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The Ventral Hippocampus, But Not the Dorsal Hippocampus Is Critical for Learned Approach-Avoidance Decision Making

Anett Schumacher,[1] Ekaterina Vlassov,[1] and Rutsuko Ito[1,2]*

ABSTRACT: The resolution of an approach-avoidance conflict induced by ambivalent information involves the appraisal of the incentive value of the outcomes and associated stimuli to orchestrate an appropriate behavioral response. Much research has been directed at delineating the neural circuitry underlying approach motivation and avoidance motivation separately. Very little research, however, has examined the neural substrates engaged at the point of decision making when opposing incentive motivations are experienced simultaneously. We hereby examine the role of the dorsal and ventral hippocampus (HPC) in a novel approach-avoidance decision making paradigm, revisiting a once popular theory of HPC function, which posited the HPC to be the driving force of a behavioral inhibition system that is activated in situations of imminent threat. Rats received pre-training excitotoxic lesions of the dorsal or ventral HPC, and were trained to associate different non-spatial cues with appetitive, aversive and neutral outcomes in three separate arms of the radial maze. On the final day of testing, a state of approach-avoidance conflict was induced by simultaneously presenting two cues of opposite valences, and comparing the time the rats spent interacting with the superimposed 'conflict' cue, and the neutral cue. The ventral HPC-lesioned group showed significant preference for the conflict cue over the neutral cue, compared to the dorsal HPC-lesioned, and control groups. Thus, we provide evidence that the ventral, but not dorsal HPC, is a crucial component of the neural circuitry concerned with exerting inhibitory control over approach tendencies under circumstances in which motivational conflict is experienced. © 2015 Wiley Periodicals, Inc.

KEY WORDS: hippocampus; decision making; incentive motivation; conditioned cue preference; conditioned cue avoidance

INTRODUCTION

Decision making that involves the successful resolution of an approach-avoidance conflict is fundamental to the survival and reproduction of organisms. Approach-avoidance decisions require individuals to evaluate the incentive value of novel or familiar stimuli based on previously learned experiences or innate knowledge, in order to orchestrate the most appropriate behavioral response. More often than not, these stimuli are ambivalent in nature (imbued with both positive and negative valence), giving rise to a motivational conflict, which must be resolved before an action can be taken. Converging animal and human studies using delayed discounting (choose between immediate smaller rewards versus delayed larger rewards) and effort-based decision making tasks (choose between easily attainable rewards vs. working harder to obtain rewards) have implicated the amygdala and medial prefrontal cortex pathway as being crucial to decision making involving uncertainty (Kopchia et al., 1992; Winstanley et al., 2004; Rudebeck et al., 2006; Cohen et al., 2008; Floresco et al., 2008; Cohen and Cavanagh, 2011; Guitart-Masip et al., 2013). However, these studies have not clearly differentiated the neural substrates involved in learning about the value of outcomes, from those that mediate the resolution of approach-avoidance conflict in the moment of decision making. Furthermore, while much work has been dedicated to delineating the neural basis of approach and avoidance responses separately, very few studies have directly investigated the neural substrates of approach-avoidance decision making under circumstances in which opposing motivational processes are experienced simultaneously.

Traditionally, the hippocampus (HPC) is thought of as a key center of learning and memory (O'Keefe and Nadel, 1978; Morris et al., 1982; Eichenbaum, 2000). However, more recently, compelling evidence has demonstrated that the HPC is involved in aspects of inhibitory response control (Chan et al., 2001; Trivedi and Coover, 2004; Cheung and Cardinal, 2005; Ito et al., 2005; McHugh et al., 2008; Abela et al., 2013), supporting the once popular behavioral inhibition theory of HPC function (Gray, 1982; Gray and McNaughton, 2000), that the HPC is involved in the suppression of prepotent responses under conditions of environmental instability (approach-avoidance conflict). Indeed, HPC-lesioned rats are typically unable to inhibit learnt approach responses in the face of punishment (Isaacson and Wickelgren, 1962), or in the absence of reward (Jarrard and Isaacson, 1965; Ito et al., 2005). Further evidence has indicated that it is the ventral aspect of the HPC, and not the dorsal

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TABLE 1.

Dorsal and Ventral Hippocampus 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	−2.8 ±1.6 −3.3	0.4	4
	(0.09 M)	−4.2 ±2.6 −3.0	0.4	4
HPC (ventral)	N-Methyl-D-Aspartic acid	−4.8 ±4.8 −6.0	0.2	2
	(0.09 M)	−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

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

aspect (which instead preferentially mediates spatial processes), that mediates behavioral inhibition and anxiety-related behavior, substantiating the notion that the HPC is an anatomically and functionally heterogeneous structure along a dorsal-ventral (septo-temporal) axis (Bannerman et al., 2004; Bast et al., 2009). Ventral HPC lesions have well-documented anxiolytic effects in ethological tests measuring innate expressions of anxiety, such as the elevated plus maze, light/dark box, successive alley and hyponeophagia (Bannerman et al., 2002; Kjelstrup et al., 2002; Bannerman et al., 2003; McHugh et al., 2004; Trivedi and Coover, 2004), and reduce unconditioned defensive behaviors induced by predatory odour stimuli (Pentkowski et al., 2006).

The present study sought to test the hypothesis that the ventral, but not the dorsal, HPC is important in approach-avoidance decisions induced by motivationally ambivalent cues. To this end, we examined the effects of pre-training selective excitotoxic lesions of the dorsal or ventral HPC on a novel concurrent mixed valence conditioning paradigm, which allows the disambiguation of neural substrates involved in (1) the acquisition of incentive values of cues (appetitive and aversive), and (2) the expression of a motivational conflict induced by the simultaneous presentation of appetitive and aversive cues. More specifically, animals were initially trained to associate three pairs of visuo-tactile bar cues placed along the sides of three different arms of a radial maze, with differential outcomes (appetitive, aversive and neutral). The rate of cue-outcome learning was assessed by the administration of three conditioned cue preference/avoidance tests (interspersing 9 conditioning sessions). Upon successful acquisition of cue-outcome associations, animals then performed a conflict test in extinction, in which they were presented with a combination of the appetitive and aversive cue in one arm, and the neutral cue in another arm. The conflict test created a scenario in which animals would face a number of approach/avoidance decisions; first, in whether they chose to enter/re-enter the conflict arm, and second, in whether they chose to stay/leave the conflict arm once inside. Importantly, the small dimension (width) of the arm ensured that animals experienced the appetitive and aversive cues simultaneously upon entry, giving the animals the opportunity to appraise and generate an incentive value for the compound cue, which they could subsequently use to guide their decisions to re-enter, or stay/exit the arm. We used a number of measures to assess the outcome of the decision making process. First, the amount of time that animals spent in the conflict arm (as opposed to the neutral arm) was taken as an indicator of the ability of animals to make a decision to stay (approach), or leave (avoid) the arm once inside. Second, the number of full entries was measured to indicate the decision to re-enter the conflict arm. Additionally, we observed and recorded the number of "retreat" behaviors, defined as head only, or half body entries into the arm that did not result in full entries, which was taken to be an expression of motivational conflict.

