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Selective excitotoxic lesions of the hippocampus and basolateral amygdala have dissociable effects on appetitive cue and place conditioning based on path integration in a novel Y-maze procedure

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Abstract

The hippocampus and amygdala are thought to be functionally distinct components of different learning and memory systems. This functional dissociation has been particularly apparent in pavlovian fear conditioning, where the integrity of the hippocampus is necessary for contextual conditioning, and of the amygdala for discrete cue conditioning. Their respective roles in appetitive conditioning, however, remain equivocal mainly due to the lack of agreement concerning the operational definition of a 'context'. The present study used a novel procedure to measure appetitive conditioning to spatial context or to a discrete cue. Following selective excitotoxic lesions of the hippocampus (HPC) or basolateral amygdala (BLA), rats were initially trained to acquire discrete CS-sucrose conditioning in a Y-maze apparatus with three topographically identical chambers, the chambers discriminated only on the basis of path integration. The same group of animals then underwent 'place/contextual conditioning' where the CS presented in a chamber assigned as the positive chamber was paired with sucrose, but the same CS presented in either of the other two chambers was not. Thus, spatial context was the only cue that the animal could use to retrieve the value of the CS. HPC lesions impaired the acquisition of conditioned place preference but facilitated the acquisition of cue conditioning, while BLA lesions had the opposite effect, retarding the acquisition of cue conditioning but leaving the acquisition of conditioned place preference intact. Here we provide strong support for the notion that the HPC and BLA subserve complementary and competing roles in appetitive cue and contextual conditioning.

Introduction

The hippocampus (HPC) and the amygdala are thought to contribute differentially to mammalian learning and memory systems, the former being integral to providing a spatiotemporal contextual 'tag' to episodic information (O'Keefe & Nadel, 1978; Squire *et al*., 1993) and the latter being critical for the formation of associations between emotive experiences and discrete stimuli (Aggleton, 1992, 2000; Phillips & LeDoux, 1992; LeDoux & Muller, 1997; Fanselow & LeDoux, 1999; Amorapanth *et al*., 2000; Huff & Rudy, 2004). However, this functional dissociation has not always been unequivocally demonstrated in appetitive conditioning.

Thus, conditioned place preference (CPP) to food and drugs of abuse is a form of appetitive conditioning that can be assumed to reflect spatial learning, and thus HPC-dependent processes (O'Keefe & Nadel, 1978; Morris *et al*., 1982; Sutherland *et al*., 1982; McDonald & White, 1995). However, although dorsal HPC lesions do impair CPP (Ferbinteanu & McDonald, 2001; Meyers *et al*., 2003), it may even be enhanced following ventral HPC or fornix lesions (White & McDonald, 1993; Ferbinteanu & McDonald, 2001). By contrast, amygdala lesions produce profound impairments in CPP (Everitt *et al*., 1991; Hiroi & White, 1991; Brown & Fibiger, 1993; White & McDonald, 1993; Fuchs *et al*., 2002; Hsu *et al*., 2002). Moreover, HPC lesions spare simple contextual discrimination learning whilst impairing the ability to retrieve associative information based on contextual cues (Good & Honey, 1991).

To explain the apparent paradox of amygdala dominance in CPP, the possibility of elemental cues comprising the context forming associations with the reinforcer has been advanced (Everitt *et al*., 1991; White & McDonald, 1993; McDonald & White, 1995). A question then arises of the nature of hippocampal involvement in CPP, and the nature of contextual conditioning itself. Much evidence points to the representation of spatial context in the HPC being configural (i.e. the integration of discrete environmental cues – Rudy & Sutherland, 1989; Rudy & Sutherland, 1995; Holland & Bouton, 1999; Jeffrey *et al*., 2004). However, spatial context is likely to be defined by other information, including place and idiothetic (movement-related) cues, the role of which in contextual learning is not well investigated.

The present study thus sought to disambiguate the competing influence of spatial context and discrete cues in appetitive conditioning by using a novel Y-maze procedure. The reward-related spatial context was defined by path integration: the rat's internal sense of direction based on idiothetic cues in the absence of any discrete differentiating

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environmental cues. Neurophysiological investigations have shown that spatially correlated hippocampal neuron population activity can be reliably updated on the basis of path integration alone (McNaughton *et al*., 1996) and that when a rat locomotes between two identical boxes containing otherwise identical sensory stimuli, the HPC forms orthogonal codes for the two boxes, provided that they are not orientated in parallel at some angle greater than approximately 90 degrees (Knierim *et al*., 1998; Fuhs *et al*., 2005). Thus, the HPC can transmit information about spatial context in the absence of disambiguating discrete or global sensory cues. Accordingly, the present study tested the hypothesis that the BLA and HPC subserve specific and complementary functions in discrete cue and spatial context appetitive learning, as well as context-dependent cued retrieval.

Materials and methods

Subjects

Subjects were 30 male Lister Hooded rats (Charles River Ltd, UK) weighing between 300 and 330 g at the time of surgery. They were housed in pairs in a room held at a temperature of 21 °C under a reversed 12-h light : 12-h dark cycle (lights off 09:00 h). Water was available *ad libitum,* but following recovery from surgery, food (laboratory chow, Purina) was restricted to 18 g laboratory chow/day, sufficient to maintain preoperative body weight and growth. All experiments were carried out during the dark phase, between 09:00 and 19:00 h and in accordance with the United Kingdom 1986 Animals (Scientific Procedures) Act Project License no. 80/1767.

Surgery

Rats were divided into four groups ($n = 8$ HPC lesions, $n = 5$ HPC shams, $n = 12$ BLA lesions, $n = 5$ BLA shams). In all surgical procedures, animals were anaesthetized with Avertin (10 g 99% 2,2,2-tribromoethanol (Sigma–Aldrich Company, Dorset, UK) in 5 g tertiary amyl alcohol and 450 mL phosphate buffered saline (Dulbecco 'A', Unipath Ltd, Basingstoke, Hampshire, UK) in 40 mL absolute alcohol; 1 mL/100 g body weight, i.p.), and placed in a stereotaxic frame (Kopf, USA) with the incisor bar set at −3.3 mm below the interaural line. A 1 μL SGE syringe (SGE, Baton Rouge, USA) was then lowered into either the HPC or BLA, and the neurotoxin was infused bilaterally. The type and volume of toxin infused, and the sites of infusions are listed in Table 1. Sham control groups were treated identically to the lesion groups, except that they received injections of 0.1 M sterile phosphate buffer (sterile PB), instead of the toxin. Following surgery, rats were allowed a recovery period of at least 14 days prior to behavioural testing, with food available *ad libitum*.

