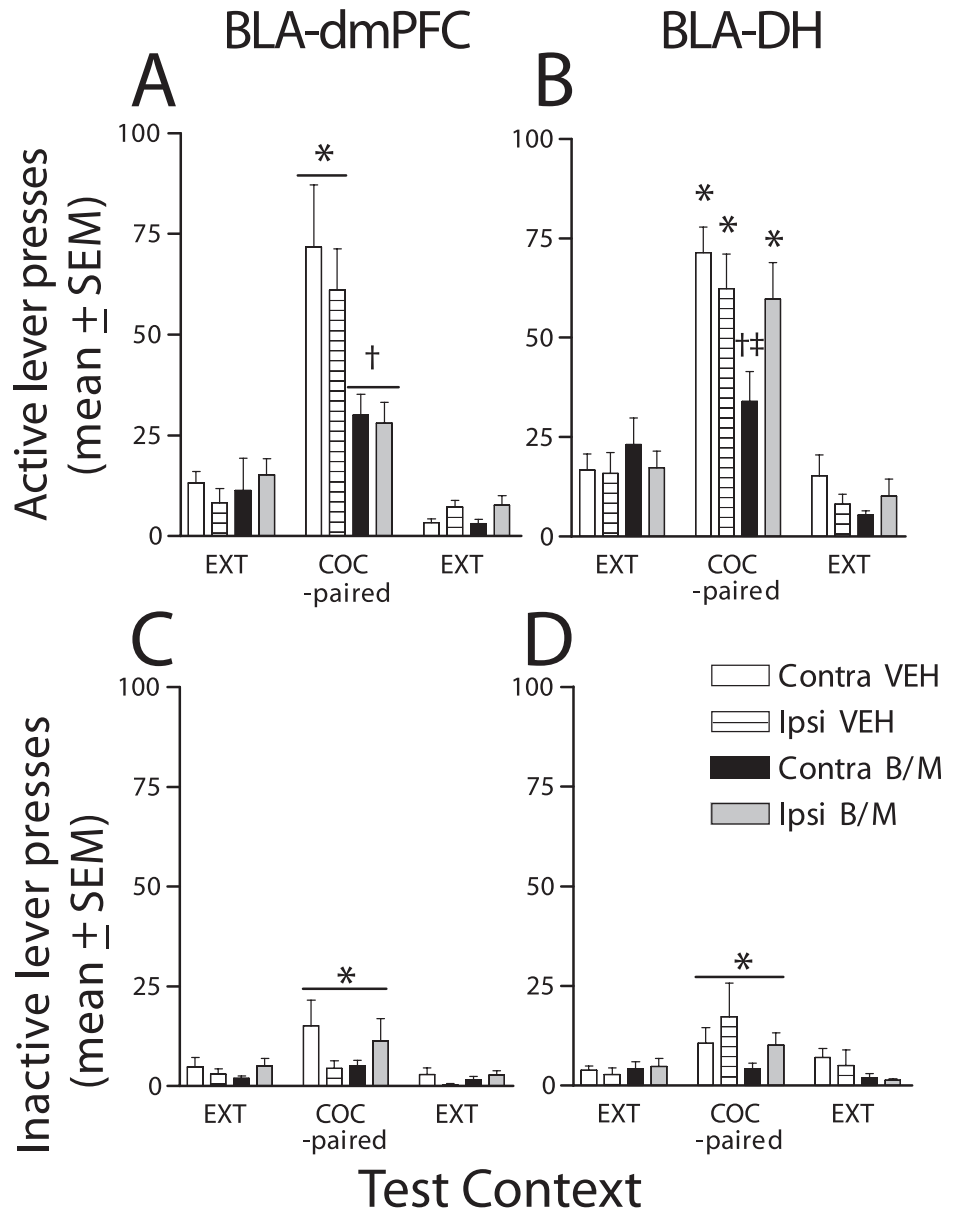


FIG. 3. Responses on the active and inactive levers (mean + SEM in 2 h) in the extinction and cocaine-paired contexts during the tests sessions. On the test day, rats received pretreatment with B/M (1.0 and 0.1 mM, respectively; 0.5  $\mu$ L/site) or VEH into the BLA and the ipsilateral or contralateral (A and C) dmPFC ( $n = 10$ – $11$ /group) or (B and D) DH ( $n = 10$ – $13$ /group). They were then exposed to the extinction (EXT) context on test days 1 and 3 and to the previously cocaine-paired (COC-paired) context on test day 2, using an ABA test design. Lever pressing was assessed in the absence of cocaine reinforcement or response-contingent stimulus presentation. Symbols represent significant difference relative to responding in the extinction context on test day 1 (\* $P < 0.05$ , Tukey's test), relative to VEH pretreatment ( $\dagger P < 0.05$ , Tukey's test) and relative to ipsilateral B/M pretreatment ( $\ddagger P < 0.05$ , Tukey's test).

$F_{2,74} = 8.63$ ,  $P = 0.0001$ ; Tukey's test,  $P < 0.05$ ; no surgery type  $\times$  treatment  $\times$  test day interaction,  $F_{2,74} = 0.99$ ,  $P = 0.38$ ). This is a well-documented effect that reflects motivated behaviour, an adaptive shift toward less discriminating drug-seeking strategy under extinction conditions (Fuchs *et al.*, 1998) or conditioned arousal (Stewart, 1992), because it is not observed in either an extinction or a novel context (Fuchs *et al.*, 2005). Despite a significant surgery type  $\times$  treatment interaction effect ( $F_{1,37} = 4.51$ ,  $P = 0.04$ ), B/M failed to affect inactive lever responding relative to VEH following contralateral (ANOVA treatment simple main effect,  $t_{18} = 1.06$ ,  $P = 0.30$ ) or ipsilateral infusion into the BLA and dmPFC (ANOVA treatment simple main effect,  $t_{19} = 1.91$ ,  $P = 0.07$ ). In addition, there was no difference between the two B/M-infused (ANOVA surgery type simple main effect,  $t_{17} = 1.84$ ,  $P = 0.08$ ) or between the two VEH-infused groups (ANOVA surgery type simple main effect,  $t_{20} = 1.03$ ,  $P = 0.32$ ). Thus, neither treatment nor surgery type affected responding on the inactive lever.

