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The Hippocampus and Appetitive Pavlovian Conditioning: Effects of Excitotoxic Hippocampal Lesions on Conditioned Locomotor Activity and Autoshaping

Rutsuko Ito,* Barry J. Everitt, and Trevor W. Robbins

ABSTRACT: The hippocampus (HPC) is known to be critically involved in the formation of associations between contextual/spatial stimuli and behaviorally significant events, playing a pivotal role in learning and memory. However, increasing evidence indicates that the HPC is also essential for more basic motivational processes. The amygdala, by contrast, is important for learning about the motivational significance of discrete cues. This study investigated the effects of excitotoxic lesions of the rat HPC and the basolateral amygdala (BLA) on the acquisition of a number of appetitive behaviors known to be dependent on the formation of Pavlovian associations between a reward (food) and discrete stimuli or contexts: (1) conditioned/anticipatory locomotor activity to food delivered in a specific context and (2) autoshaping, where rats learn to show conditioned discriminated approach to a discrete visual CS+. While BLA lesions had minimal effects on conditioned locomotor activity, hippocampal lesions facilitated the development of both conditioned activity to food and autoshaping behavior, suggesting that hippocampal lesions may have increased the incentive motivational properties of food and associated conditioned stimuli, consistent with the hypothesis that the HPC is involved in inhibitory processes in appetitive conditioning. © 2005 Wiley-Liss, Inc.

KEY WORDS: rat; inhibition; amygdala; motivation; context

INTRODUCTION

The hippocampus (HPC) and amygdala are thought to be functionally distinct components of different learning and memory systems. The HPC is critically involved in the formation of associations between contextual/spatial information and behaviorally significant events (Selden et al., 1991; Kim and Fanselow, 1992; Phillips and LeDoux, 1992; Rudy et al., 2002; Jeffery et al., 2004) as well as in the retrieval of such associations (Hirsch, 1974; Good and Honey, 1991). The amygdala is an integral part of the brain's emotional processing system, particularly as a site where information about conditioned stimuli (when discrete in nature) and unconditioned effects of emotive experiences (LeDoux et al., 1990; Parkinson et al., 2000) are integrated and given emotional value to gain control over behavior.

Department of Experimental Psychology, University Cambridge, Downing Street, Cambridge, CB2 3EB UK
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*Correspondence to: Rutsuko Ito, Department of Experimental Psychology, University of Cambridge, Downing St, CB2 3EB.
E-mail: ri204@cam.ac.uk
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A role that is not so readily associated with the HPC is its contribution to basic motivational processes, and yet numerous studies have reported alterations in food-related appetitive behavior following selective lesions of the HPC (Tracy et al., 2001). Thus, changes in feeding patterns, including increased number of food contacts, and frequency of eating (Davidson and Jarrard, 1993; Clifton et al., 1998), increased breakpoints in progressive-ratio schedules of responding for food (Schmelzeis and Mittleman, 1996), perseveration of conditioned responding in the absence of reward, or in the face of changes in reward magnitude (Jarrard and Isaacson, 1965; Flaherty et al., 1998), and increased behavioral activity in environments consistently associated with food (Benoit et al., 1999), are some of the effects linked with HPC lesions. These diverse observations have given rise to the hypothesis of the involvement of the HPC in the formation of simple inhibitory associations between behaviorally significant events where there is a concurrent formation of excitatory associations (Chan et al., 2001; Davidson and Jarrard, 2004).

The present study aimed to further investigate the role of the HPC and BLA in appetitive processes by making excitotoxic lesions of each structure and evaluating their effects on the acquisition of two Pavlovian conditioning tasks: (1) conditioned locomotor activity, which is a form of incentive-motivational or appetitive conditioning (Beninger, 1983; Poncelet et al., 1987; Brown and Fibiger, 1993) where environmental stimuli are endowed with incentive properties through association with the unconditioned stimulus (US) (context). Rats have been consistently shown to exhibit greater activity (anticipatory responses) in a context previously associated with the effects of psychostimulants and natural reinforcers (Amsel et al., 1962; Bolles and Stokes, 1965) and (2) autoshaping, in which repeated pairings of a conditioned stimulus (CS) and reward give rise to the acquisition of a Pavlovian discriminated approach response to the CS+, an action that does not, in itself, affect the reinforcement schedule. The robustness of the acquisition of the CS–US association can subsequently be tested in the reward omission phase, where animals will continue to approach the CS+ even though the responses are now punished (cancellation of reward) (Brown and Jenkins, 1968; Bussey et al., 1997).

TABLE 1.