Apparatus

All behavioural training and testing took place in a purpose-built automated Y-maze apparatus (Medical Associates), placed on a rotatable table elevated 1 m above the floor (Fig. 1). The maze consisted of a central, white PVC floored compartment in the shape of an equilateral triangle (side 38.1 cm), and three connecting chambers identical in size (38.1 × 38.1 × 20 cm) and other physical features. An archway (10 cm high × 9 cm wide) served as the entrance to each chamber, and could be closed off with a manually operated guillotine door. Each wall (0.7-mm thick grey PVC) of the chamber contained a centrally located receding well consisting of a tray into which sucrose could be delivered, a 1.8 W, 17 V light, and nose-poke sensor. Each well was connected to its own individual software-operated infusion pump (Semat Technical Ltd, St Albans, UK) placed outside the apparatus. Other features of the chamber included a stainless steel grid rod floor consisting of 3/16″ (4.8 mm) rods, placed above a 38.0 × 38.0 cm stainless steel tray, three infrared beams placed at the entrance, 10 cm and 28 cm away from the entrance, and a 2.5 W, 24 V house light located in the top left corner of the entrance wall. The entire maze was covered with red translucent Plexiglas lids, to allow recording of behaviour (via a video camera mounted above the apparatus) whilst preventing rats from seeing extra-maze cues once inside the Y-maze. The apparatus was controlled and behavioural data collection achieved using Whisker software version 10.1.

The Y-maze itself was placed in a testing room containing a bright reference light (60 W) in the right corner as the sole light source (Fig. 1A). All other extra-maze objects remained in the same position in the room for the duration of the experiment. Radio noise was played beneath the Y-maze to mask any unwanted (inadvertently salient) auditory cues. Furthermore, the noise emanating from infusion pumps was presented in every trial (concurrently with every CS) including nonrewarded trials to ensure that it did not become a salient directional cue. The floor and walls of the apparatus were wiped down with ethanol solution following each session to eliminate any odour traces.

General procedure

At the start of each testing day, all rats were transferred from their home cage to a holding room located approximately 15 ft away from the testing room. Each rat was then transported in an opaque carrier box to the testing room. Once inside the room, each rat was placed on

TABLE 1. Lesion parameters

Lesion	Excitotoxin	Co-ordinates for injections sites			Volume per site (μL)	Diffusion time before removal of injector (min)
		AP	L	DV		
HPC (dorsal)	*N*-methyl-D-aspartic acid (0.09 M)	−2.8	±1.6	−3.3	0.4	4
		−4.2	±2.6	−3.0	0.4	4
HPC (ventral)	*N*-methyl-D-aspartic acid (0.09 M)	−4.8	±4.8	−6.0	0.2	2
		−5.3	±4.6	−4.2	0.2	2
		−5.3	±4.6	−6.0	0.2	2
		−5.8	±4.6	−4.2	0.2	2
BLA	Quinolinic acid (0.09 M)	−2.3	±4.6	−7.6	0.2	3
		−3.0	±4.6	−7.8	0.1	3

AP, anterior-posterior axis; L, lateral, DV, dorsal-ventral axis (co-ordinates all taken from dura).

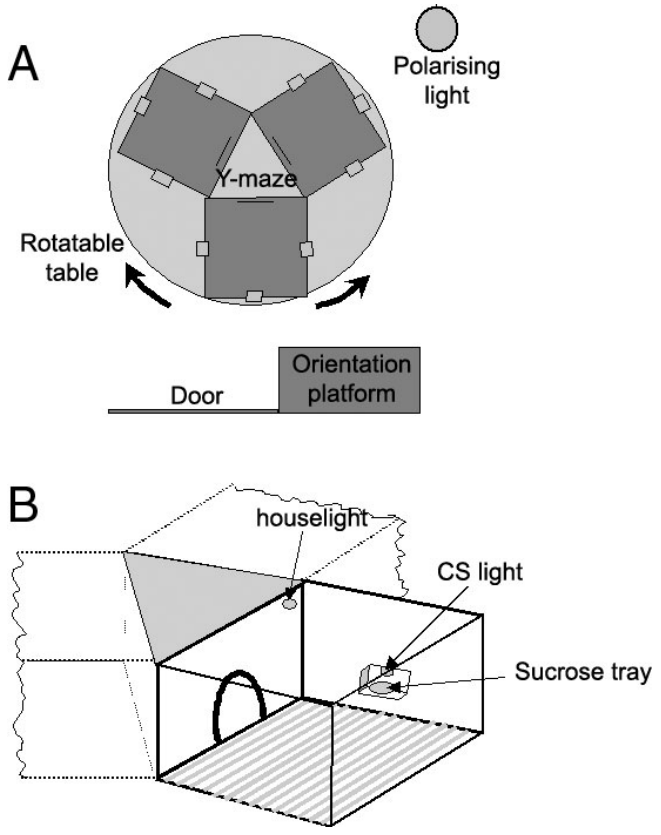

FIG. 1. Schematic diagrams of the Y-maze apparatus used in the study showing (A) layout of testing room and (B) partial view of one of the three chambers. Each chamber was equipped with three sets of CS light and sucrose well, one houselight, and three infrared beams (not shown).

an elevated platform to the right of the door for 1 min where it could freely view the room. The rat was then placed into the central compartment from one of three directions (south, north-east, north-west) chosen at random, whilst ensuring that it did not have the same entry direction for two consecutive sessions. After 1 min in the central compartment, all three guillotine doors were opened to let the rat explore all chambers. The polarizing light was then switched off, with the houselights in the chambers providing the only illumination in the testing room. The experimenter then left the room, closed the door and observed the rat from an adjacent control room. This rigid orientation procedure ensured that a stable, unique association was formed between an external visual landmark (polarizing light) and an internal directional reference system (cells of the path integration system), that would serve to initialize the rat's location within an appropriate spatial reference frame on subsequent visits. It has been shown that once initialized, the rat's place field could be preserved and maintained on the basis of path integration alone, even if the landmarks were removed, or room lights were extinguished (see McNaughton *et al*., 1996). Indeed, a control conditioned place preference experiment had revealed that animals that were re-trained and tested in the absence of the reference light and disoriented before being placed in the Y-maze no longer showed place preference (R. Ito, T. W. Robbins and B. J. Everitt, unpublished observations), pointing to the importance of the orientation procedure and presence of polarizing light in the formation of a stable spatial reference frame, which is used subsequently to differentiate three otherwise identical chambers.

At the end of each training day, the Y-maze was rotated 120° clockwise whilst occupying exactly the same position in relation to the rest of the room. This ensured that conditioning to individual chambers was minimized. These procedures were strictly adhered to from day to day as it was important that perceptual constancy between the Y-maze and external environment was maintained, such that the rats would learn to use the reference light as a consistent source of directional information.

Experimental procedure

Habituation (day 1)

All rats were given one 20-min habituation session in which they were free to explore all three chambers.