#### Experiment 2. Effects of BLA–DH asymmetrical inactivation on context-induced cocaine-seeking

##### Self-administration and extinction

Rats exhibited stable responding on the active lever during the last three self-administration days with a within-subject variability of  $< 10\%$  in daily cocaine intake. Collapsed across groups, the mean  $\pm$  SEM daily active lever responding and cocaine intake were  $81.14 \pm 13.28$  lever presses and  $34.74 \pm 2.74$  infusions ( $\sim 11.58 \pm 0.91$  mg/kg per session), respectively. There was no pre-existing difference in active lever responding ( $F_{7,36} = 0.64$ ,  $P = 0.72$ ) or cocaine intake ( $F_{7,36} = 0.51$ ,  $P = 0.82$ ) between the eight groups that subsequently received VEH or B/M into the right or left BLA and the contralateral or ipsilateral DH. Responding declined upon removal of cocaine reinforcement on extinction day 1 ( $65.23 \pm 8.10$  lever presses per session). The microinfusion adaptation procedure failed to alter responding on extinction day 2 (data not shown). Subsequently, responding gradually extinguished to criterion

( $\leq 25$  responses per day on the last two consecutive days with a minimum of seven extinction days) in all groups prior to reinstatement testing (test 2). Mean active lever responding on the day preceding test 2 was  $9.30 \pm 1.00$  lever presses per session.

#### Cocaine-seeking behaviour

B/M treatment selectively altered responding on the active lever in the cocaine-paired context depending on surgery type (surgery type  $\times$  treatment  $\times$  test day interaction effect,  $F_{2,80} = 4.06$ ,  $P = 0.02$ ; treatment  $\times$  test day interaction effect,  $F_{2,80} = 4.20$ ,  $P = 0.02$ ). In the VEH control groups and in the group that received B/M into the BLA and the ipsilateral DH, exposure to the cocaine-paired context on test day 2 (Fig. 3B) produced a significant increase in responding on the active lever relative to exposure to the extinction context on test days 1 and 3 (Tukey's test,  $P < 0.01$ ). In contrast, in the group that received B/M into the BLA and the contralateral DH, active lever responding on test day 2 failed to exceed responding on test day 1 but it exceeded responding on test day 3 (Tukey's test,  $P < 0.05$ ). Pretreatment with B/M into the BLA and the ipsilateral DH failed to alter active lever pressing in the cocaine-paired or the extinction context relative to VEH. However, pretreatment with B/M into the BLA and the contralateral DH attenuated active lever pressing in the cocaine-associated context relative to VEH (Tukey's test,  $P < 0.01$ ) and relative to ipsilaterally infused B/M (Tukey's test,  $P < 0.05$ ) but failed to alter responding in the extinction context. This effect was independent of the specific hemisphere in which B/M was administered into the BLA or the dmPFC ( $t_{11} = 0.52$ ,  $P = 0.61$ ). Furthermore, a separate ANOVA indicated that responding in the extinction context significantly decreased from test day 1 to test day 3 regardless of treatment or

surgery type ( $F_{1,40} = 11.8$ ,  $P = 0.001$ ). It is thus unlikely that nonspecific effects of B/M in the extinction context on test day 1 were masked by a floor effect.

All groups exhibited an increase in inactive lever responding (Fig. 3D) upon exposure to the cocaine-paired context relative to exposure to the extinction context (test day main effect only,  $F_{2,80} = 7.69$ ,  $P = 0.001$ ; Tukey's test,  $P < 0.05$ ). However, as in experiment 1, neither treatment nor surgery type affected responding on the inactive lever.

#### Effects of unilateral inactivation

Unilateral B/M-induced inactivation of the BLA coupled with inactivation of a structure just dorsal to the dmPFC or the DH, or unilateral inactivation of the dmPFC or DH coupled with inactivation of a structure just dorsal to the BLA, failed to alter context-induced cocaine-seeking relative to VEH treatment administered into the BLA and the DH or dmPFC (Fig. 4A). All groups exhibited an increase in active lever-responding upon exposure to the cocaine-paired context relative to exposure to the extinction context (test day main effect only,  $F_{2,136} = 76.20$ ,  $P = 0.0001$ ; Tukey's test,  $P < 0.01$ ). There was no difference in active lever responding between the groups that received unilateral inactivation of the BLA, DH or dmPFC vs. VEH controls from experiments 1 and 2 (ANOVA group main effect,  $F_{3,68} = 2.55$ ,  $P = 0.06$ ; group  $\times$  test day interaction,  $F_{6,136} = 1.64$ ,  $P = 0.14$ ). Thus, it is unlikely that the behavioural impairments seen in experiments 1 and 2 were due to ipsilateral or contralateral inactivation of one target region and an outlying brain area. Furthermore, the effects of ipsilateral inactivation indeed appeared to approximate the sum of two separate unilateral inactivations. All groups exhibited an increase in inactive lever responding (Fig. 4B) upon exposure to the cocaine-paired context relative to exposure to the extinction context (test day main effect only,  $F_{2,136} = 4.28$ ,  $P = 0.02$ ; Tukey's test,  $P < 0.05$ ). However, there was no difference between the groups in responding on the inactive lever (ANOVA group main effect,  $F_{3,68} = 0.28$ ,  $P = 0.84$ ; group  $\times$  test day interaction,  $F_{6,136} = 0.74$ ,  $P = 0.62$ ).

#### Effects of BLA–DH asymmetrical inactivation on food-reinforced operant responding

Food reward elicited high levels of responding (data not shown). The total number of active lever presses and food pellets obtained ( $\pm$  SEM) did not differ following B/M administration into the BLA and contralateral DH ( $235.6 \pm 70.4$  responses per session,  $87.4 \pm 23.7$  pellets per session) relative to VEH administration ( $225.8 \pm 85.6$  responses per session,  $80.3 \pm 24.9$  pellets per session;  $t_8 = 0.130$ ,  $P = 0.90$ ; and  $t_8 = 0.299$ ,  $P = 0.77$ , respectively). Thus, BLA–DH asymmetrical inactivation did not selectively impair the ability to lever press.