Lesion Parameters

Lesion	Excitotoxin	Co-ordinates for injections sites AP	L	DV	Volume per site (μl)	Diffusion time before removal of injector (min)
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	−7.3	0.3	3
		−3.0	±4.6	−7.3	0.3	3

AP, anterior-posterior axis relative to bregma; L, lateral; DV, dorsal–ventral axis (from dura).

METHODS

Subjects

Subjects were 51 male Lister Hooded rats (Charles River, UK) weighing 300–330 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/dark cycle (lights off 0900 h). Water was available ad libitum, but following recovery from surgery, food (laboratory chow, Purina) was restricted to 18 g lab chow/day, sufficient to maintain preoperative body weight and growth. All experiments were carried out during the dark phase, between 0900 and1900 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 = 22 HPC lesions; n = 8 HPC sham-operated controls; n = 14 basolateral amygdala (BLA) lesions; n = 10 BLA sham-operated controls). In all surgical procedures, animals were anesthetized with 10 g Avertin (99% 2,2,2-tribromoethanol, Sigma-Aldrich, Dorset, UK) prepared with 5 g tertiary amyl alcohol and 450 ml phosphate buffered saline (PBS) (Dulbecco "A", Unipath, Basingstoke, Hampshire, UK) and 40 ml absolute alcohol; 1 ml/100 g body weight, IP), 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 types and volumes 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 behavioral testing, with food available ad libitum.

EXPERIMENT 1: CONDITIONED LOCOMOTOR ACTIVITY

Apparatus

Locomotor activity was tested in sixteen photobeam activity cages each measuring 25 × 40 × 18 cm^3 and equipped with two photobeams spaced equally along the length of the cage, 1 cm above the grid floor. Each session lasted 90 min, and beam breaks were measured in 10-min bins and stored in an Acorn Archimedes microcomputer (Acorn Computers, Cambridge, UK).

Behavioral Procedure

Prelesion locomotor activity

A 90-min locomotor activity session was given before surgery.

Habituation (5 days)

Rats were placed in the activity cages for 90 min at the same time each day until stable levels of activity were achieved. During this period, water was available ad libitum in the activity cages, but feeding occurred at varying intervals (2–4 h) after testing in the home cages.

Conditioning (12 days)

Feeding was switched from the home cages to the activity cages. Plastic bowls containing food pellets were presented 30 min after the commencement of the activity session. Rats were then given 60 min to consume the food (with water ad libitum), and were returned to their home cages, where no further food was given.

Prefeeding (5 days)

Rats were allowed free access to food for 60 min prior to the activity session in their home cages, and then transferred to the

activity cages. Food was still presented 30 min into the session, as with conditioning sessions.

Extinction (5 days)

Following the prefeeding manipulation, rats were given extinction sessions in which food was no longer presented in the activity cages, but was instead presented at varying times between 1 and 3 h after the activity tests.

EXPERIMENT 2: AUTOSHAPING

This part of the study was conducted only in the HPC-lesioned and sham-operated groups, as the effects of BLA lesions on autoshaping have previously been assessed (Parkinson et al., 2000).

Apparatus

Six test chambers (45 × 32 × 30 cm^3; Cambridge Cognition, Cambridge, UK) contained within a sound-attenuating box with a ventilating fan were used in the experiment. Each chamber consisted of a centrally positioned 3-W house light, a front wall with a video display unit (VDU) (Intrasolve, UK), two food magazine hoppers attached to an external pellet dispensing device for the controlled delivery of 45 mg sucrose pellets (Noyes, UK), and a pressure sensitive floor panel at the rear. The first food magazine was positioned centrally within a vertical chimney 6 cm away from the VDU. The second food magazine was located at the rear of the testing chamber, directly above the floor panel. Stimuli were white rectangles (10 × 28 cm^2) presented on the right or left hand side of the VDU. Approaches to these stimuli were detected by photocell beams located to the right and left of the central food magazine.

Behavioral Procedure

Habituation

Rats were given two 30-min habituation sessions in which sucrose pellets were delivered under a variable interval 40-s schedule (VI40) in the central food magazine. Pellets were also delivered in the rear food magazine, but only following the depression of the rear floor panel. This food magazine was used only during the habituation sessions.