Cue conditioning

Rats were trained to associate a 15-s flashing light stimulus with the availability of 0.7 mL sucrose solution delivered to a well. On the first day of training, a small amount (≈0.5 mL) of sucrose was placed in all the wells to encourage subsequent exploration of the wells.

Days 2–4

In each daily session, rats received 30 contingent pairings of the sucrose solution (US) and the flashing light stimulus (CS) positioned directly above the well, under a variable interval 20–60 s schedule. The 30 CS–US presentations took place in a different 'place' each day, with the starting place counterbalanced across rats (e.g. day 2, chamber 1; day 3, chamber 2; day 4, chamber 3), and with each pairing being presented in one of three locations (wells within the chamber) in random order. Nevertheless, each trial could only be initiated by the first photobeam interruption in a chamber that was not rewarded in the previous trial so as to encourage exploration of all chambers during the training session. In order to increase the salience of the flashing CS, all houselights were turned off during a trial. The session terminated at the end of the 30 th trial, and typically lasted 35 min.

Days 5–7

In the second phase of cue conditioning, the CS–US presentations were no longer confined to one chamber. Instead, they could be located in any one of nine possible locations within the Y-maze apparatus (3 × 3 wells in each chamber) in each trial. Although the presentation of the CS–US occurred in random order across the nine locations, it was ensured that the number of CS–US presentations per chamber was equal, and that not more than two consecutive pairings occurred in one chamber within each session. Other session parameters remained the same as before.

CS probe test (day 8)

This day was designed to test the acquisition of cue conditioning. Thirty CSs were presented on a VI20–40-s schedule with the absence of sucrose, again in random but calculated order across the nine different locations. The trials in which the rat made a nose-poke response in the well whilst the CS was still flashing (15 s) were classified as 'correct' trials.

Place conditioning (days 9–17)

In this part of the experiment, rats were trained to associate CSs presented in only one of three places (compartments) with the sucrose

reward, i.e. CSs presented in the other two places were not. As before, each daily session involved the presentation of 30 CSs in random order between the nine possible locations under a VI20–60-s schedule. This time however, only the CSs presented in a preassigned 'positive' place ($n = 10$) were rewarded. The other 20 CS presentations were no longer associated with reward. The session terminated after the 30th CS presentation and typically lasted 45 min. The number of approaches to the CS presented in the positive place (CS+) and the CS presented in the negative places (CS–) were recorded for each session.

Conditioned place preference test (day 18)

Rats were given 20 min to explore the entire apparatus in the absence of the CS and sucrose presentations. The time spent, locomotor activity and the number of nose-pokes made in each place were recorded in 5 min bins.

CS probe test

After 4 weeks, rats were given six re-training sessions of contextual conditioning under exactly the same parameters and conditions, except that this time, the noise of the pump that normally accompanied the delivery of sucrose was presented even in the unpaired contexts, to eliminate the possibility that it could have acted as an inadvertent discriminative stimulus. Rats were then given an extinction session to assess whether they showed discriminatory nose-poking in the three places. Thirty CSs were presented on a VI20–40-s schedule in the absence of sucrose. As before, the number of 'correct' trials in each context was recorded.

Histological procedure and assessment of lesions

After completion of testing, all rats were anaesthetized with sodium pentobarbitone (1.5 mL/animal, 200 mg/mL Euthatal, Rhone Merieux, UK) and perfused intracardially via the ascending aorta with 0.01 M phosphate-buffered saline (PBS) for 4 min, followed by formaldehyde solution (4% paraformaldehyde in PBS) for 6 min. Brains were then removed, stored in paraformaldehyde solution and transferred to a 20% sucrose cryoprotectant solution on the day before sectioning. Coronal sections (60 μm) of the brain were cut using a freezing microtome and then stained with Cresyl Violet to be viewed under a Leitz microscope for the verification of lesion placements, which were mapped onto a standardized stereotaxic atlas of the rat brain (Paxinos & Watson, 1997).

Data analysis

All data were analysed using the SPSS statistical package version 9.0 (SPSS, Chicago, IL). The total number of correct trials, mean latency to approach the CS during correct and incorrect trials, nose-pokes and beam breaks recorded in each 35-min session during cue conditioning were subjected to repeated measures analysis of variance (ANOVA) with lesion group as the between-subjects factor (lesion: HPC, BLA and sham) and session as the within-subjects factor. Data obtained during the context conditioning phase consisted of the number of approaches to CS+ (maximum ten) and CS– (maximum 20 divided by two for equivalence with the CS+ approach) for each session. These were subjected to a repeated measures ANOVA with lesion group as the between-subjects factor (lesion: HPC and sham or BLA and sham) and CS (CS+/CS–) and session as the within-subject factors. Conditioned place preference was expressed as percentage time spent in each of the three chambers and the central compartment, but repeated measures ANOVA was conducted on the actual time spent (min) with lesion group as the between subject factor (lesion: HPC and sham or BLA and sham) and chamber as the within subjects factor. The same ANOVA was conducted for CS extinction data following re-training sessions. Where there was a significant violation of homogeneity of variance across groups for a repeated measure design, as assessed by the Mauchly Sphericity Test, the Greenhouse-Geisser Epsilon was used to calculate a more conservative P-value for each F ratio. Where simple one-way ANOVAs were conducted upon confirmation of significant interactions, the α was adjusted using Sidak's method:

$$\alpha' = 1 - (1 - \alpha)^{1/C},$$

where c is the number of within-experiment analyses.

Results

Lesion assessment

The extents of the excitotoxic lesions of the HPC and BLA are shown schematically in Fig. 2, based on the stereotaxic atlas of the rat brain (Paxinos and Watson, 1997). Excitotoxic lesions of the HPC induced by NMDA infusions extended rostrally from −1.8 to −6.7 mm posterior to the bregma, encompassing all the hippocampal subfields and dentate gyrus of the dorsal and ventral HPC. Minor mechanical damage from the cannulae was evident in both sham and lesioned subjects in the primary somatosensory and lateral secondary visual cortical areas, and neuronal damage occasionally extended unilaterally to the ventral subiculum. One rat was excluded from the study on the basis of extensive bilateral damage to the overlying cortex. The final group numbers were seven HPC lesions and five sham-operated controls.

Excitotoxic lesions of the BLA induced by quinolinic acid resulted in significant neuronal damage to the anterior and posterior basal amygdaloid nucleus, and the lateral nucleus of amygdala, but did not extend to the central nucleus and medial nucleus of the amygdala. The lesion typically extended from −1.8 mm to −3.6 mm posterior to bregma. Three rats were excluded from data analysis on the basis of bilateral damage extending into the central nucleus of the amygdala. The final group numbers were nine BLA lesions and five sham-operated controls.