We also performed an additional behavioral assay, the delayed non matching to place T maze working memory task that has previously been shown to be selectively sensitive to dorsal hippocampus lesions (Dudchenko et al., 2000; Bannerman et al., 2002), to assess the effectiveness of the excitotoxic lesions. We report that ventral HPC lesion, but not dorsal HPC lesion enhanced approach tendencies in the conflict test, indicating the importance of ventral HPC in approach-avoidance decision making.

METHODS

Subjects

Subjects were 30 male Long Evans rats (Charles River Laboratories, QC, Canada) weighing ∼330–400 g at the time of surgery. They were housed in groups of two in a room held at a constant temperature of 21°C, under a 12 h light/dark cycle (lights on at 7:00 A.M.). Water was available ad libitum, but before the start of behavioral testing, food was restricted to 20 g of lab chow/day, sufficient to maintain preoperative/treatment body weight and growth. All experiments were conducted during the light phase and in accordance with the guidelines of Canadian Council of Animal Care, and approved by the University Animal Care Committee of the University of Toronto.

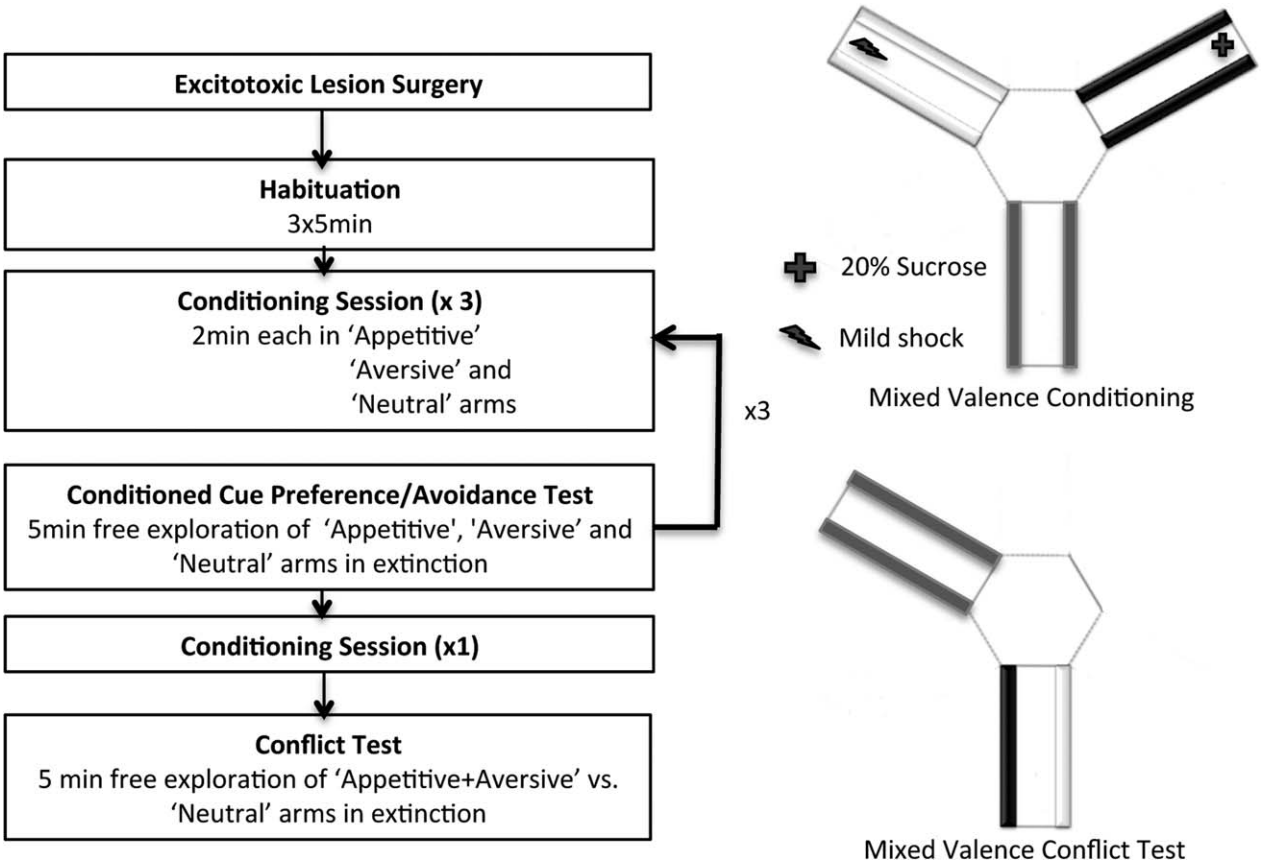

FIGURE 1. Schematic diagram showing the sequence of events (A), and the apparatus and cues used (B) in the novel concurrent mixed valence conditioning paradigm for rats. Animals were trained to associate three different cues (whisker-bar inserts) with an appetitive outcome (sucrose), aversive outcome (mild shock), or no outcome (neutral) in conditioning sessions. The rate of acquisition was then monitored by conditioned cue preference/avoidance tests occurring after every three conditioning sessions. Once the acquisition of incentive learning was established, animals underwent a "conflict test," in which they were simultaneously presented with an aversive and appetitive cue within one arm (conflict arm). Their willingness/reluctance to enter (retreat), and the time spent in the "conflict" arm were compared to entering and spending time in the arm with the neutral cue, as measures of approach-avoidance conflict.

Surgery

Rats were divided into four groups ($n = 10$ Ventral hippocampus (HPC) lesions; $n = 5$ Ventral HPC sham-operated controls; $n = 10$ Dorsal HPC lesions; $n = 5$ Dorsal HPC sham-operated controls). All rats were anesthetized with isoflurane (Benson Medical, ON, Canada) and placed in a stereotaxic frame (Steolting Co, IL) with the incisor bar set at −3.3 mm below the interaural line. A midline incision along the skull was made, and the fascia retracted by small skin clips to reveal the bregma. Small burr holes were then created at the lesion sites using a dental drill, and a 1 μL Hamilton syringe was lowered into the dorsal, or ventral HPC for a bilateral infusion of neurotoxin 0.09M N-Methyl-D-Aspartic acid (NMDA). The volume of NMDA infused, and the sites of infusions are listed in Table 1 (as in Ito et al., 2005). Sham control groups were treated identically to the lesion groups, except that they received injections of 0.1M sterile phosphate buffer (sterile PB), instead of the toxin. Following surgery, rats were allowed a recovery period of at least 10 days before behavioral testing with food available ad libitum.