Acquisition

During the acquisition phase, rats were trained to associate a 10-s white rectangle stimulus presented on one side of the VDU with the availability of a sucrose pellet in the central magazine. Rats were given a total of 100 trials (across 2 days), each trial consisting of one 10-s CS+ presentation (followed immediately by reward) and one 10-s CS– presentation (never followed by reward) in a randomized order, with a minimum of 10 s between the stimulus presentations. The CS+ was discriminable from the CS– by its spatial location (right or left hand side of the VDU), with the assignment of CS+/– counterbalanced across rats. Each trial was initiated by the depression of the rear floor panel (ensuring equal stimulus sampling as well as elimination of chance approaches), and stimuli were presented on a VI40s schedule. The first beam break made during the stimulus presentation at either the left or right photocell beam locations directly in front of the stimulus was scored as an approach. The number of approaches to the CS+ and CS– per block of 10 trials, as well as the mean latencies to approach the stimuli across 10 trials were determined for data analysis. A difference score (approach to the CS+ minus approach to the CS– across 10 trials) was also calculated for each block as an index of the level of discrimination learning achieved during acquisition.

Omission

Following acquisition, rats were given 50 omission trials, in which all parameters remained the same as in acquisition, except for a change in contingency such that approaches to the CS+ prevented the delivery of a food pellet.

Data Analysis

All data were analyzed using the SPSS statistical package version 9.0 (SPSS, Chicago, IL). The total number of beam breaks recorded in the first 30 min of a session was subjected to repeated measures analysis of variance (ANOVA) with lesion group as the between-subjects factor (lesions: HPC, BLA, and sham) and session as the within-subjects factor. The autoshaping data were subjected to a repeated measures ANOVA with lesion group as the between-subjects factor (lesions: HPC and sham) and block as the within-subject factor. 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).

Histological Procedure and Assessment of Lesions

Within a week after the completion of the testing, all rats were anesthetized 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 PFA and transferred to a 20% sucrose cryoprotectant solution on the day before sectioning. For the verification of lesions, coronal sections (60 μm) of the brain were cut using a freezing microtome.

RESULTS

Lesion Assessment

The extents of the excitotoxic lesions of the HPC and BLA are shown schematically in Figure 1, based on Paxinos and Watson's stereotaxic atlas of the rat brain (1998). Excitotoxic lesions of the HPC induced by NMDA infusions extended rostrally from 1.8 mm to 6.7 mm posterior to the bregma. Mechanical damage from the cannula was evident in the primary somatosensory and lateral secondary visual cortical areas of both sham and lesioned subjects, and neuronal damage occasionally extended unilaterally to the ventral subiculum. Three rats were excluded from the study on the basis of extensive bilateral damage to the overlying cortex and/or extrahippocampal structures. The final group numbers were 19 for HPC lesions and 8 for 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, in the majority of cases leaving the central nucleus and medial nucleus of the amygdala intact. 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 to the central nucleus of the amygdala. The final group numbers were 11 for BLA lesions and 10 for sham-operated controls.

Experiment 1: Conditioned Locomotor Activity

There were no significant changes in spontaneous locomotor activity prior to and following surgery in sham-operated and HPC lesioned rats. There was, however, a significant decrease in spontaneous locomotor activity following surgery in the BLA-lesioned group ($F(1,10) = 78.22$, $P < 0.0001$, Fig. 2a).

Overall ANOVA on activity levels of the three groups across five habituation sessions revealed a significant main effect of session ($F(4.180) = 20.35$, $P < 0.0001$) and lesion × session interaction ($F(8.180) = 5.82$, $P < 0.0001$). Further one-way ANOVAs revealed that the activity level recorded on the first habituation session postsurgery remained unchanged across the 5 days of habituation in the BLA-lesioned group ($F(4,40) = 1.22$, ns), but showed significant decline over the 5 sessions in sham- and HPC-lesioned rats (sham: $F(4,68) = 17.56$, $P < 0.0001$; HPC: $F(4.72) = 17.31$, $P < 0.0001$). By the end of habituation however, all groups had reached the same level of baseline activity (one-way ANOVA: $F(2,47) = 0.23$, $P = 0.80$, Fig. 2a).

During conditioning, activity levels significantly increased across 12 sessions in all groups (main effect of session: $F(11,495) = 17.38$, $P < 0.0001$, Fig. 2b). However, the level of increased activity was significantly higher in the HPC-lesioned rats compared with both the sham and BLA-lesioned rats (lesion effect: $F(2,45) = 23.53$, $P < 0.0001$; day × lesion interaction: $F(22,495) = 6.07$, $P < 0.0001$). Separate ANOVA for each lesion group revealed that the rate of acquisition of conditioned activity in the BLA-lesioned rats was significantly slower compared with the sham-operated rats (session × lesion interaction: $F(11,297) = 2.16$, $P < 0.02$; independent sample t-test on day 2: $t = 2.34$, $P < 0.03$). Point by point comparisons of activity levels in the HPC-lesion group revealed that the HPC-lesioned rats showed potentiated conditioned locomotor activity compared with sham-operated rats from session 3 onwards (independent samples t-test: $t = 3.14$, $P < 0.003$).