Cue conditioning

This phase of the experiment assessed the ability of sham-operated, BLA- and HPC-lesioned rats to acquire an association between a discrete CS and sucrose availability (US). The acquisition data from the two sham-lesioned groups (HPC and BLA) were not significantly different (no group effect $F_{1,8} = 0.851$, $P = 0.383$) and as planned *a priori*, were pooled together for subsequent analyses. Overall two-way ANOVA including the acquisition data from all lesion groups (Fig. 3A) revealed a significant lesion effect ($F_{2,23} = 12.62$, $P < 0.0001$), day effect ($F_{6,138} = 92.26$, $P < 0.0001$) and day–lesion interaction ($F_{12,138} = 9.52$, $P < 0.0001$) across the 6 days of cue conditioning. *Posthoc* analyses comparing all data points (days 1–5) to performance on day 6 for each lesion group revealed that the HPC-lesioned group reached asymptote (30 correct trials) significantly earlier (day 1 vs. day 6, $P < 0.003$), than the sham group (days 1 and 2 vs. day 6, $P < 0.003$). This was not associated with a shorter latency to approach the CS/US during the correct trials in the HPC-lesioned group (no lesion effect $F_{5,75} = 0.09$, $P = 0.76$). In contrast, the rate of acquisition of cue conditioning was significantly slower in the

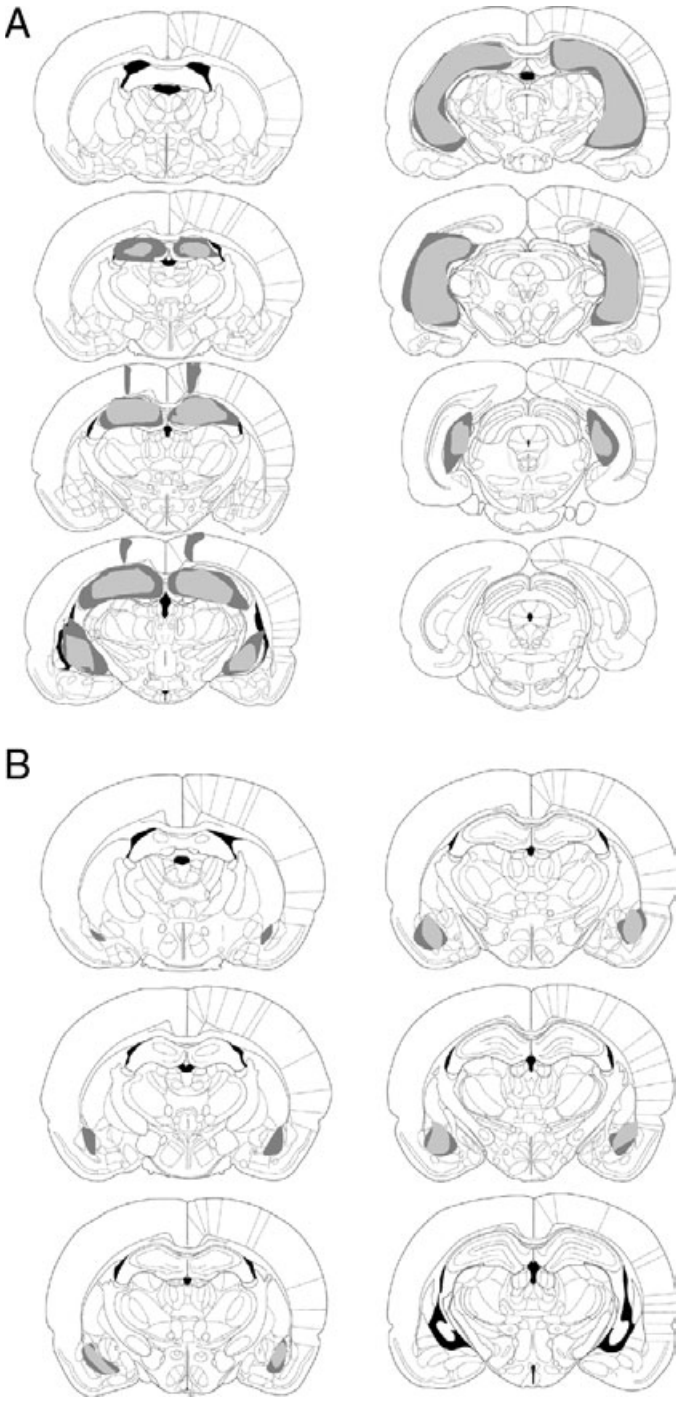

FIG. 2. Schematic representation of NMDA lesions of the HPC (A) and quinolinic acid lesions of the BLA (B). Areas shaded in grey and black represent the largest and smallest extent of neuronal damage in a single animal, respectively. Coronal sections are −2.2 mm through −6.5 mm posterior to bregma.

BLA-lesioned group compared to the sham group (days 1, 2 and 3 vs. day 6, $P < 0.003$). This was accompanied by increased latencies (slower approach) to the CS/US location during correct trials in the BLA-lesioned group across the six sessions of conditioning, compared to the sham group (see Fig. 3B, lesion $F_{1,17} = 5.01$, $P < 0.04$).

The performance of the BLA-lesioned and HPC-lesioned groups on the CS probe day, however, was not significantly different from that of the sham group (independent samples *t*-test; HPC $t = 0.51$, $P < 0.62$; BLA $t = -0.08$, $P < 0.94$), suggesting that all groups including the BLA group eventually acquired the CS–US association.

There were no significant differences between the levels and pattern of nose-poke behaviour of sham-operated and HPC-lesioned rats across the six conditioning sessions (lesion $F_{1,15} = 1.06$, $P =$ ns; lesion–day interaction $F_{6,90} = 0.68$, $P =$ ns; Fig. 3C). Both groups showed a reduction in the total number of nose-pokes made within a session as conditioning progressed (day $F_{6,90} = 14.98$, $P < 0.0001$). Although there was no overall significant difference between the levels of nose-pokes between the sham-operated group and BLA-lesioned group across the 6 days (lesion $F_{1,17} = 0.21$, $P =$ ns), there was a significant day–lesion interaction ($F_{6,102} = 3.31$, $P < 0.01$). Subsequent analyses showed, however, that the difference in the levels of nose-poke behaviour between the BLA-lesioned group and sham-lesioned group on the first two days of conditioning was not significant (day 1 $F_{1,17} = 4.89$, $P < 0.04$; day 2 $F_{1,17} = 1.86$, $P < 0.19$).

Locomotor activity (see Fig. 3D) was consistently and significantly elevated in the HPC-lesioned group compared to the sham-operated group during cue conditioning (lesion $F_{1,15} = 16.21$, $P < 0.001$), except for the CS probe day. The locomotor activity levels of the BLA-lesioned group were not significantly different from those of the sham-operated group at any point during cue conditioning (lesion $F_{1,17} = 0.27$, $P =$ ns).