Radial Arm Maze Apparatus

Behavioral testing for the approach avoidance conflict task took place in an automated six-arm radial maze (Med Associates, VT) placed on a rotatable table elevated 80 cm from the floor. The maze consisted of six enclosed arms [45.7 cm (L) × 16.5 cm (H) × 9.0 cm (W)] emanating from a central hexagonal hub compartment with six automatic stainless steel guillotine doors allowing access to the arms. Arms were enclosed by Plexiglas walls and a removable Plexiglas lid, and contained a stainless grid floor, which was connected to a shock generator (Med Associates, VT). At the end of each arm was a receding well consisting of a stainless steel tray that could be connected up to a syringe pump for the delivery of sucrose solution. Each arm was also equipped with two sets of infrared beams located 2 and 3 cm away from the entrance of the arm to monitor an

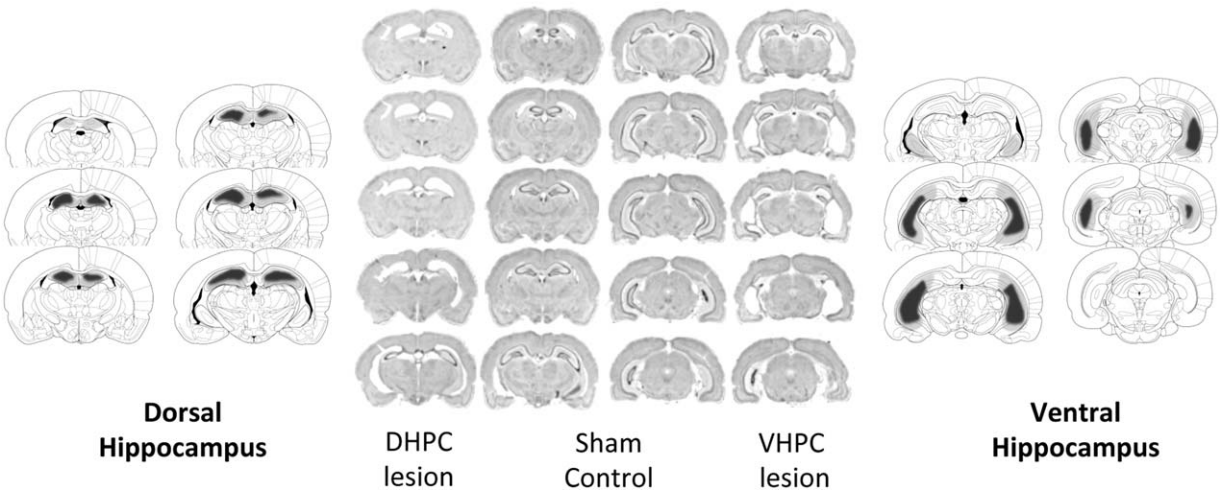

FIGURE 2. Schematic diagram and representative photomicrographs showing the extent of excitotoxic lesions of the dorsal and ventral hippocampus.

animal's entry into and exit out of the arm. The entire maze was covered in red cellophane paper to block visibility of extra-maze cues, while enabling video recording of behavior via a video camera mounted above the apparatus. Only three out of six arms (forming a Y maze, see Fig. 1) were used at any one time in an experimental session. The maze was wiped down with ethanol solution after each session to eliminate odor traces, and the maze was randomly rotated left or right by varying degrees (60°, 120°, or 180°) at the end of the testing day to minimize conditioning to intra-maze cues.

T-Maze Apparatus

Rewarded alternation was conducted in a T-maze consisting of 3 opaque Plexiglas arms arranged in a T configuration [60 cm (L) × 12 cm (W) × 18 cm (H)], with a start arm and 2 goal arms containing wells at the far end for the delivery of sucrose solution. The wells were specially-adapted stainless steel 'double' wells, comprising a small well embedded in a bigger well and half-filled with 20% sucrose solution (but inaccessible to the animal) to ensure that the animals did not solve the task on the basis of odor cues. Entrance into the goal arms was controlled by the manual opening of guillotine doors.

Behavioral Procedure: Mixed Valence Cue Conditioning (See Fig. 1)

Preconditioning habituation

All rats were given three 6 min pre-conditioning habituation periods. For each 6min session, they were initially placed in the central hub of the apparatus. After an adaptation time of 1 min in the hub, three guillotine doors were opened and the rats were free to explore three arms for a further 5 min. In the first habituation session, the rats explored the three arms without the presence of any cue inserts. In the second habituation session, the rats were exposed, for the first time, to three sets of bar cues [45 cm (L) × 4 cm (W) × 0.5 cm (D)] that were placed along the entire length of the sides (walls) of each of the three arms, and that differed in texture and color. The cues were all made of wood, two pairs of which were covered in their entirety by black felt or white rough linen. Following this habituation session, each set of bar cues was assigned an affective value: appetitive, aversive, or neutral. The valence assignment was counterbalanced across rats, with the exception of rats exhibiting strong preferences for one set of stimuli over the others during the habituation session. In such cases, the preferred stimulus was assigned as the aversive cue, and the least preferred stimulus as the appetitive cue. In the third habituation session, animals were presented with two sets of cues in two arms—one of which contained the "neutral" cue. The other arm contained a combinatorial cue—one bar of opposite valence (appetitive vs. aversive) placed on either side of the arm, to mirror the conditions of the final conflict test. This session ensured that the combination of stimuli presented in the conflict test was not novel to the animals. During the habituation sessions, the time spent in each of the arms was measured.

Mixed valence cue conditioning

The animals underwent a total of 9 conditioning sessions to concurrently acquire appetitive and aversive cue conditioning. Each daily conditioning session commenced with a 30 s adaptation period in the hub, followed by a 2 min confinement in each of the 3 arms. In the arm containing the appetitive cue (e.g., coarse white bars), the animals received 4 × 0.4 mL aliquots of 20% sucrose solution delivered at 15 s intervals. In the arm with the aversive cue (e.g., soft black bars), the animals received 4 mild shocks (0.5 s, 0.25 − 0.30 mA), also delivered at 15 s intervals. In order to minimize the effect of individual differences, the shock level was calibrated for each rat in the first conditioning session, and fixed at a value that elicited a

mild startle and defensive treading behavior for the rest of the conditioning sessions. There was no notable difference in the magnitude of the shock required to induce mild startle and defensive treading between lesion groups. In the arm with the "neutral" cue (e.g., rough wood), the animals did not experience any reward or shock. Extensive piloting was conducted to optimize the magnitudes of the sucrose reward and shock required to facilitate the development of conditioned approach and avoidance respectively, while preventing the induction of generalized fear of the whole apparatus, and the development of freezing responses to the cue paired with shock.