Prefeeding significantly suppressed conditioned locomotor activity in all groups (session: $F(2,90) = 55.6$, $P < 0.0001$ and

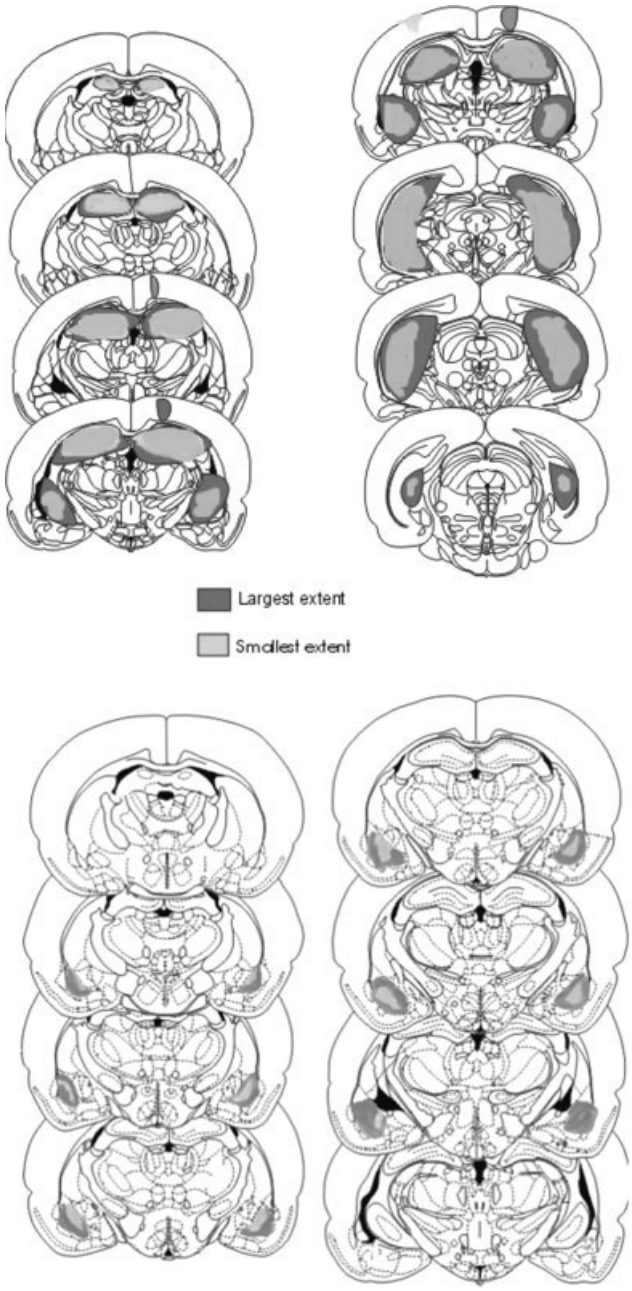

FIGURE 1. Schematic representation of NMDA lesions of the HPC (top) and quinolinic acid lesions of the BLA (bottom). Areas shaded in gray and black represent the largest and smallest extent of neuronal damage in a single animal, respectively. Coronal sections are 2.2 mm through 6.5 mm posterior to bregma.