In summary, the HPC-lesioned group was significantly faster, whereas the BLA-lesioned group was significantly slower in the acquisition of cue conditioning compared to the sham-operated group.

Place-cue retrieval

This phase of the experiment assessed the ability of sham-operated, BLA- and HPC-lesioned rats to use contextual information to guide discriminative approach behaviour towards the CS (Fig. 4). Upon confirmation that there was no significant difference in the acquisition data of place-cue retrieval in BLA-sham and HPC-sham animals (no lesion effect $F_{1,8} = 0.224$, $P < 0.649$), two-way ANOVA conducted on the pooled sham-operated group data revealed that the rats successfully acquired discriminated approach behaviour towards a CS+, by decreasing the number of approaches to the CS– whilst maintaining asymptotic numbers of approach to the CS+ within a session, across 8 days of conditioning (CS $F_{1,9} = 39.08$, $P < 0.0001$, day $F_{7,63} = 11.64$, $P < 0.0001$, CS × day: $F_{7,63} = 16.67$, $P < 0.0001$). A significant level of discrimination was achieved from day 6 ($P < 0.01$) onwards.

Overall ANOVA comparing the acquisition data of the HPC-lesioned and sham-operated groups (Fig. 4A) revealed significant main effects of CS ($F_{1,15} = 44.57$, $P < 0.0001$) and day ($F_{7,105} = 14.5$, $P < 0.0001$) with the main effect of lesion just failing to attain significance ($F_{1,15} = 4.06$, $P = 0.07$); however, there was a significant difference in the pattern of CS+/CS– discriminative approach behaviour between the HPC-lesioned and sham-operated groups (CS–lesion interaction $F_{1,15} = 4.17$, $P < 0.05$, CS × day–lesion interaction $F_{7,105} = 2.37$, $P < 0.03$). Further analyses showed this effect to be attributable to (i) a significantly higher level of approach responses to the CS– in the HPC-lesioned group across the 8 days of place conditioning (one-way ANOVA on CS– data; day–lesion interaction $F_{1,15} = 6.48$, $P < 0.02$, lesion effect $F_{1,15} = 5.45$, $P < 0.03$) and (ii) significantly slower attainment of significant levels of discrimination between the CS+ and CS– in the HPC-lesioned group (day 8, $P < 0.01$), compared to those of the sham-operated group. Thus, HPC-lesioned rats were significantly disrupted in the ability to use spatial

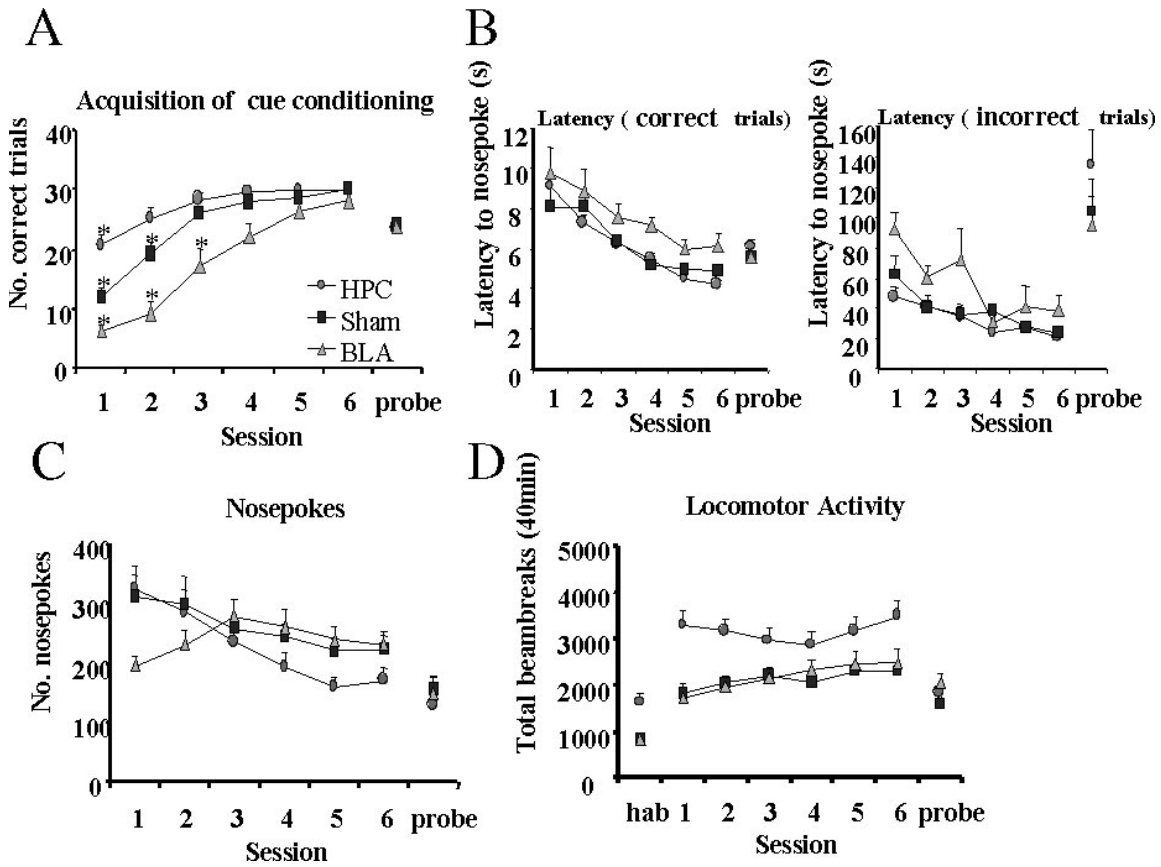

FIG. 3. Mean ± SEM performance of sham-operated controls and HPC- or BLA-lesion groups in discrete cue conditioning. (A) Acquisition expressed as number of approaches to the CS within 15 s (correct trial). *$P < 0.003$ vs. day 6; (B) latency to approach CS/US in correct and incorrect trials; (C) general nose-poke activity; (D) locomotor activity.

context in guiding discriminative approach behaviour towards a CS+ whilst inhibiting approach to a CS–.

Overall ANOVA of the acquisition performance of BLA-lesioned and sham-operated groups during place conditioning (Fig. 4B) showed significant main effects of lesion ($F_{1,17} = 7.26$, $P > 0.02$) as well as CS ($F_{1,17} = 75.13$, $P < 0.0001$) and day ($F_{7,119} = 12.58$, $P < 0.0001$). Separate analyses of the number of approaches made to the CS+ and CS– following a significant lesion–day interaction ($F_{7,119} = 2.78$, $P < 0.01$) revealed that the BLA-lesioned group showed significantly lower levels of approach behaviour towards the CS– compared to the sham group (lesion effect $F_{1,17} = 6.49$, $P < 0.02$) between days 4 and 6. Moreover, a significant level of discrimination between the CS+ and CS– was present from day 5 ($P < 0.01$) in the BLA-lesioned group, indicating that discriminative approach behaviour based on spatial context was established more rapidly in the BLA-lesioned group compared to the sham-operated group.