Several measures were taken to ensure that the animals did not associate the outcomes with the sequence of arm presentation, the spatial location of the arms or other intra-maze cues. First, the order of entry into each arm was varied across sessions. Second, the placement (spatial locations) of the bar cues was counterbalanced between animals and varied between sessions for each animal. Lastly, the relative orientation of the arms was held constant (same Y-maze configuration), but the maze was rotated left and right by varying degrees between training sessions, such that all 6 arms were used throughout the experiment.

Conditioned cue preference/avoidance test

The day after the third conditioning session, rats underwent a conditioned cue preference/avoidance test to assess whether they had learnt the cue contingencies. In the test, rats were allowed to explore the appetitive, aversive and neutral cues for 5 min, in the absence of the sucrose or footshock. The time spent exploring each of the cues/arms was recorded in the test. A successful acquisition of conditioned cue preference and avoidance was indicated by (1) the time spent interacting with the appetitive cue being longer than that spent interacting with the neutral and aversive cues (conditioned cue preference), and (2) the time spent interacting with the aversive cue being shorter than that spent with the appetitive and neutral cues (conditioned cue avoidance). The rats received two further tests, with each test occurring after the completion of three conditioning sessions.

Refresher conditioning session

Following the final conditioned preference/avoidance test, animals received a refresher conditioning session, procedurally identical to the mixed valence conditioning session.

Approach avoidance conflict test

This test was administered on the day after the refresher session. During this 5 min session, a state of approach-avoidance conflict was induced in the rats by superimposing two stimuli of opposite valences (reward- or shock-associated cue) in one arm, and presenting the neutral cue in another arm. The time spent in, and the latency to enter the conflict and neutral arms were recorded for each animal. In addition, the number of entries, and the number of retreats (head only, or half body entries into an arm that did not result in full entries) in the conflict arm relative to the neutral arm, was recorded for each animal.

Behavioral Procedure: Delayed Rewarded Alternation

Habituation

On the day before testing began, the rats were habituated to the T-Maze apparatus, by being allowed to freely explore the maze for 10 min and to sample sucrose solution (20%) in the wells located at the end of the 2 goal arms.

Behavioral procedure

Animals received a total of 50 trials, across 10 days (5 trials/day). Each trial consisted of 2 phases: the sucrose sampling phase and the choice phase. The sample phase involved placing the animal in the start arm facing away from the entrance to the goal arms, and then being allowed to enter one of the goal arms to consume sucrose (by blocking off the entrance to the non-rewarded goal arm). When the animal had consumed the sucrose solution, it was removed from the goal arm, and then confined in the start arm for a delay of 60 s. The choice phase of the trial commenced at the end of the 60-s delay period, upon which animals were given a choice to enter the left or right goal arms. The animals were rewarded for alternating, and for choosing the previously unvisited arm in the sample phase. If the animal revisited the previously rewarded arm, it was promptly removed from the maze after it had reached the end of the arm. It was ensured that the location of the reward (left or right) in the sample phase was not in the same arm for more than three consecutive trials.

Histological Procedure

All rats were anesthetized with chloral hydrate (1200mg/kg, Sigma-Aldrich, St Louis, MO) and perfused intracardially via the ascending aorta with 0.9% saline, followed by 4% paraformaldehyde (PFA) solution. Brains were then removed, stored in 4% PFA, and transferred to a 30% sucrose cryoprotectant solution before sectioning. Coronal sections (50 μm) of the brain were cut using a freezing microtome and were then stained with cresyl violet, to be viewed under the microscope for the verification of excitotoxic lesions. We conducted a quantitative analysis of the lesion extent, using a Lasso tracing method (Park et al., 2013). Twenty-six equidistant brain sections were scanned at 3600 dpi (Pathscan Enabler IV, Meyer Instruments, TX) and the perimeter of intact tissue for each section was traced using the Lasso tool in Adobe Photoshop CS5 (Adobe Systems Inc, CA). The surface area inside the selection was then calculated by dividing the number of pixels within the selection by the number of pixels within a unit area (200.8 pixels/mm^2). The volume of intact tissue was then estimated by the application of the cylindrical computation rule, summing the multiplication of the surface area of the section by the distance between the sections (0.2 mm).

The overall percentage of cell loss in the respective target regions (dorsal vs. ventral HPC) was calculated by comparing the volume of intact tissue in the lesioned animals with the mean volumes of the whole HPC, dorsal or ventral HPC of sham controls.

Data Analysis

All data were analysed using the SPSS statistical package version 20 (SPSS Inc., Chicago, IL). Data generated for each acquisition test session (5 min) consisted of the absolute time spent in each of the 3 arms of the radial maze as well as the time spent in the hub. A two-way repeated measures analysis of variance (ANOVA) was conducted on the raw data (time spent) obtained from each of the conditioned cue preference/avoidance tests with Lesion (Sham; Dorsal HPC lesion; Ventral HPC lesion) as the between-subject factor and Arm (Appetitive; Aversive; Neutral Cue) as the within-subjects factor. All data (time spent, change in time spent, latency to enter, number of entries, number of retreats) from the conflict test day were subjected to a two-way ANOVA with Lesion as the between-subject factor, and Arm (Combined Cue; Neutral Cue) as the within-subjects factor. The change in time spent was calculated by comparing the time spent with the combined and neutral cues during the third habituation session conducted prior to the commencement of conditioning, and the data generated on the conflict test day following conditioning sessions. Any significant two-way interactions were further explored using simple effects analyses. Subsequent post-hoc comparisons for simple effects were performed with a Bonferroni correction. Rewarded alternation data were collected for 50 trials, and the number of correct and incorrect responses was collapsed across the 50 trials to generate an overall percentage of correct responses. A one way ANOVA was conducted on this data set, and post hoc Bonferroni tests were applied to compare differences in the performance of the lesioned and sham groups.