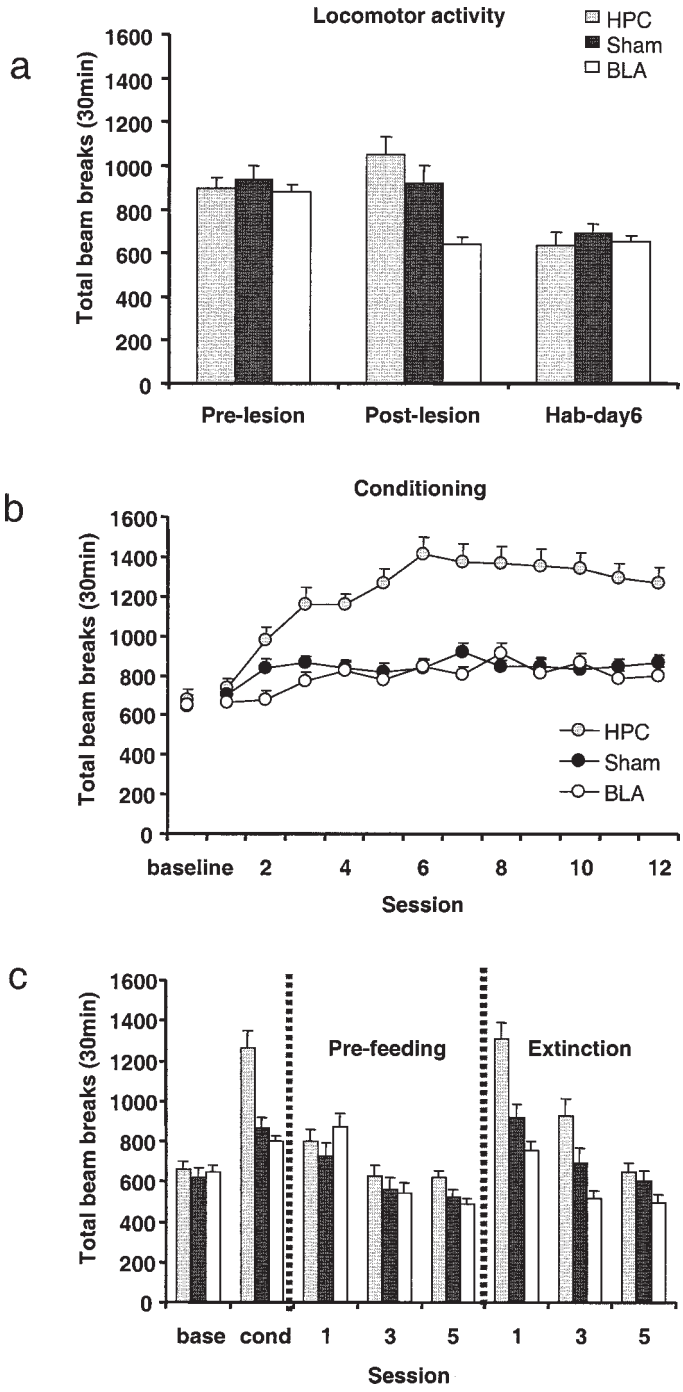

FIGURE 2. a: Effects of HPC and BLA lesions on spontaneous locomotor activity and locomotor activity on the last day of habituation, b: the acquisition of conditioned locomotor activity, c: prefeeding prior to conditioning sessions and extinction of conditioned locomotor activity. All data are presented as mean ± standard error of mean (SEM).

no lesion effect: $F(2,44) = 0.79$, ns, Fig. 2c), reaching a level equal to (session: HPC $F(1,18) = 0.53$, ns), or lower (session: BLA; $F(1,10) = 13.45$, $P < 0.01$; and Sham: $F(1,17) = 6.72$, $P < 0.02$) than baseline activity by the third day.

On the first day of extinction, activity returned to prefeeding conditioned levels in all three groups (Session sham: $F(1,17) = 2.64$, ns; HPC:$F(1,10) = 0.33$, ns, BLA: $F(1,18) = 1.34$, ns). With further extinction sessions, activity levels declined to baseline levels in all groups (Session: $F(2,90) = 603.44$, $P < 0.0001$), although the HPC-lesioned rats took significantly longer time (by day 4 as opposed to day 3 in the sham-group) to return to the baseline level of locomotor activity (day × lesion interaction: $F(4,140) = 61.9$, $P < 0.0001$).

Experiment 2: Autoshaping

Sham-operated and HPC-lesioned groups both acquired discriminated approach behavior toward the CS+ across 10 blocks of 10 stimulus presentations (CS: $F(1,12) = 27.42$, $P < 0.0001$, CS × block: $F(9,108) = 3.86$, $P < 0.0001$, Fig. 3a). The pattern of acquisition, however, was significantly different in the HPC-lesioned group compared with the sham group (lesion: $F(1,12) = 11.43$, $P < 0.005$, lesion × block: $F(9,108) = 1.91$, $P < 0.05$) as analysis of difference scores (Fig. 3b) indicated that a significant level of discrimination was achieved at an earlier stage in the HPC-lesioned animals (lesion: $F(1,12) = 6.53$, $P < 0.03$).

All animals became significantly faster in approaching the CS+ as training progressed ($F(9,108) = 5.55$, $P < 0.0001$), but the HPC-lesioned group had consistently faster approach latencies than did the sham group at all stages of training (lesion: $F(1,12) = 60.17$, $P < 0.0001$, Fig. 3d).