Conditioned place preference

As depicted in Fig. 5, the sham-operated group exhibited a strong preference for the paired place on the CPP test day (no significant difference between BLA-sham and HPC-sham groups; $F_{1,8} = 1.32$, $P < 0.283$), as indicated by the increased level of time spent in the paired place, compared to the two other unpaired places ($F_{2,18} = 18.89$, $P < 0.0001$). The BLA-lesioned group also showed a significant preference for the paired place ($F_{2,16} = 15.75$, $P < 0.0001$), a pattern of results that was not significantly different from the performance of the sham group (no place–lesion interaction, $F_{2,34} = 0.29$, $P =$ ns). The HPC-lesioned group however, failed to show a significant preference for the paired place ($F_{2,12} = 1.39$, $P = 0.29$), their performance being significantly different from that of the sham-operated group ($F_{2,30} = 3.64$, $P < 0.04$). Thus, lesions of the HPC, but not BLA, abolished the acquisition of place conditioning.

CS preference based on contextual information

On re-training after four weeks (Fig. 6), both sham-operated and BLA-lesioned groups quickly returned to the level of CS+/CS– discrimination attained just prior to the break (CS–day interaction sham $F_{5,45} = 13.84$; BLA $F_{5,40} = 14.25$, $P < 0.0001$). The groups' overall performance did not significantly differ (no lesion effect $F_{1,15} = 1.93$, $P =$ ns), however, the BLA-lesioned group showed consistently better discrimination levels across the 6 days of retraining (CS–lesion interaction $F_{1,15} = 5.00$, $P < 0.04$; day–lesion interaction $F_{5,75} = 2.66$, $P < 0.03$). In contrast, the HPC-lesioned group failed to achieve significant discrimination between the CS+ and CS– (no CS–day interaction, $F_{5,30} = 2.20$, $P = 0.09$), their overall performance being significantly different from that of the sham-operated group (lesion $F_{1.13} = 26{,}22$, $P < 0.0001$).

ANOVA conducted on the CS preference performance (Fig. 7) revealed that all the sham-operated and BLA-lesioned groups made significantly more approaches to the CS+ (sham $F_{1,9} = 41.61$, $P < 0.0001$; BLA $F_{1,8} = 16.03$, $P < 0.004$). The difference between

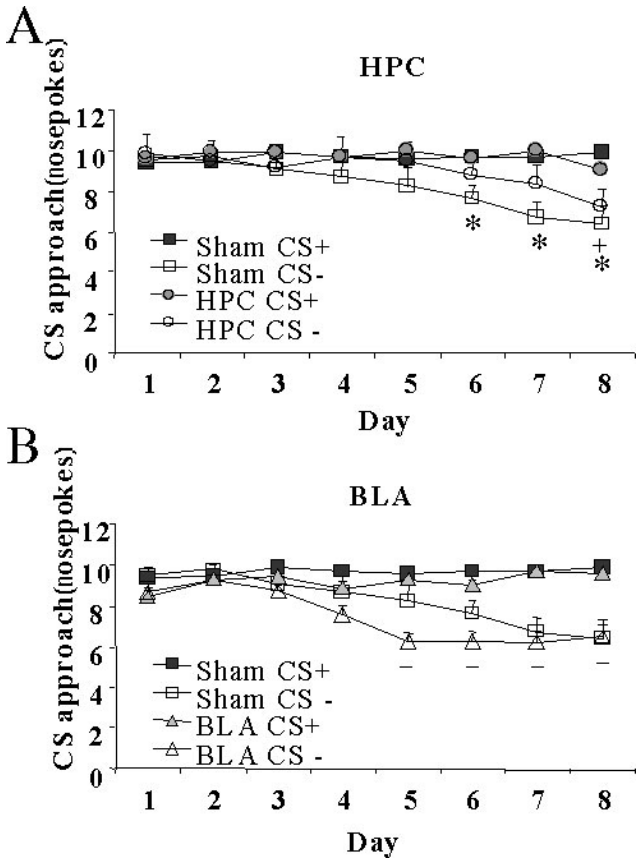

FIG. 4. Mean ± SEM performance in acquisition of place conditioning (context/place-cue retrieval) expressed as number of approaches to CS+ and CS–. (A) HPC-lesion group compared to sham-operated controls; (B) BLA-lesion group compared to sham-operated controls. *$P < 0.01$ vs. CS+ in sham; +$P < 0.01$ vs. CS+ in HPC; –$P < 0.01$ vs. CS+ in BLA.

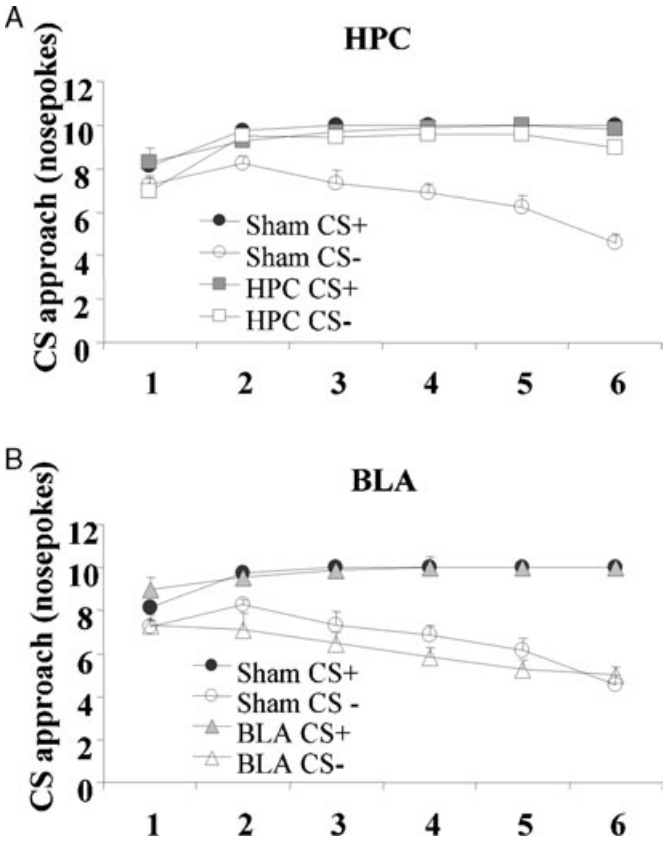

FIG. 6. Mean ± SEM performance during re-training of place-cue retrieval expressed as number of approaches to CS+ and CS–. (A) HPC-lesion group and sham-operated controls; (B) BLA-lesion group compared to sham-operated controls.