#### Locomotor activity

B/M administered into the BLA and dmPFC failed to significantly alter locomotor activity in a novel context (Fig. 5A; treatment and surgery type main effects and interaction ranges,  $F_{1,37} = 0.45$ – $2.95$ ,  $P = 0.50$ – $0.09$ ). In contrast, B/M administered into the BLA and DH significantly increased locomotor activity, independent of surgery type (Fig. 5B; treatment main effect only,  $F_{1,40} = 16.78$ ,  $P = 0.0001$ ). Finally, there was no difference between the unilateral B/M-treated control groups and the VEH-pretreated control group in locomotor activity ( $F_{3,68} = 0.19$ ,  $P = 0.91$ ; Fig. 5C).

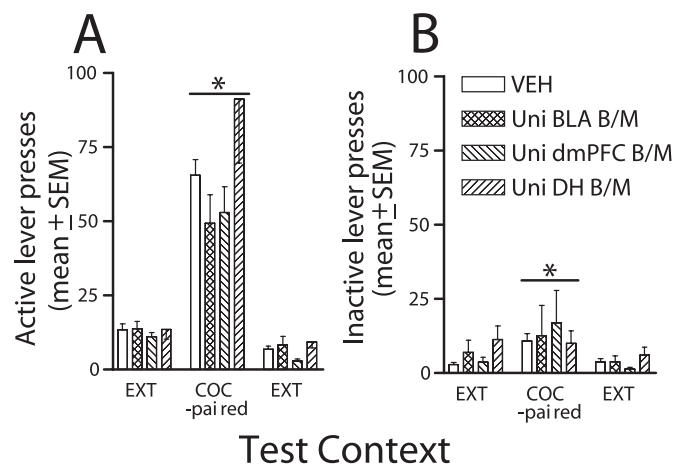


FIG. 4. Responses on the active (A) and inactive (B) levers (mean  $\pm$  SEM in 2 h) exhibited by the unilateral control groups in the extinction and cocaine-paired contexts during the test sessions. On the test day, rats received pretreatment with B/M (1.0 and 0.1 mM, respectively;  $0.5 \mu\text{L}/\text{site}$ ) into the BLA ( $n = 5$ ), dmPFC ( $n = 16$ ) or DH ( $n = 8$ ) and into an ipsilateral or contralateral site just dorsal to one of these target brain regions due to cannula misplacement (see placements in Fig. 1, bottom). Control rats received pretreatment with VEH into the BLA and the ipsilateral or contralateral DH or dmPFC in experiments 1 and 2 ( $n = 43$ ). They were then exposed to the EXT context on test days 1 and 3 and to the previously COC-paired context on test day 2, using an ABA test design. Lever pressing was assessed in the absence of cocaine reinforcement or response-contingent stimulus presentation. Symbols represent significant difference relative to responding in the extinction context on test day 1 ( $*P < 0.05$ , Tukey's test).

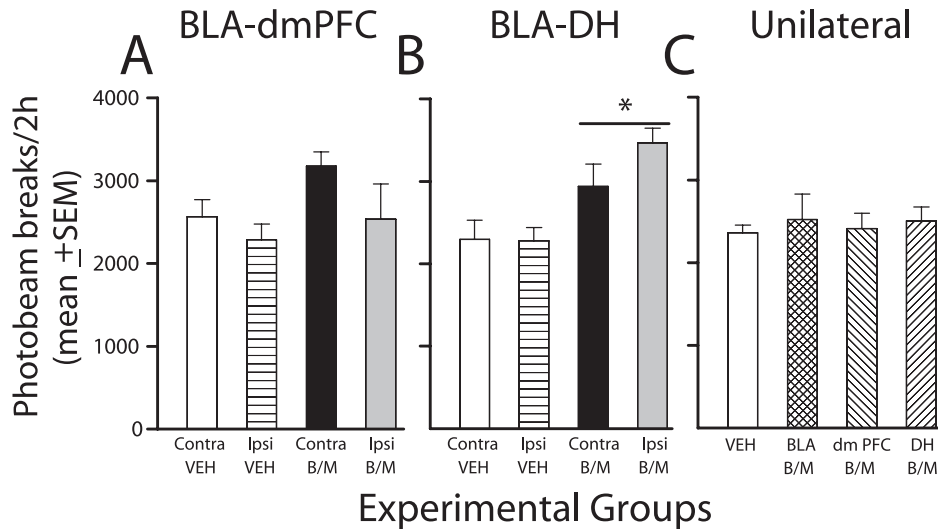


FIG. 5. Effects of B/M (1.0 and 0.1 mM, respectively; 0.5  $\mu$ L/site) on locomotor activity in a novel environment (mean  $\pm$  SEM photobeam breaks in 2 h). Microinfusions of B/M or VEH were administered immediately before the test session unilaterally into the BLA and into the ipsilateral or contralateral (A) dmPFC ( $n = 10$ – $11$ /group) or (B) DH ( $n = 10$ – $13$ /group), or (C) unilaterally into the BLA, dmPFC or DH and into an ipsilateral or contralateral site just dorsal to one of these target brain regions due to cannula misplacement ( $n = 5$ – $16$ ; unilateral control groups). Control rats in C received pretreatment with VEH into the BLA and the ipsilateral or contralateral DH or dmPFC in experiments 1 and 2 ( $n = 43$ ). An automated photocell system recorded the number of times photobeams were broken by an animal moving in the chamber (\* $P = 0.0001$  relative to VEH, ANOVA treatment main effect).

## Discussion

The present study represents the first attempt to examine functionally significant interactions between three elements of the neural circuitry of drug context-induced cocaine-seeking behaviour: the BLA, dmPFC and DH (Fuchs *et al.*, 2005). For this purpose, the effects of BLA–dmPFC and BLA–DH asymmetrical inactivation were compared with those of ipsilateral and unilateral inactivation of the same brain regions. These comparisons revealed that the nature of BLA–dmPFC and BLA–DH interactions is distinctly different with respect to drug context-induced cocaine-seeking behaviour. The findings did not fully support the hypothesis that inputs from forebrain structures, including the BLA and DH, converge at the level of the dmPFC before cocaine-seeking behaviour is initiated (Kalivas & McFarland, 2003).