During omission, both sham-operated and HPC-lesioned animals continued to show significant discrimination between the CS+ and CS– (CS: $F(1,12) = 49.8$, $P < 0.0001$, Fig. 3c). There was no significant difference in the omission performance of the HPC-lesioned and sham groups (no lesion effect; $F(1,12) = 0.02$, ns).

DISCUSSION

The present study provides further insight into the nature of the involvement of the HPC in appetitive behavior. Excitotoxic lesions of the HPC induced potentiation of conditioned anticipatory locomotor activity for food, consistent with previous observations that food-deprived hippocampal lesioned rats show "exaggerated" conditioned anticipatory stereotypies and locomotion in situations predictive of food reward (Schmaltz and Isaacson, 1966; Devenport et al., 1981). The present study also provides the first demonstration of hippocampal lesions having a facilitatory effect on the acquisition of Pavlovian conditioned discriminative approach behavior, in contrast to the lack of effect following damage to the ventral or dorsal subiculum (Parkinson et al., 2000). Taken together, lesions to the HPC resulted in the disinhibition of Pavlovian appetitive behavioral responses, highlighting the role of the HPC in inhibiting behavioral processes.

The enhancement of conditioned locomotor activity and facilitation of the acquisition of autoshaping behavior as a result of HPC lesions is unlikely to be a product of increased general behavioral activation. HPC-lesioned rats in the present study were not spontaneously hyperactive, habituated normally to the