RESULTS

Lesion Assessment (Fig. 2)

Bilateral excitotoxic lesions of the dorsal HPC extended rostrally from −1.6 to −4.2 mm posterior to bregma (Paxinos and Watson, 1998), and generated an average cell loss of 78% (±2.2 SEM) of dorsal tissue compared to sham controls, and a corresponding mean loss of 40% (±1.1) of the entire HPC. Sparing of the dorsal HPC occurred largely at more posterior sites. Bilateral excitotoxic lesions of the ventral HPC extended from −4.2 to −6.7mm posterior to bregma, consistently encompassing the anterior ventral tip and the intermediate region of the HPC, but distinctly demarcated from the dorsal HPC. The ventral HPC-lesioned rats sustained a mean cell loss of 66% (±1.9) of ventral tissue compared to sham controls, and a mean loss of 35% (±0.65) of the entire HPC. Sparing of tissue typically occurred at more posterior sites of the ventral HPC, and anteriodorsal sites of the ventral HPC. In all animals included in the data analysis, there was minimal damage to the cortical areas overlying the HPC, and the dorsal and

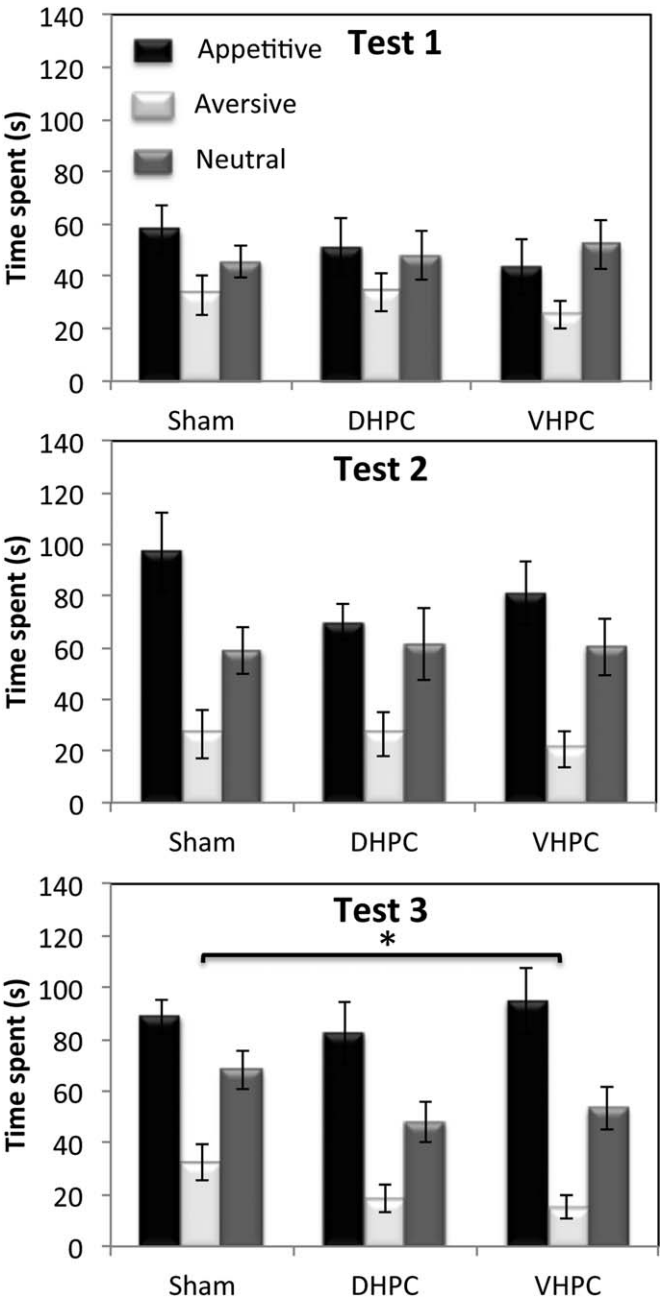

FIGURE 3. The acquisition of concurrent mixed valence conditioning in sham-operated, dorsal hippocampus (DHPC)- and ventral hippocampus (VHPC)-lesioned rats, as shown by the mean time spent (±SEM) in arms with "appetitive," "aversive," and "neutral" cues in three conditioned cue preference/avoidance tests (Tests 1 − 3), under extinction conditions (5 min). In Test 3, the ventral HPC-lesioned group spent significantly less time in the aversive arm, compared to that of the Sham control group (*$P < 0.05$).