### Contributions of the BLA and dmPFC to context-induced cocaine-seeking

Asymmetrical inactivation of the BLA and dmPFC produced a significant impairment in cocaine-seeking behaviour. Remarkably, however, this effect was similar in magnitude to the impairment produced by ipsilateral inactivation (Fig. 3A). Furthermore, unilateral inactivation of the BLA or dmPFC produced only a trend toward a decrease in responding (Fig. 4A), but cumulatively approximated the amount of impairment produced by ipsilateral or contralateral inactivation. In other studies, unilateral BLA manipulations similarly failed to alter sucrose-conditioned place preference (Everitt *et al.*, 1991) but impaired instrumental learning, memory for reward reduction or devaluation, and fear conditioning (Coleman-Meschers *et al.*, 1996; LaBar & LeDoux, 1996; Baldwin *et al.*, 2000; Izquierdo & Murray, 2004; Blair *et al.*, 2005). These findings may reflect the fact that unilateral BLA function is sufficient for conditioned appetitive behaviour, including reinstatement, but bilateral BLA function is necessary for primary reward processing and aversive learning and memory.

Attenuation in cocaine-seeking behaviour was not due to nonspecific performance deficits because the effects of B/M occurred

selectively in the cocaine-paired context and on the active lever (Fig. 3C). In addition, inactivation of the BLA and dmPFC failed to attenuate general activity in a novel context (Fig. 5A). Thus, B/M-induced attenuation of cocaine-seeking behaviour in the present study clearly reflected impairment in context-induced motivation for cocaine, and the pattern of findings points toward two possible interpretations regarding the relationship between the BLA and dmPFC.

On one hand, similar impairment following contralateral, ipsilateral, and unilateral inactivation may reflect the fact that there is no obligatory interdependence between the BLA and PFC, without ruling out the existence of functionally significant interactions between these brain regions. One possibility is that inputs from the BLA that are sufficient for cocaine-seeking behaviour bypass the PFC and vice versa. This implies the existence of parallel loops of information processing within the relapse circuitry. These loops probably converge at the level of the NAC, the input structure of the basal ganglia that is involved in the execution of context-induced drug-seeking behaviour (Bossert *et al.*, 2006) and other forms of drug-seeking behaviour (Everitt *et al.*, 1999; Parkinson *et al.*, 2000a; Di Ciano & Everitt, 2004). Consistent with this, both the BLA and dmPFC densely innervate the NAC and influence the activity of medium spiny neurons (Sesack *et al.*, 1989; Johnson *et al.*, 1994; O'Donnell & Grace, 1995; Groenewegen *et al.*, 1996). Moreover, disconnection of the NAC from either the BLA or dmPFC impairs cocaine-primed and explicit cue-induced drug-seeking behaviour and other goal-directed behaviours (Everitt *et al.*, 1991; Parkinson *et al.*, 2000b; McFarland & Kalivas, 2001; Di Ciano & Everitt, 2004). The effects of similar manipulations on context-induced cocaine-seeking behaviour have yet to be investigated using the reinstatement model. However, findings from a recent study support the proposal that the BLA can bypass the dmPFC to control cocaine-conditioned place preference (Miller & Marshall, 2005). In this study, BLA efferents to the prelimbic cortex and NAC exhibited Fos-mediated neural activation concomitant with cocaine-conditioned place preference, whereas prelimbic cortex efferents to the BLA or NAC did not

(Miller & Marshall, 2005). These findings suggest that the BLA is a source of direct excitatory input to the NAC for drug-seeking behaviour (Miller & Marshall, 2005). The probable existence of parallel pathways of information processing at the level of the BLA and dmPFC is inconsistent with the prediction that the BLA accesses the relapse circuitry exclusively via the dmPFC (Kalivas & McFarland, 2003), and implies somewhat limited neocortical control over context-induced goal-directed behaviour. This, in turn, is consistent with the limited success of therapeutic approaches that rely on strengthening cognitive control in order to reduce the propensity for relapse in cocaine users (Jentsch & Taylor, 1999).

On the other hand, it is noteworthy that ipsilateral inactivation of the BLA and dmPFC bilaterally disrupts potential functionally significant interhemispheric interactions between these brain regions while sparing intrahemispheric interactions in one hemisphere. Hence, equal impairment following contralateral vs. ipsilateral inactivation can possibly occur if bilateral communication via intra- and interhemispheric connections between the BLA and dmPFC is necessary for context-induced cocaine-seeking. Importantly, direct connections between the BLA and dmPFC are topographically organized. These consist of bilateral descending projections from the dmPFC to the rostral BLA and ipsilateral ascending projections from the caudal BLA to the dmPFC (Sesack *et al.*, 1989; Kita & Kitai, 1990; Conde *et al.*, 1995; McDonald *et al.*, 1996; Floresco & Ghods-Sharifi, 2007). In the present study, B/M and VEH microinfusions were administered into the rostral BLA (Figs 1 and 2). Therefore, equal impairment in reinstatement following ipsilateral and contralateral B/M treatment may reflect the fact that bilateral input from the dmPFC to the rostral BLA is necessary for reinstatement, and unilateral input is insufficient to maintain this behaviour. The apparent functional significance of corticofugal projections from the dmPFC to the BLA is inconsistent with the prediction that cocaine-seeking behaviour relies on sequential information processing by the BLA then the dmPFC (Kalivas & McFarland, 2003). However, the possibility that context-induced reinstatement requires bilateral interaction between the PFC and BLA is somewhat mitigated by the fact that the impairments seen after ipsilateral or contralateral inactivation did not appear to exceed the additive effect of individual unilateral inactivation of the BLA plus the dmPFC.