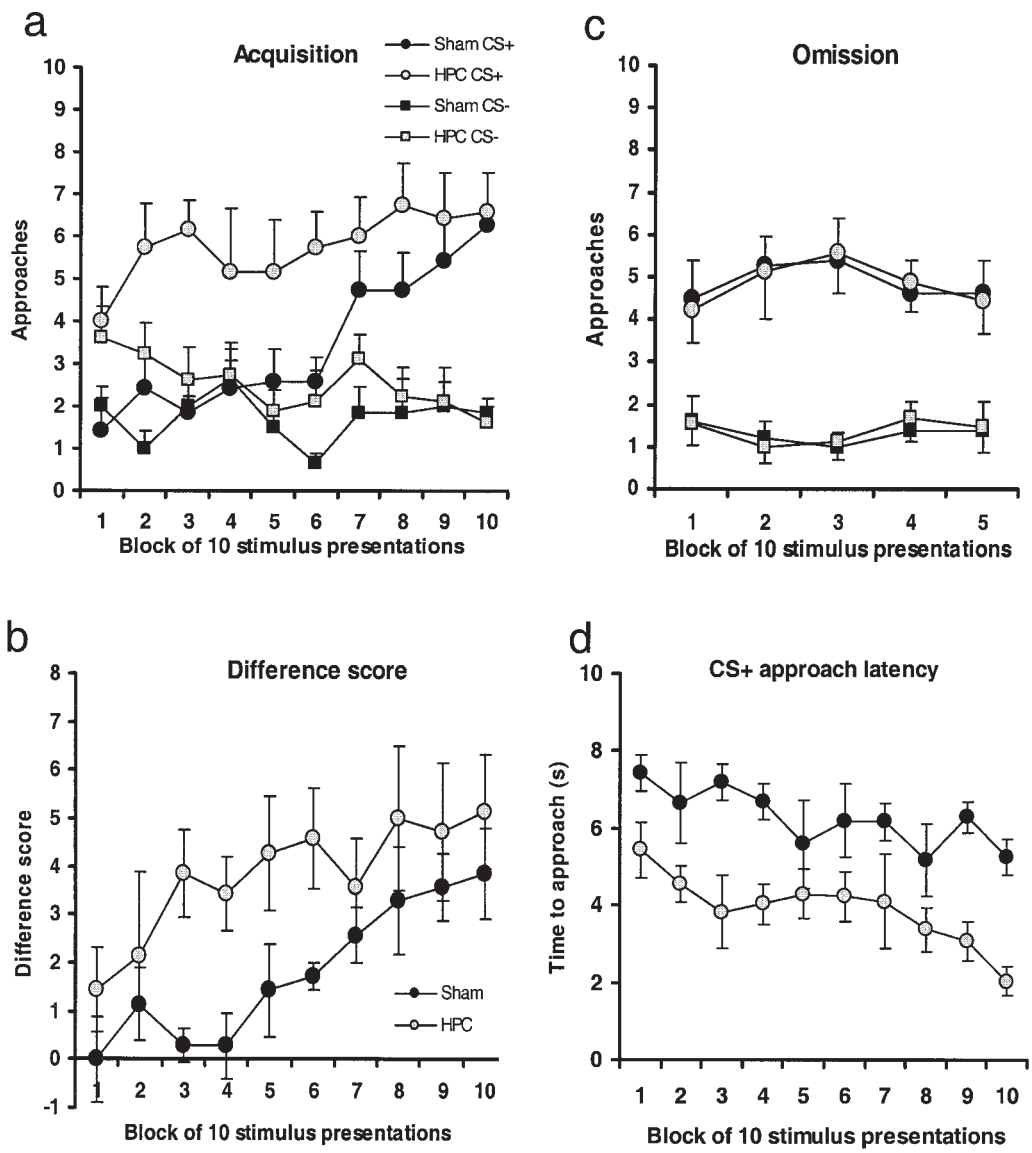

FIGURE 3. Effects of HPC lesions on the acquisition of autoshaping, as shown by a: the number of approaches to the CS+ and CS–, b: the difference score for each block (CS+ approaches−CS– approaches), c: the number of CS+ and CS–1; approaches during the omission phase, and d: the latency to approach the CS+. All data are presented as mean ± SEM.

activity cage, and showed satiety-induced suppression of conditioned locomotor activity, the increase in activity being specific to the anticipation of food within the cage. Benoit et al., (1999) similarly reported that the expression of increased anticipatory behavioral activity in HPC-lesioned rats compared with sham-operated rats was specific to the context that reliably predicted the availability of reward, as opposed to a novel context, or a context in which the context–US association was weak. HPC-lesioned rats also learned to discriminate their conditioned response to a CS+ and a CS–, indicating that their ability to selectively suppress inappropriate behaviors remained intact.

One explanation for the enhanced conditioned locomotor activity and facilitation of the acquisition of autoshaping behavior is that lesions of the HPC had increased the incentive motivational properties of the reward, and of any conditioned stimuli associated with the reward. Indeed, Schmelzeis and Mittleman (1996) reached a similar conclusion after it was found that hippocampal lesions increased breakpoints in food-deprived rats responding for food under a progressive-ratio schedule relative to controls, and that sucrose substitution augmented this effect, while a prefeeding challenge did not.

The neurobiological mechanism by which the HPC exerts its influence on incentive motivation may be through its inhibitory influence on the dopamine-dependent functions of the nucleus accumbens (NAc). The expression of food-conditioned locomotor activity is enhanced following systemic amphetamine

administration (Jones et al., 1990), an effect that is dependent on NAc dopamine (DA) transmission (Jones and Robbins, 1992). Such conditioned locomotor activity is attenuated in adult rats subjected to repeated maternal separation, which also exhibit a blunted response to d-amphetamine and increased response-attenuation effects of the D2 receptor antagonist sulpiride and the α_1/α_2 adrenoceptor agonist clonidine (Matthews et al., 1996). These findings together highlight the catecholaminergic mediation of conditioned locomotor activity. Moreover, amphetamine-induced locomotion has been shown to increase following hippocampal damage (Mittleman et al., 1998), an effect that is accompanied by larger elevations in extracellular NAc DA levels compared with that in controls (Wilkinson et al., 1993), and blocked by 6-OHDA lesions of the NAc (Whishaw and Mittleman, 1991).

Given the prominence of the role of the HPC in contextual conditioning (Selden et al., 1991; Phillips and LeDoux, 1992; Fanselow, 2000) and the fact that conditioned activity is presumably dependent on the formation of an association between the delivery of food and the context, it is perhaps surprising that lesions of the HPC did not abolish conditioned activity. It may be argued that conditioned activity is engendered instead by elemental cue–food associations, but the present data also demonstrate that lesions of the BLA, an area highly implicated in discrete cue conditioning, do not attenuate conditioned locomotor activity for food either, a finding consistent with previous data suggesting that amphetamine-induced conditioned activity is not dependent on the integrity of the amygdala (Brown and Fibiger, 1993; Ahmed et al., 1995). Indeed, the absence of any effects of BLA lesions on conditioned activity in the present study, and autoshaping as previously reported (Parkinson et al., 2000), supports the view that the BLA is not concerned with the simple elicitation of a Pavlovian conditioned response by a CS (Hall et al., 2001; Holland and Gallagher, 2003), but is critical for mediating Pavlovian influences over more complex and outcome-specific forms of goal-directed instrumental behavior (Cador et al., 1989; Burns et al., 1993; Cardinal et al., 2002a; Corbit and Balleine, 2005).