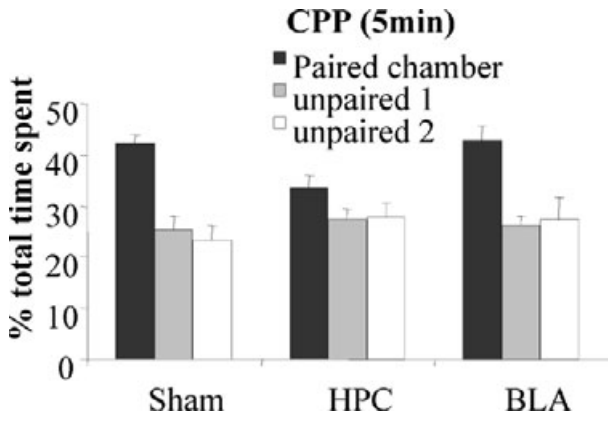

FIG. 5. CPP performance expressed as per cent of total time spent in each of the chambers of sham controls, HPC- and BLA-lesion groups.

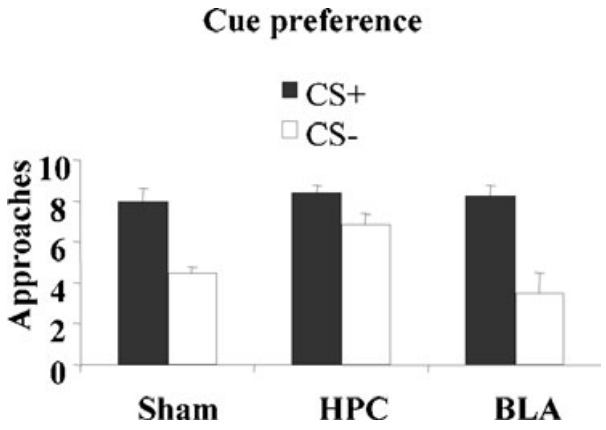

FIG. 7. Mean ± SEM number of approaches to CS+ and CS– during extinction in sham controls, HPC and BLA-lesion groups.

the number of approaches made to the CS+ and CS– in the HPC-lesioned group just failed to reach significance ($F_{1,6} = 5.45$, $P < 0.06$); however, their approach behaviour to the CS+/CS– was significantly different compared to that of the sham-operated group (lesion $F_{1,13} = 8.57$, $P < 0.01$), an effect that was attributable to the increased level of approach responses to the CS– in the HPC-lesioned group (*t*-test, $t = -3.82$, $P < 0.001$).

In summary, HPC-lesioned rats were significantly impaired in the use of spatial context to retrieve information about the CS. This was accompanied by poor conditioned preference for (i) the place embedded within the CS–US association, and (ii) for the CS associated with sucrose in a specific place. In contrast, BLA-lesioned rats were significantly better in their use of spatial context for CS retrieval.

Discussion

By using a novel CPP procedure devoid of visual landmarks to guide the animals' behaviour, the present study has demonstrated a truly HPC-dependent CPP, the acquisition of which was not only unaffected, but even enhanced in BLA-lesioned rats. When an explicit, discrete cue was deliberately made available for conditioning however, the task became BLA-sensitive, and HPC-independent. These findings not only demonstrate a double dissociation of the effects of BLA and HPC lesions on appetitive discrete CS and place conditioning based on path integration (rather than on the basis of differential sensory cues available during the trials), but also importantly demonstrate that the HPC and BLA subserve associative mechanisms that compete to gain control over appetitive behaviour. Furthermore, these findings help to resolve the issue of what constitutes a spatial 'context', in showing that a context need not necessarily be defined solely by a collection or configuration of elemental cues, but also by idiothetic cues.

Appetitive visual cue conditioning

The slower rate of acquisition of appetitive discrete cue conditioning following BLA lesions in the present study is consistent with the small but significant deficits in the acquisition of conditioned approach behaviour towards a CS signalling availability of sucrose in BLA-lesioned rats reported by Burns *et al*. (1993). The same CS then failed to support the acquisition of a new instrumental response as a conditioned reinforcer. The slowed acquisition of discrete cue conditioning seen in BLA-lesioned rats in the present study was accompanied by consistently increased latencies to approach the CS suggesting that an intact BLA is necessary for a CS to retrieve the value (affective) of a US (Hatfield *et al*., 1996; Cardinal *et al*., 2002) only early in training, perhaps akin to the observations that overtraining can ameliorate conditioned freezing deficits to contextual cues in BLA-lesioned rats (Parent *et al*., 1992; Maren, 1999). The transient nature of the effects of BLA lesions on cue conditioning here may reflect the presence of a dorsal striatal-mediated stimulus-response learning process (Packard & Knowlton, 2002). However, this does not adequately explain the enhanced ability of BLA-lesioned rats subsequently to acquire place-cue retrieval. The establishment of stimulus–response associations in the cue conditioning phase should have rendered the BLA-lesioned rats more resistant to extinction, but this was not observed in the present study.

BLA lesions do not impair the acquisition of pavlovian approach behaviour to a discrete cue (as seen in an autoshaping task, Parkinson *et al*., 2000), indicating that an intact BLA is not necessary for pavlovian CS-reward learning *per se*, except when pavlovian CS–US (affective) information has to be translated into instrumental and outcome-specific goal-directed action (Cador *et al*., 1989; Burns *et al*., 1993; Corbit & Balleine, 2005), or when the acquired motivational value of a CS supports the acquisition of conditioned behaviour to a second-order stimulus (Hatfield *et al*., 1996; Setlow *et al*., 2002). One critical procedural feature in the present study and the study of Burns *et al*. (1993) that could have rendered the acquisition of CS–US associations sensitive to BLA lesions is that the visual CS and US were presented in exactly the same location, giving rise to the possibility that the conditioned approach behaviour was under the influence of both pavlovian and instrumental contingencies. The same possibility is often raised in relation to the exact nature of conditioned responses elicited in CPP; although largely regarded as a pavlovian phenomenon, it likely also contains an instrumental component (Everitt *et al*., 1991; Tzschentke, 1998). This interpretation alone, however, does not account for the lack of deficit in first-order visual CS–US conditioning following neurotoxic BLA lesions in the study by Hatfield *et al*. (1996) in which the CS and US were also presented in a similar location. It is possible, however, that the present procedure placed more cognitive demands on the rats, as they would have had to attend to, and seek out the CS/reward in any one of three or nine locations, rather than in a single location in a confined space.