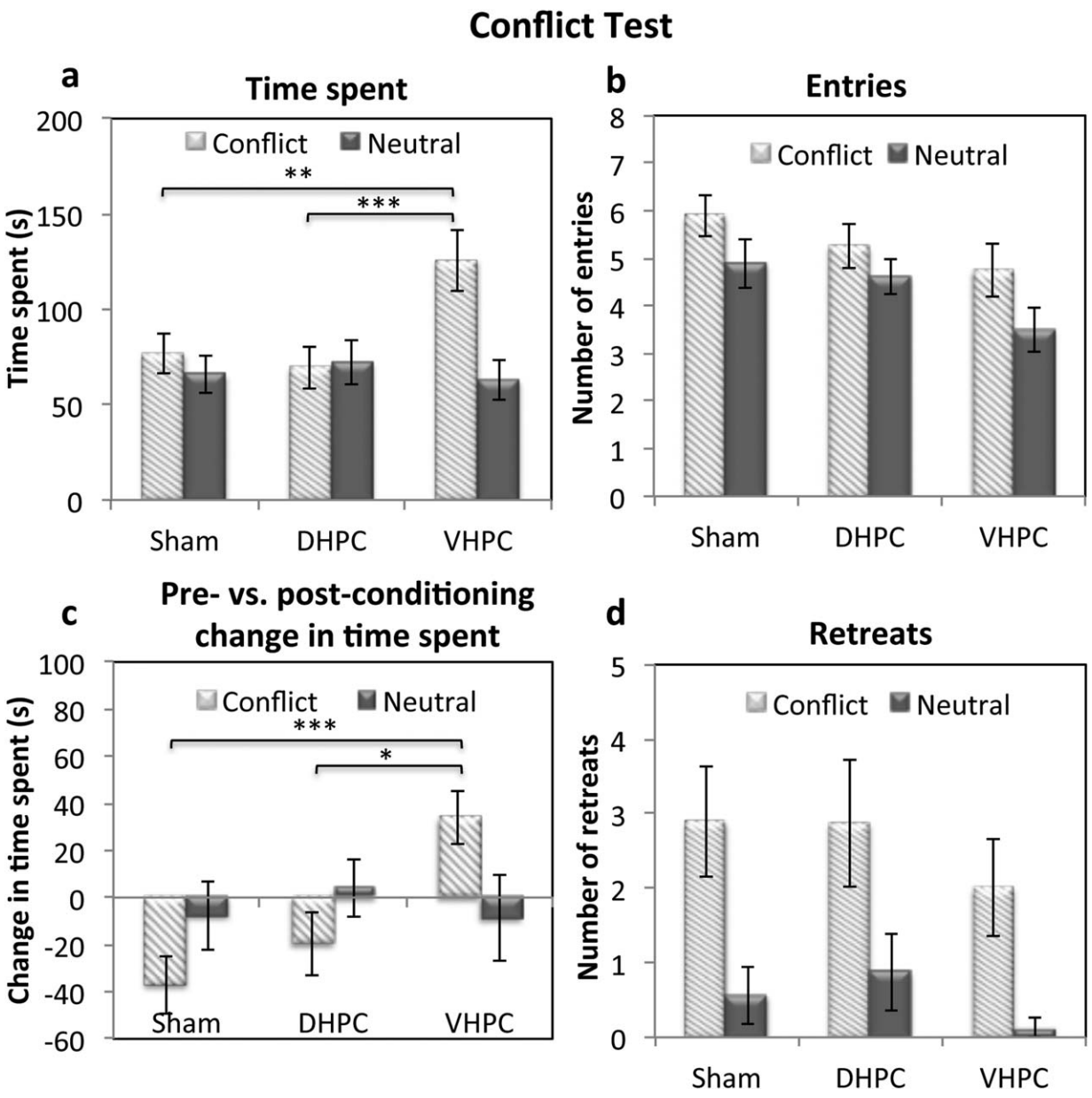

FIGURE 4. (a) Mean time spent in (±SEM), (b) mean number of entries (±SEM) into, c) mean change (pre vs. post conditioning, ±SEM) in the time spent in, and d) the number of retreats measured in the "conflict arm" (superimposed appetitive and aversive cues) and "neutral arm" (neutral cues) in the approach avoidance conflict test (5 min) in sham-operated, dorsal hippocampus (DHPC)- and ventral hippocampus (VHPC)-lesioned rats. Sham control rats spent equal times in the arms with the conflict cues, and neutral cues. The VHPC-lesioned rats, however, spent significantly more time in the conflict arm than in the neutral arm (*$P < 0.001$), and significantly more time in the conflict arm than those of the Sham- and DHPC-lesioned rats (*$P < 0.05$).**

ventral subiculum were spared. Data from two animals in the dorsal HPC lesion group, and 2 animals in the ventral HPC lesion group were excluded from statistical analyses due to the estimated lesion volume falling below 20% of the entire HPC (compared to sham controls). In addition, data from 1 animal in the ventral HPC sham control group was excluded due to incomplete data records. The final group numbers were: dorsal and ventral HPC sham controls ($n = 9$), dorsal HPC-lesioned group ($n = 8$) and ventral HPC-lesioned group ($n = 8$).

Acquisition of Conditioned Cue Preference and Avoidance

Animals received a total of nine conditioning sessions in which they were trained to associate discrete, non-spatial cues with an appetitive or aversive outcome. Learning was assessed by conducting a conditioned cue preference (CCP)/conditioned cue avoidance (CCA) test (under extinction conditions) after every three conditioning sessions. The acquisition test data from the two sham-operated control groups (dorsal HPC and

ventral HPC) were not significantly different across the three tests (no Group effect, $F(1,7) = 0.49$, $P = 0.51$; no Test x Group effect, $F(2,14) = 0.34$, $P = 0.72$; no Test x Arm x Group effect, $F(4,28) = 0.49$, $P = 0.74$) and as planned a priori, were pooled together for subsequent analyses. It was also observed that the animals spent a large proportion of their time (43.5% ± 2.5 SEM,) in the hub during the acquisition tests. However, since there were so significant differences in the time spent in the hub between groups (no Group effect, $F(2,22) = 1.22$, $P = 0.31$, no Group × Test interaction, $F(4,44) = 0.64$, $P = 0.64$), and no other significant trends of interest, the data were not included in subsequent analyses.

ANOVA conducted on the time spent in the arms containing the appetitive, aversive and neutral cues in the first CCP/CCA test (Fig. 3a) revealed significant differences in the overall time spent in the three arms (Arm effect, $F(2,44) = 4.95$, $P < 0.05$), but no significant difference in the performance of conditioned cue preference/avoidance between the lesion groups (no effect of Lesion, $F(2,22) = 0.31$, $P = 0.74$; no Lesion × Arm effect $F(4,44) = 1.12$, $P = 0.36$). Pairwise comparisons of the overall time spent in the arms revealed that there was a significant discrimination of the appetitive and aversive arms ($t(24) = 3.44$, $P = 0.002$), but no preference for the appetitive over the neutral arm ($t(24) = 1.24$, $P = 0.23$, ns), nor avoidance of the aversive arm over the neutral arm ($t(24) = -1.92$, $P = 0.07$).

ANOVA conducted on the time spent in the three arms in the second CCP/CCA test (Fig. 3b) revealed significant differences in the overall time spent in the appetitive, aversive, and neutral arms (Arm effect, $F(2,44) = 21.47$, $P < 0.0001$), but no difference in the performance of the test between the lesion groups (no effect of Lesion $F(2,22) = 0.36$, $P = 0.70$; No Lesion x Arm interaction $F(4,44) = 1.06$, $P = 0.39$). Paired sample t-tests of the time spent in the three arms revealed that there was a significant discrimination of the appetitive and aversive arms ($t(24) = 6.72$, $P < 0.0001$), avoidance of the aversive arm over the neutral arm ($t(24) = -4.46$, $P < 0.0001$), and preference of the appetitive over neutral arm ($t(24) = 2.19$, $P = 0.04$), indicating significant learning of the cue-outcome contingencies.

ANOVA conducted on the time spent exploring the three arms in the third, and final CCP/CCA test (Fig. 3c) not only revealed significant differences in the overall time spent in the appetitive, aversive and neutral arms (Arm effect, $F(2,44) = 47.74$, $P < 0.0001$), but also a significant effect of Lesion group (Lesion effect: $F(2,22) = 4.31$, $P < 0.03$) and a significant Lesion x Arm interaction ($F(4,44) = 2.66$, $P < 0.05$). Subsequent simple main effects analyses revealed the significant interaction effect to be attributable to a significant difference in the time spent in the aversive arm between the lesion groups ($F(2,22) = 4.46$, $P < 0.03$), but not in the appetitive ($F(2,22) = 2.79$, $P = 0.08$) or neutral arms ($F(2,22) = 2.52$, $P = 0.10$). Bonferroni corrected post hoc comparisons revealed that the time spent in the aversive arm was significantly lower in the ventral HPC- lesioned group, compared to the sham-lesioned group ($P < 0.03$), indicating an increased level of conditioned cue avoidance in the ventral HPC-lesioned group compared to the sham group.