#### *Interaction between the BLA and DH is necessary for drug context-induced cocaine-seeking*

Interestingly, asymmetrical inactivation of the BLA and DH disrupted cocaine-seeking behaviour in the cocaine-paired context selectively, whereas ipsilateral inactivation (Fig. 3B and D) or individual unilateral inactivation of the BLA plus the DH (Fig. 4) did not alter responding relative to vehicle treatment. This outcome was unexpected because direct anatomical connections between the BLA and DH proper are sparse (Pikkarainen *et al.*, 1999), relative to those between the BLA and ventral hippocampus (VH; Moser & Moser, 1998). Stimulation of the VH is sufficient to alter electrophysiological activity in the BLA and PFC (Ishikawa & Nakamura, 2003, 2006) and elicit cocaine-seeking behaviour (Vorel *et al.*, 2001). Furthermore, temporary inactivation of the VH impairs cue-induced and cocaine-primed reinstatement of cocaine-seeking behaviour (Sun & Rebec, 2003; Atkins *et al.*, 2006; Rogers & See, 2007). However, in the present study, B/M could not have spread into the VH due to distance, nor could it have altered VH function via the fimbria–fornix because it does not inhibit the conductance of action potentials in fibers of passage. It is unlikely that contralateral

inactivation of the BLA and DH elicited a nonspecific performance effect as it attenuated cocaine-seeking behaviour selectively on the active lever and in the cocaine-paired context (Fig. 3B and D), and it did not alter operant responding for a natural reinforcer (data not shown). Furthermore, although both contralateral and ipsilateral inactivation of the BLA and DH increased general activity in a novel context (Fig. 5B), only contralateral inactivation interfered with context-induced reinstatement.

Based on the above evidence, serial information processing by the BLA and DH is necessary for drug context-induced cocaine-seeking behaviour. Interaction between these brain regions may occur through direct connection as the density of connections does not necessarily correspond to their functional significance. Alternatively, interaction may occur via the lateral entorhinal cortex, where dense hippocampal-entorhinal projections are in close proximity to dense entorhinal-amygdaloid projections (van Groen & Wyss, 1990; McDonald & Mascagni, 1997; Pikkarainen *et al.*, 1999). It remains to be determined whether information sharing between the BLA and DH is unidirectional or reciprocal. One possibility is that the DH conveys mnemonic information regarding contextual stimuli (i.e. spatial or configural representations) to the BLA. Upon context exposure, the BLA may utilize this information to assess the motivational significance of the context (Baxter *et al.*, 2000; Gallagher, 2000; Fuchs *et al.*, 2002). Additionally, it may send feedback to the DH in order to update the cognitive representation of the context or of context–cocaine associations in long-term memory.

#### *Conclusions*

In the present study, B/M-induced contralateral inactivation of the BLA and the dmPFC or DH impaired context-induced cocaine-seeking behaviour. As the spread of B/M was not assessed directly, it is possible that some of these effects were mediated by adjacent brain regions alone or together with the target brain regions. However, the present findings were consistent with our previous report (Fuchs *et al.*, 2005) that tetrodotoxin-induced bilateral inactivation of these brain regions disrupts context-induced cocaine-seeking behaviour whereas inactivation of adjacent brain regions (i.e. barrel fields, ventromedial prefrontal cortex and trunk region of the somatosensory cortex) has no effect. These studies provide converging evidence that the functional integrity of the BLA, dmPFC and DH is necessary for context-induced motivation for cocaine. Importantly, the present study yields further insight into the dynamics of information processing within the relapse circuitry by revealing that the BLA and DH are functionally interdependent with respect to drug context-induced cocaine-seeking behaviour. Serial information processing by these structures is intrahemispheric and is not characterized by laterality in rats. The pattern of interaction between the BLA and dmPFC is more complex. It involves parallel loops of information processing and/or interhemispheric input from the dmPFC to the BLA, probably in addition to functionally significant intrahemispheric interactions between these brain regions. The BLA, DH and dmPFC probably assume distinctly different functions related to cocaine-seeking behaviour, as described above. Together, these findings strongly support the hypothesis (Di Ciano & Everitt, 2004) that distinct limbic corticostriatal subcircuits make up the relapse circuitry and underlie various elements of context-induced drug-seeking behaviour.

Future studies will need to expand the scope of investigation by using complementary techniques (e.g. administration of selective antagonists; Di Ciano & Everitt, 2004; Bossert *et al.*, 2005), by investigating the temporal dynamics and direction of information

processing, and by including additional brain regions. Other brain regions of interest include the NAC and ventral tegmental area, based on their critical contribution toward context-induced renewal of motivated behaviour (Crombag *et al.*, 2002; Bossert *et al.*, 2004, 2006; Hamlin *et al.*, 2006). While the DH is selectively involved in contextual drug-seeking behaviour (Fuchs *et al.*, 2005), there is significant overlap at the systems level in the neural substrates of drug-seeking behaviour elicited by contextual stimuli (Crombag *et al.*, 2002; Bossert *et al.*, 2004, 2006), other discriminative stimuli (Weiss *et al.*, 2000), explicit conditioned stimuli (Weissenborn *et al.*, 1996; Whitelaw *et al.*, 1996; Kantak *et al.*, 2002; McLaughlin & See, 2003; Fuchs *et al.*, 2004; Vanderschuren *et al.*, 2005), stress (Shaham *et al.*, 2000; Capriles *et al.*, 2003; McFarland *et al.*, 2004), and cocaine priming (Neisewander *et al.*, 1996; McFarland & Kalivas, 2001; Park *et al.*, 2002). Thus, this line of research has the potential to result in the generation of new hypotheses regarding computational processes that give rise to drug relapse elicited by various triggers and in the development of therapeutic strategies that will disrupt these processes.

## Acknowledgements

The authors would like to thank Julian Duda, Dr Donna Ramirez, Heather Lasseter and Stephanie Traina for excellent technical assistance and for insightful comments on an earlier version of this manuscript. This work was supported by National Institute on Drug Abuse grant R01 DA017673 (R.A.F.).

## Abbreviations

B/M, baclofen + muscimol; BLA, basolateral amygdala; DH, dorsal hippocampus; dmPFC, dorsomedial prefrontal cortex; FR, fixed ratio; NAC, nucleus accumbens; VH, ventral hippocampus.