The conditioning to the context during the acquisition of conditioned locomotor activity in the present study may have involved the concurrent formation of both excitatory and inhibitory associations, as food was presented with a 30-min delay. Thus, conditioned activity could be thought of as the net outcome of a balance between the invigorating and suppressive effects of a reward-associated context. Lesions of the HPC may have led to the loss of contextual inhibition, releasing the excitatory control over conditioned locomotor activity. This view, together with the finding of the present study that the conditioned locomotor activity in HPC-lesioned rats returned to baseline significantly more slowly than in the shams (slowed extinction), would seem to support the simple associative model of HPC function which suggests that the HPC is selectively involved in mediating inhibitory learning in situations (e.g., in extinction) where there is a concurrent, previously established excitatory association (Chan et al., 2001). Indeed, the observed facilitation of autoshaping to a CS+ in the present study could also reflect the loss of contextual inhibition over the approach response.

Another way of interpreting the present pattern of results is to view the HPC-lesioned rats as being prone to impulsive action. The autoshaping response and conditioned locomotor activity have both been proposed to be expressions of impulsive responding (Tomie et al., 1998a,b; Winstanley et al., 2004). Performance on autoshaping has been reported to show strong within-subject correlations with other measures of impulsive responding such as intolerance to delayed reinforcement, as indicated by increased choice for smaller (as opposed to larger) delayed rewards (Tomie et al., 1998a). Presession administration of low doses of ethanol, a manipulation known to increase the level of impulsive responses in delayed reinforcement procedures (Evenden and Ryan, 1996; Tomie et al., 1998a), also facilitates the acquisition of an autoshaped conditioned response (Tomie et al., 1998b). Moreover, Winstanley et al., (2004) found that the potentiative effect of forebrain serotonin depletion on autoshaping was paralleled by an elevation in the level of conditioned activity for food. However, the HPC lesion-induced impulsivity seen here could again simply be a manifestation of the loss of contextual inhibition over Pavlovian behavioral processes.

The present autoshaping data importantly point to a very specific inhibitory role of the HPC within a network of brain structures implicated in acquiring Pavlovian approach behavior. Autoshaping deficits following damage to the nucleus accumbens core (Parkinson et al., 2000; Cardinal et al., 2002b), central nucleus of the amygdala (Parkinson et al., 2000), anterior cingulate cortex (Bussey et al., 1997), and the orbitofrontal cortex (Chudasama and Robbins, 2003) have been previously reported. The exact mechanism by which the HPC may exert its inhibitory influence upon Pavlovian approach behavior is not immediately obvious. However, from the lack of effect of subiculum lesions on autoshaping (Parkinson et al., 2000), it is clear that there may be an alternative, extra-subicular route by which HPC projections exert their influence. Anatomical studies demonstrate that there are reciprocal connections between the CA1 region of the HPC and medial and lateral entorhinal cortices that are more or less independent of the subiculum (Naber et al., 2001). The dorsolateral entorhinal area also sends projections to the anterior cingulate cortex which in turn has indirect links with the HPC via parahippocampal regions in rats (Van Eden et al., 1992). Taken together with these anatomical data, the present study may point to a possible functional interaction between the HPC and anterior cingulate cortex in the control over the acquisition of appetitive Pavlovian approach behavior. Other extra-subicular routes further downstream of the HPC could also contribute to the control over Pavlovian approach behavior; for example, the connection between the CA1 region of the HPC and specific hypothalamic nuclei that are concerned with the control of feeding and ingestive behaviors, or with the lateral septal nucleus and the nucleus reunions of the dorsal thalamus (Herkenham, 1978; Swanson, 2000). However, the hypothalamus is perhaps more specifically concerned with homeostatic mechanisms of inges-

tive behavior, than with the conditioned appetitive responses studied here (Robbins and Everitt, 1996; Cardinal et al., 2002a).

In summary, excitotoxic lesions of the HPC facilitated the acquisition of autoshaped responses, while also enhancing the acquisition of conditioned locomotor activity for food. These data thus support the notion that the HPC is not exclusively involved in learning and memory processes, but may also play a more fundamental role in the regulation of motivated behavior, in the form of contextual inhibition, specifically in situations where the presentation of expected reward is delayed, or even absent. The exact mechanism by which the inhibitory control is exerted is open to speculation, but is likely to involve the dopaminergic and noradrenergic innervation of the NAc. Moreover, the fact that the acquisition of autoshaping responses is unaffected by subiculum lesions (Parkinson et al., 2000) indicates the operation of an alternative, extra-subicular route by which HPC projections may exert their influence, perhaps involving an indirect interaction between the HPC and anterior cingulate cortex. It remains to be seen whether the removal of the HPC has similar effects on other indices of impulsivity.

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