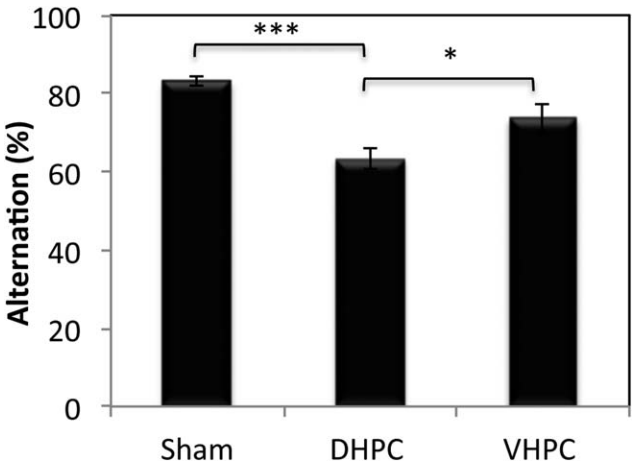

FIGURE 5. Delayed T maze rewarded alternation performance in sham-operated, dorsal hippocampus (DHPC)- and ventral hippocampus (VHPC)-lesioned rats, shown as the mean percentage of correct responses (±SEM) made across a total of 50 trials.

Cue-Induced Approach-Avoidance Conflict Expression

Time spent in conflict vs. neutral arms (Figs. 4a,c)

During the approach-avoidance conflict test, animals were presented with superimposed appetitive and aversive cues in one arm of the radial maze (conflict arm), and a neutral cue in another arm (Fig. 4a). ANOVA conducted on the time spent in the conflict and neutral arms revealed a significant main effect of Arm ($F(1,22) = 5.52$, $P < 0.05$) and a significant Lesion x Arm interaction ($F(2,22) = 4.91$, $P < 0.02$), but no significant main effect of Lesion ($F(2,22) = 2.50$, $P = 0.11$). Subsequent simple main effects analyses revealed the significant interaction to be due to the time spent in the conflict arm being significantly higher in the ventral HPC-lesioned group, compared to both the sham and dorsal HPC-lesioned groups ($F(2,22) = 7.53$, $P < 0.01$, pairwise comparisons: $P < 0.01$ for ventral HPC vs. Sham, $P < 0.001$ for ventral HPC vs. dorsal HPC). In addition, the ventral HPC-lesioned animals spent significantly more time in the conflict arm over the neutral arm ($F(1,22) = 14.70$, $P < 0.001$), whereas no such differences were seen in the dorsal HPC-lesioned group ($F(1,22) = 0.12$, $P = 0.74$), nor in the sham control group ($F(1,22) = 0.28$, $P = 0.60$).

This pattern of results was confirmed using another measure of the expression of approach-avoidance conflict, which is the comparison of the time spent with the conflict-inducing cues prior to, and after, the mixed valence conditioning sessions (Fig. 4c). ANOVA conducted on the change in the time spent interacting with the conflict and neutral cues revealed a significant Lesion x Arm interaction ($F(2,22) = 3.99$, $P < 0.05$) but no significant main effects of Lesion ($F(2,22) = 3.22$, $P = 0.06$) or Arm ($F(1,22) = 0.003$, $P = 0.95$). The significant interaction was due to the change in time spent in the conflict arm being significantly higher than that in the neutral arm in the ventral HPC-lesioned group ($F(1,22) = 5.27$, $P < 0.04$), but

not in the sham-lesioned ($F(1,22)=1.91$, $P=0.18$) or dorsal HPC-lesioned group ($F(1,22)=0.80$, $P=0.38$). Furthermore, the time spent in the conflict arm changed in opposite directions in the ventral HPC-lesioned and sham-lesioned groups (simple main effect of lesion group $F(2,22)=8.93$, $P<0.001$), increasing after the conditioning sessions in the former group, and decreasing after conditioning sessions in the latter group. Bonferroni corrected post-hoc tests confirmed the difference in the change in time spent in the conflict arm to be significant between ventral HPC- and sham-lesioned groups ($P<0.001$), and between the ventral HPC- and dorsal HPC-lesioned groups ($P<0.05$), but not between sham- and dorsal HPC-lesioned groups ($P=1.0$).

Number of entries into conflict and neutral arms (Fig. 4b)

ANOVA of the total number of full body entries made into the conflict and neutral arms during the 5min session revealed a significant main effect of Arm ($F(1,22)=10.20$, $P<0.01$), with animals making more entries into the conflict arm over the neutral arm. However, there was no significant group differences in this performance measure (Lesion x Arm interaction ($F(2,22)=0.35$, $P=0.71$); Lesion ($F(2,22)=2.70$, $P=0.09$).

Number of retreats (Fig 4d)

ANOVA of the total number of retreats (half body entry, or head poking followed by retreat) into the conflict and neutral arms revealed a significant effect of Arm ($F(1,22)=39.88$, $P<0.0001$), but no significant Lesion x Arm interaction ($F(2,22)=0.18$, $P=0.84$) or main effect of Lesion ($F(2,22)=0.66$, $P=0.53$), indicating that all lesion and sham groups exhibited some degree of hesitation in entering the conflict arm, but not in entering the neutral arm.

Latency to enter arms (not shown)

ANOVA of the time taken to make a full body entry into the conflict and neutral arm revealed no significant main effect of Arm ($F(1,22)=0.30$, $P=0.59$), Arm $\times$ Lesion interaction ($F(2,22)=0.33$, $P=0.72$) or Lesion ($F(2,22)=0.40$, $P=0.70$).

Delayed Rewarded Alternation

Animals underwent the rewarded alternation task to provide evidence of a positive (but debilitating) effect of dorsal HPC lesions upon a task that assays spatial working memory. The dorsal HPC-lesioned rats were significantly impaired in the performance of the rewarded alternation T-maze task when compared to the sham control and ventral HPC-lesioned groups ($F(2,21)=9.66$, $P<0.001$, Dorsal HPC vs. Sham control, $P<001$; Dorsal HPC vs. Ventral HPC, $P<0.05$). The performance of the ventral HPC-lesioned group was not significantly different to that of the sham control group (Ventral HPC vs. Sham control; $P=0.15$).