## References

- Alleweireldt, A.T., Weber, S.M. & Neisewander, J.L. (2001) Passive exposure to a contextual discriminative stimulus reinstates cocaine-seeking behavior in rats. *Pharmacol. Biochem. Behav.*, **69**, 555–560.
- Arikan, R., Blake, N.M., Erinjeri, J.P., Woolsey, T.A., Giraud, L. & Highstein, S.M. (2002) A method to measure the effective spread of focally injected muscimol into the central nervous system with electrophysiology and light microscopy. *J. Neurosci. Meth.*, **118**, 51–57.
- Atkins, A.L., Mashhoon, Y. & Kantak, K.M. (2006) Hippocampal regulation of context-induced cocaine-seeking behavior. *CPDD 68th Annual Scientific Meeting Program*. College of Problems of Drug Dependence, Philadelphia, PA, Scottsdale, Arizona, June 21, 2006, pp. 82.
- Baker, D.A., Khroyan, T.V., O'Dell, L.E., Fuchs, R.A. & Neisewander, J.L. (1996) Differential effects of intra-accumbens sulpiride on cocaine-induced locomotion and conditioned place preference. *J. Pharmacol. Exp. Ther.*, **279**, 392–401.
- Baldwin, A.E., Holahan, M.R., Sadeghian, K. & Kelley, A.E. (2000) N-methyl-D-aspartate receptor-dependent plasticity within a distributed corticostriatal network mediates appetitive instrumental learning. *Behav. Neurosci.*, **114**, 84–98.
- Baptista, M.A., Martin-Fardon, R. & Weiss, F. (2004) Preferential effects of the metabotropic glutamate 2/3 receptor agonist LY379268 on conditioned reinstatement versus primary reinforcement: comparison between cocaine and a potent conventional reinforcer. *J. Neurosci.*, **24**, 4723–4727.
- Baxter, M.G., Parker, A., Lindner, C.C., Izquierdo, A.D. & Murray, E.A. (2000) Control of response selection by reinforcer value requires interaction of amygdala and orbital prefrontal cortex. *J. Neurosci.*, **20**, 4311–4319.
- Blair, H.T., Sotres-Bayon, F., Moita, M.A. & Ledoux, J.E. (2005) The lateral amygdala processes the value of conditioned and unconditioned aversive stimuli. *Neuroscience*, **133**, 561–569.
- Bossert, J.M., Ghitza, U.E., Lu, L., Epstein, D.H. & Shaham, Y. (2005) Neurobiology of relapse to heroin and cocaine seeking: an update and clinical implications. *Eur. J. Pharmacol.*, **526**, 36–50.
- Bossert, J.M., Gray, S.M., Lu, L. & Shaham, Y. (2006) Activation of group II metabotropic glutamate receptors in the nucleus accumbens shell attenuates context-induced relapse to heroin seeking. *Neuropsychopharmacology*, **31**, 2197–2209.
- Bossert, J.M., Liu, S.Y., Lu, L. & Shaham, Y. (2004) A role of ventral tegmental area glutamate in contextual cue-induced relapse to heroin seeking. *J. Neurosci.*, **24**, 10726–10730.
- Capriles, N., Rodaros, D., Sorge, R.E. & Stewart, J. (2003) A role for the prefrontal cortex in stress- and cocaine-induced reinstatement of cocaine seeking in rats. *Psychopharmacology (Berl.)*, **168**, 66–74.
- Ciccocioppo, R., Sanna, P.P. & Weiss, F. (2001) Cocaine-predictive stimulus induces drug-seeking behavior and neural activation in limbic brain regions after multiple months of abstinence: Reversal by D1 antagonists. *Proc. Natl Acad. Sci. USA*, **98**, 1976–1981.
- Coleman-Meschke, K., Salinas, J.A. & McGaugh, J.L. (1996) Unilateral amygdala inactivation after training attenuates memory for reduced reward. *Behav. Brain Res.*, **77**, 175–180.
- Conde, F., Maire-Lepoivre, E., Audinat, E. & Crepel, F. (1995) Afferent connections of the medial frontal cortex of the rat. II. Cortical and subcortical afferents. *J. Comp. Neurol.*, **352**, 567–593.
- Crombag, H.S., Grimm, J.W. & Shaham, Y. (2002) Effect of dopamine receptor antagonists on renewal of cocaine seeking by reexposure to drug-associated contextual cues. *Neuropsychopharmacology*, **27**, 1006–1015.
- Crombag, H.S. & Shaham, Y. (2002) Renewal of drug seeking by contextual cues after prolonged extinction in rats. *Behav. Neurosci.*, **116**, 169–173.
- Di Ciano, P. & Everitt, B.J. (2004) Direct interactions between the basolateral amygdala and nucleus accumbens core underlie cocaine-seeking behavior by rats. *J. Neurosci.*, **24**, 7167–7173.
- Di Pietro, N.C., Black, Y.D. & Kantak, K.M. (2006) Context-dependent prefrontal cortex regulation of cocaine self-administration and reinstatement behaviors in rats. *Eur. J. Neurosci.*, **24**, 3285–3298.
- Ehrman, R.N., Robbins, S.J., Childress, A.R. & O'Brien, C.P. (1992) Conditioned responses to cocaine-related stimuli in cocaine abuse patients. *Psychopharmacology*, **107**, 523–529.
- Everitt, B.J., Morris, K.A., O'Brien, A. & Robbins, T.W. (1991) The basolateral amygdala-ventral striatal system and conditioned place preference: further evidence of limbic-striatal interactions underlying reward-related processes. *Neuroscience*, **42**, 1–18.
- Everitt, B.J., Parkinson, J.A., Olmstead, M.C., Arroyo, M., Robledo, P. & Robbins, T.W. (1999) Associative processes in addiction and reward. The role of amygdala-ventral striatal subsystems. *Ann. N Y Acad. Sci.*, **877**, 412–438.
- Ferbinteanu, J. & McDonald, R.J. (2001) Dorsal/ventral hippocampus, fornix, and conditioned place preference. *Hippocampus*, **11**, 187–200.
- Floresco, S.B. & Ghods-Sharifi, S. (2007) Amygdala-prefrontal cortical circuitry regulates effort-based decision making. *Cereb. Cortex*, **17**, 251–260.
- Foltin, R.W. & Haney, M. (2000) Conditioned effects of environmental stimuli paired with smoked cocaine in humans. *Psychopharmacology (Berl.)*, **149**, 24–33.
- Fuchs, R.A., Branham, R.K. & See, R.E. (2006a) Different neural substrates mediate cocaine seeking after abstinence versus extinction training: a critical role for the dorsolateral caudate-putamen. *J. Neurosci.*, **26**, 3584–3588.
- Fuchs, R.A., Evans, K.A., Ledford, C.C., Parker, M.P., Case, J.M., Mehta, R.H. & See, R.E. (2005) The role of the dorsomedial prefrontal cortex, basolateral amygdala, and dorsal hippocampus in contextual reinstatement of cocaine seeking in rats. *Neuropsychopharmacology*, **30**, 296–309.
- Fuchs, R.A., Evans, K.A., Parker, M.C. & See, R.E. (2004) Differential involvement of the core and shell subregions of the nucleus accumbens in conditioned cue-induced reinstatement of cocaine seeking in rats. *Psychopharmacology (Berl.)*, **176**, 459–465.
- Fuchs, R.A., Feltstein, M.W. & See, R.E. (2006b) The role of the basolateral amygdala in stimulus-reward memory and extinction memory consolidation and in subsequent conditioned cued reinstatement of cocaine seeking. *Eur. J. Neurosci.*, **23**, 2809–2813.
- Fuchs, R.A., Tran-Nguyen, L.T., Specio, S.E., Groff, R.S. & Neisewander, J.L. (1998) Predictive validity of the extinction/reinstatement model of drug craving. *Psychopharmacology (Berl.)*, **135**, 151–160.
- Fuchs, R.A., Weber, S.M., Rice, H.J. & Neisewander, J.L. (2002) Effects of excitotoxic lesions of the basolateral amygdala on cocaine-seeking behavior and cocaine conditioned place preference in rats. *Brain Res.*, **929**, 15–25.
- Gaffan, D., Murray, E.A. & Fabre-Thorpe, M. (1993) Interaction of the amygdala with the frontal lobe in reward memory. *Eur. J. Neurosci.*, **5**, 968–975.
- Gallagher, M. (2000) The amygdala and associative learning. In Aggleton, J.P. (ed.), *The Amygdala: a Functional Analysis*. Oxford University Press, Oxford, pp. 311–329.