Excitotoxic lesions of the HPC resulted in the opposite effect of facilitating the acquisition of discrete cue conditioning. Spared discrete cue conditioning in HPC-lesioned rats showing clear deficits in contextual conditioning has also been reported in aversive tasks (Selden *et al*., 1991; Kim & Fanselow, 1992; Phillips & LeDoux, 1992; Anagnostaras *et al*., 2001; Rudy *et al*., 2004). The present finding accords with a number of appetitive conditioning studies showing a similar facilitation of acquisition of an amygdala-dependent CPP following fornix lesions (White & McDonald, 1993) and pavlovian discriminated approach behaviour towards a discrete CS+ following HPC lesions (Ito *et al*., 2005). The enhanced discrete cue learning observed here in HPC-lesioned rats is unlikely to be explained by increased general behavioural activity, as elevated locomotor activity levels in HPC-lesioned rats were not accompanied by equivalent increases in the levels of general nose-poke responding, or decreases in the latency to nose-poke in response to CS presentations. Instead, the enhanced learning of a task that is sensitive to BLA lesions in the present study is more likely to reflect the absence of a competing and parallel inhibitory HPC-based contextual learning system (Gold, 2004).

Place-cue retrieval and place learning

The second part of the present study required rats either to approach or to inhibit their approach to the same CS depending on its spatial location. Thus, the CS was accompanied by reward only when presented in a specific spatial context. The data show that HPC-lesioned rats were impaired in using spatial context to guide their conditioned approach responses, consistent with the notion that the HPC is critical in context-dependent memory retrieval (Hirsh, 1974; Good & Honey, 1991; Davidson & Jarrard, 1993; Corcoran & Maren, 2001; Kennedy & Shapiro, 2004). The impaired use of place to disambiguate CS information marked by increased levels of approach towards the CS- in the HPC-lesioned rats in the present study could be argued to be due to simple deficits in inhibitory learning that can follow damage to the HPC, specifically in situations where there is a concurrent, previously established excitatory association (Chan *et al*., 2001; Davidson & Jarrard, 2004; Ito *et al*., 2005). The place-cue retrieval learning in the present study did have an extinction component, requiring the formation of an inhibitory association between a previously excitatory CS and place, and possibly another between the US and place. However, this account does not adequately explain why the HPC-lesioned rats were so clearly facilitated in discrete cue-reward learning, when an inhibitory component was absent, and why they showed normal performance during the extinction session.

A more plausible explanation for the impaired place-cue retrieval and acquisition of place conditioning (as evident in the failure to express conditioned preference for the paired place in the absence of CS presentations), is that the HPC-lesioned rats were unable to form effective associative spatial representations based primarily on idiothetic (self-motion) cues, in the absence of any other visually guiding cues. This hypothesis is supported by several studies that have shown the HPC to be a component of the path integration neural system,

which processes converging input from head direction cells in the postsubiculum and associated neocortical and thalamic areas to HPC place cells (McNaughton *et al*., 1996; Whishaw & Maaswinkel, 1998; Taube, 1999; Save *et al*., 2001). HPC cells are able to differentiate two identical chambers if the animals can walk between the two chambers connected by a passageway (Skaggs & McNaughton, 1998), or if the chambers are aligned at a large angle relative to one another (Fuhs *et al*., 2005), as in this study. Together with these data, the present results support the view that the HPC is not only important for processing spatial contextual information derived from distinctive visual cues in most test environments, but also for integrating information derived from self-motion cues in the absence of external sensory input.

It could be suggested that the HPC-lesioned rats were beginning to demonstrate discriminative approach behaviour towards the CS+ in the final days of place conditioning, as well as showing a tendency (although nonsignificant) to spend somewhat more time in the paired compartment on the day of CPP testing, indicating a residual capacity to discriminate the three compartments. Despite rigorous efforts to eliminate the possibility of any extraneous visual, auditory and odour cues providing directional information, it seems likely that highly motivated rats would actively seek alternative (cue-based) strategies to compensate for the absence of a HPC-dependent, spatial strategy. Odour cues, for example, may acquire discriminative stimulus properties, but their effect must have been confined entirely to within-session changes in behaviour, as the apparatus was thoroughly cleaned with ethanol between each session, the assignment of the rewarding compartment was counterbalanced across rats, and the whole apparatus underwent a 120° rotation at the end of each training day. Moreover, any non-HPC dependent strategy that HPC-lesioned rats may adopt is likely to vary considerably among rats and remains indeterminate within the context of this study. What is clear, however, is that the acquisition of place conditioning under the precisely controlled conditions used here required the animals to adopt a predominantly HPC-dependent strategy, and that the effect of any other, emergent strategy adopted by lesioned rats was minimal, and ineffective on its own.

Although not as well documented as the improved learning effect of HPC lesions upon amygdala-dependent tasks (White & McDonald, 1993; McDonald & White, 1995; Ferbinteanu & McDonald, 2001), it has been shown that lateral amygdala lesions also facilitate the acquisition of a HPC-dependent version of CPP; learning to discriminate between two adjacent arms of a radial maze (Chai & White, 2004). These findings further indicate a competitive interaction between amygdala-mediated and HPC-mediated learning and memory systems. However, some evidence suggests that this relationship is modulatory, rather than competitive, with post-training intra-BLA infusions of *d*-amphetamine enhancing memory in an HPC-dependent stationary hidden platform version of the water maze task (Packard *et al*., 1994; Packard & Teather, 1998).

Competition and interaction between hippocampus and amygdala-mediated learning and memory systems

The present study has shown how a CPP procedure can be made HPC-dependent, and BLA-independent by requiring the animals to rely primarily on idiothetic spatial cues to discriminate between three identical chambers. In contrast, the task was BLA-dependent and HPC-independent if an explicit cue was made available for conditioning. These findings help to resolve apparent discrepancies in previous data showing variable effects of HPC and BLA lesions on CPP. The present results also provide evidence for a complementary and competitive involvement of the HPC and BLA in learning and memory, raising the important issue of how these two streams of information processing are integrated to gain control over behaviour. One possible neural locus of convergence is in the nucleus accumbens to which both the HPC and BLA project (Groenewegen *et al*., 1997, 1999), and where neurophysiological evidence suggests that HPC afferents play a permissive role in the gating of BLA and prefrontal cortical inputs to NAc medium spiny neurons (O'Donnell & Grace, 1995). The balance between limbic and cortical inputs to the NAc is in turn modulated differentially by D1 and D2 dopamine receptors in the NAc (Goto & Grace, 2005). Thus, associative information dependent upon the HPC and BLA may gain control over goal-directed behaviour through dopamine–modulated interactions with NAc neurons that receive converging input from these structures (Pennartz *et al*., 1994).

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Abbreviations

BLA, basolateral amygdala; CCP, conditioned place preference; HPC, hippocampus.

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