- van Groen, T. & Wyss, J.M. (1990) Extrinsic projections from area CA1 of the rat hippocampus: olfactory, cortical, subcortical, and bilateral hippocampal formation projections. *J. Comp. Neurol.*, **302**, 515–528.
- Groenewegen, H.J., Wright, C.I. & Beijer, A.V.J. (1996) The nucleus accumbens: gateway for limbic structures to reach the motor system. In Holstege, G., Bandler, R. & Saper, C.B. (eds), *Progress in Brain Research*. Elsevier, Oxford, pp. 485–511.
- Hamlin, A.S., Blatchford, K.E. & McNally, G.P. (2006) Renewal of an extinguished instrumental response: neural correlates and the role of D1 dopamine receptors. *Neuroscience*, **143**, 25–38.
- Ishikawa, A. & Nakamura, S. (2003) Convergence and interaction of hippocampal and amygdalar projections within the prefrontal cortex in the rat. *J. Neurosci.*, **23**, 9987–9995.
- Ishikawa, A. & Nakamura, S. (2006) Ventral hippocampal neurons project axons simultaneously to the medial prefrontal cortex and amygdala in the rat. *J. Neurophysiol.*, **96**, 2134–2138.
- Izquierdo, A. & Murray, E.A. (2004) Combined unilateral lesions of the amygdala and orbital prefrontal cortex impair affective processing in rhesus monkeys. *J. Neurophysiol.*, **91**, 2023–2039.
- Jentsch, J.D. & Taylor, J.R. (1999) Impulsivity resulting from frontostriatal dysfunction in drug abuse: implications for the control of behavior by reward-related stimuli. *Psychopharmacology (Berl.)*, **146**, 373–390.
- Johnson, L.R., Aylward, R.L., Hussain, Z. & Totterdell, S. (1994) Input from the amygdala to the rat nucleus accumbens: its relationship with tyrosine hydroxylase immunoreactivity and identified neurons. *Neuroscience*, **61**, 851–865.
- Kalivas, P.W. & McFarland, K. (2003) Brain circuitry and the reinstatement of cocaine-seeking behavior. *Psychopharmacology (Berl.)*, **168**, 44–56.
- Kantak, K.M., Black, Y., Valencia, E., Green-Jordan, K. & Eichenbaum, H.B. (2002) Dissociable effects of lidocaine inactivation of the rostral and caudal basolateral amygdala on the maintenance and reinstatement of cocaine-seeking behavior in rats. *J. Neurosci.*, **22**, 1126–1136.
- Kita, H. & Kitai, S.T. (1990) Amygdaloid projections to the frontal cortex and the striatum in the rat. *J. Comp. Neurol.*, **298**, 40–49.
- Kruzich, P.J. & See, R.E. (2001) Differential contributions of the basolateral and central amygdala in the acquisition and expression of conditioned relapse to cocaine-seeking behavior. *J. Neurosci.*, **21**, RC155, 1–5.
- LaBar, K.S. & LeDoux, J.E. (1996) Partial disruption of fear conditioning in rats with unilateral amygdala damage: correspondence with unilateral temporal lobectomy in humans. *Behav. Neurosci.*, **110**, 991–997.
- Leisen, C., Langguth, P., Herbert, B., Dressler, C., Koggel, A. & Spahn-Langguth, H. (2003) Lipophilicities of baclofen ester prodrugs correlate with affinities to the ATP-dependent efflux pump P-glycoprotein: relevance for their permeation across the blood-brain barrier? *Pharm. Res.*, **20**, 772–778.
- Martin, J.H. (1991) Autoradiographic estimation of the extent of reversible inactivation produced by microinjection of lidocaine and muscimol in the rat. *Neurosci. Lett.*, **127**, 160–164.
- McDonald, A.J. & Mascagni, F. (1997) Projections of the lateral entorhinal cortex to the amygdala: a Phaseolus vulgaris leucoagglutinin study in the rat. *Neuroscience*, **77**, 445–459.
- McDonald, A.J., Mascagni, F. & Guo, L. (1996) Projections of the medial and lateral prefrontal cortices to the amygdala: a Phaseolus vulgaris leucoagglutinin study in the rat. *Neuroscience*, **71**, 55–75.
- McFarland, K., Davidge, S.B., Lapish, C.C. & Kalivas, P.W. (2004) Limbic and motor circuitry underlying footshock-induced reinstatement of cocaine-seeking behavior. *J. Neurosci.*, **24**, 1551–1560.
- McFarland, K. & Kalivas, P.W. (2001) The circuitry mediating cocaine-induced reinstatement of drug-seeking behavior. *J. Neurosci.*, **21**, 8655–8663.
- McLaughlin, J. & See, R.E. (2003) Selective inactivation of the dorsomedial prefrontal cortex and the basolateral amygdala attenuates conditioned-cue reinstatement of extinguished cocaine-seeking behavior in rats. *Psychopharmacology*, **168**, 57–65.
- Miller, C.A. & Marshall, J.F. (2005) Altered Fos expression in neural pathways underlying cue-elicited drug seeking in the rat. *Eur. J. Neurosci.*, **21**, 1385–1393.
- Moser, M.B. & Moser, E.I. (1998) Functional differentiation in the hippocampus. *Hippocampus*, **8**, 608–619.
- Neisewander, J.L., Baker, D.A., Fuchs, R.A., Tran-Nguyen, L.T., Palmer, A. & Marshall, J.F. (2000) Fos protein expression and cocaine-seeking behavior in rats after exposure to a cocaine self-administration environment. *J. Neurosci.*, **20**, 798–805.
- Neisewander, J.L., Fuchs, R.A., O'Dell, L.E. & Khroyan, T.V. (1998) Effects of SCH-23390 on dopamine D1 receptor occupancy and locomotion produced by intraaccumbens cocaine infusion. *Synapse*, **30**, 194–204.
- Neisewander, J.L., O'Dell, L.E., Tran-Nguyen, L.T., Castaneda, E. & Fuchs, R.A. (1996) Dopamine overflow in the nucleus accumbens during extinction and reinstatement of cocaine self-administration behavior. *Neuropsychopharmacology*, **15**, 506–514.
- O'Donnell, P. & Grace, A.A. (1995) Synaptic interactions among excitatory afferents to nucleus accumbens neurons: hippocampal gating of prefrontal cortical input. *J. Neurosci.*, **15**, 3622–3639.
- Park, W.K., Bari, A.A., Jey, A.R., Anderson, S.M., Speelman, R.D., Rowlett, J.K. & Pierce, R.C. (2002) Cocaine administered into the medial prefrontal cortex reinstates cocaine-seeking behavior by increasing AMPA receptor-mediated glutamate transmission in the nucleus accumbens. *J. Neurosci.*, **22**, 2916–2925.
- Parkinson, J.A., Cardinal, R.N. & Everitt, B.J. (2000a) Limbic cortical-ventral striatal systems underlying appetitive conditioning. *Prog. Brain Res.*, **126**, 263–285.
- Parkinson, J.A., Willoughby, P.J., Robbins, T.W. & Everitt, B.J. (2000b) Disconnection of the anterior cingulate cortex and nucleus accumbens core impairs Pavlovian approach behavior: further evidence for limbic cortical-ventral striatopallidal systems. *Behav. Neurosci.*, **114**, 42–63.
- Paxinos, G. & Watson, C. (1997) *The Rat Brain in Stereotaxic Coordinates*. Academic Press, New York.
- Pikkarainen, M., Ronkko, S., Savander, V., Insausti, R. & Pitkanen, A. (1999) Projections from the lateral, basal, and accessory basal nuclei of the amygdala to the hippocampal formation in rat. *J. Comp. Neurol.*, **403**, 229–260.
- Rogers, J.L. & See, R.E. (2007) Selective inactivation of the ventral hippocampus attenuates cue-induced and cocaine-primed reinstatement of drug-seeking in rats. *Neurobiol. Learn. Mem.*, **87**, 688–692.
- Sesack, S.R., Deutch, A.Y., Roth, R.H. & Bunney, B.S. (1989) Topographical organization of the efferent projections of the medial prefrontal cortex in the rat: an anterograde tract-tracing study with Phaseolus vulgaris leucoagglutinin. *J. Comp. Neurol.*, **290**, 213–242.
- Shaham, Y., Erb, S. & Stewart, J. (2000) Stress-induced relapse to heroin and cocaine seeking in rats: a review. *Brain Res. Brain Res. Rev.*, **33**, 13–33.
- Stewart, J. (1992) Conditioned stimulus control of the expression of sensitization of the behavioral effects of opiate and stimulant drugs. In Gormezano, I. & Wasserman, E.A. (eds), *Learning and Memory: The Behavioral and Biological Substrates*. Erlbaum, Hillsdale, NJ, pp. 129–151.
- Sun, W.L. & Rebec, G.U. (2003) Lidocaine inactivation of ventral subiculum attenuates cocaine-seeking behavior in rats. *J. Neurosci.*, **23**, 10258–10264.
- Tzschentke, T.M. & Schmidt, W.J. (1999) Functional heterogeneity of the rat medial prefrontal cortex: effects of discrete subarea-specific lesions on drug-induced conditioned place preference and behavioural sensitization. *Eur. J. Neurosci.*, **11**, 4099–4109.
- Vanderschuren, L.J., Di Ciano, P. & Everitt, B.J. (2005) Involvement of the dorsal striatum in cue-controlled cocaine seeking. *J. Neurosci.*, **25**, 8665–8670.
- Vorel, S.R., Liu, X., Hayes, R.J., Spector, J.A. & Gardner, E.L. (2001) Relapse to cocaine-seeking after hippocampal theta burst stimulation. *Science*, **292**, 1175–1178.
- Weiss, F., Maldonado-Vlaar, C.S., Parsons, L.H., Kerr, T.M., Smith, D.L. & Ben-Shahar, O. (2000) Control of cocaine-seeking behavior by drug-associated stimuli in rats: effects on recovery of extinguished operant-responding and extracellular dopamine levels in amygdala and nucleus accumbens. *Proc. Natl Acad. Sci. USA*, **97**, 4321–4326.
- Weissenborn, R., Deroche, V., Koob, G.F. & Weiss, F. (1996) Effects of dopamine agonists and antagonists on cocaine-induced operant responding for a cocaine-associated stimulus. *Psychopharmacology (Berl.)*, **126**, 311–322.
- Whitelaw, R.B., Markou, A., Robbins, T.W. & Everitt, B.J. (1996) Excitotoxic lesions of the basolateral amygdala impair the acquisition of cocaine-seeking behaviour under a second-order schedule of reinforcement. *Psychopharmacology (Berl.)*, **127**, 213–224.