DISCUSSION

Using a novel non-spatial mixed valence conditioning paradigm, the present study provides evidence for a functional dissociation between the ventral and dorsal hippocampus (HPC) in approach-avoidance decision making. Selective excitotoxic lesions of the ventral HPC led to the potentiation of approach tendencies when cues of opposite valences were presented, and experiecned concurrently. This occurred despite the finding that ventral HPC-lesioned rats showed enhanced expression of conditioned cue avoidance prior to the motivational conflict test. In contrast, selective excitotoxic lesion of the dorsal HPC did not lead to significant alterations in the acquisition of conditioned cue preference or avoidance, nor in approach-avoidance decision making. However, the dorsal HPC-lesioned rats were impaired in spatial working memory performance, as tested in a delayed rewarded alternation T-maze task. Together, our findings implicate the ventral, but not dorsal HPC, in serving a crucial role in the regulation of approach-avoidance decisions in situations when two opposing motivations are experienced simultaneously.

Ventral and Dorsal HPC in the Acquisition of Conditioned Cue Preference and Avoidance

Performance of the concurrent conditioned cue preference and avoidance tests revealed that all rats, including the dorsal and ventral HPC-lesioned rats, successfully learned the incentive values of cues that had been repeatedly paired with appetitive or aversive outcomes. A significant level of conditioned cue preference was evident after three conditioning sessions, and conditioned cue avoidance expression was achieved after 6 conditioning sessions. Intact acquisition of these cue contingencies in the HPC-lesioned rats is consistent with our previous findings demonstrating HPC lesions to selectively affect conditioning of appetitive outcomes to spatial cues, and not to elemental, non-spatial cues, using the radial maze apparatus (Ito et al., 2006; Ito and Canseliet, 2010). The ventral HPC-lesioned rats also exhibited enhanced conditioned cue avoidance in the third conditioning test, which may be a reflection of the absence of a competing (inhibitory) ventral HPC-mediated associative system, which operates in parallel to another learning system (Ito et al., 2006). Indeed, we have evidence indicating that the latter system could be mediated by the basolateral amygdala (BLA), as pretraining inactivation of this structure disrupts the acquisition of conditioned cue avoidance and preference (unpublished), and the existence of a competitive relationship between a HPC-mediated and BLA-mediated learning and memory systems is well documented (White and McDonald, 1993; McDonald and White, 1995; Ito et al., 2006; Gold 2004). There also appeared to be a qualitative enhancement in conditioned cue preference in the ventral HPC-lesioned group, compared to the sham control group in Test 3, but this was not supported by statistical significance, and our present results are not able to fully explain the absence

of any augmentation in conditioned cue avoidance or preference at an earlier stage of learning.

Ventral HPC Function in Conditioned Approach-Avoidance Conflict Resolution

The successful acquisition of incentive value of the cues was crucial in ensuring that a state of motivational conflict was elicited by the superimposition of cues of opposite valence in the final approach-avoidance conflict test. A number of behavioral measures were used to examine the behavioral outcomes of approach-avoidance decisions, including the number of committed entries into (decision to re-enter the conflict arm), and time spent in the arm presenting cues of opposing valence and the neutral arm (decision to stay/leave the conflict arm). The control rats and dorsal HPC-lesioned rats spent comparable amounts of time interacting with the neutral and conflict-inducing cues, indicating that approach-avoidance tendencies were balanced during motivational conflict (McNaughton and Corr, 2004). In contrast, selective excitotoxic lesion of the ventral HPC led to a display of increased tendency to stay in the conflict arm (preference of the conflict cue) in the face of a motivational conflict, despite the fact that conditioned cue avoidance remained intact (and even potentiated). This outcome may be a consequence of two potentially non-mutually exclusive events; an increased salience of the positively valenced cue, and/or decreased salience of negatively valenced information. The latter explanation for the increased approach tendency in ventral HPC lesioned rats would be in accord with Gray and McNaughton's (2000) theory of septo-hippocampal function in behavioral inhibition, that in the face of a conflict in competing goals, the HPC serves to evaluate the risk associated with the goal alternatives, and increase the influence of negative associations so as to eliminate the motivational discord. However, the absence of a deficit in the acquisition of conditioned cue avoidance in the ventral HPC-lesioned animals in the present data, together with the observation that ventral-HPC lesioned rats spent a disproportionate amount of time in the mixed valenced arm (despite there being no significant differences in the number of entries into the conflict arm between the ventral HPC lesion and sham groups), makes the increased positive bias account equally plausible. Indeed, this would be congruent with a number of previous studies demonstrating increased incentive properties of reward, or reward-related stimuli in HPC-lesioned rats (Tracy et al., 2001; Ito et al., 2005). For instance, hippocampal damage has been shown to decrease reward thresholds, and increase rates of responding in rats undergoing intra-cranial self stimulation in the ventral tegmental area (Kelley and Mittleman, 1999). HPC lesions also cause increased breakpoints in progressive ratio schedules of reinforcement (Schmelzeis and Mittleman, 1996), potentiation of conditioned locomotor activity in a reward-paired context (Devenport et al., 1981; Ito et al., 2005), and facilitation of the acquisition of pavlovian conditioned discriminative approach behavior (autoshaping - Ito et al., 2005). However, in the present study, we did not observe a significant enhancement in the acquisition of conditioned cue preference in the preceding mixed valence conditioning phase, albeit visual inspection of the Test 3 conditioned cue preference data may suggest otherwise. Our failure to detect an enhanced conditioned cue preference in ventral HPC-lesioned rats may in part, have been due to the fact that cue preference measures are prone to ceiling effects (being a reflection of a balance between cue-elicited approach and non-reward induced frustrative behavior).

We also measured the number of 'retreat' behaviors that the animals exhibited in the conflict and neutral arms, as a behavioral index of the ability of animals to detect/perceive a conflict situation when cues of opposite valence were presented simultaneously. It is of note, that the ventral HPC lesioned rats in the present study exhibited "retreat" behavior in the mixed valenced cue arm (conflict arm) to a similar degree to that exhibited by the sham control and dorsal HPC-lesioned groups, indicating that the ability of the ventral HPC-lesioned animals to discern the conflicting nature of the mixed valenced cue remained intact. This observation again lends support to the notion that incentive properties of the reward-associated cue were enhanced as a result of ventral HPC lesions.

Thus, the present findings extend the behavioral inhibition theory of HPC function (Gray and McNaughton, 2000; McNaughton and Corr, 2004) in a number of ways, by proposing that it is the ventral HPC, and not the dorsal HPC that plays a critical role in mobilizing the necessary behavioral response in situations of motivational conflict. Furthermore, the present results raise the possibility that the ventral HPC decreases the positive value of ambivalent cues (as well as increasing negative bias) when a motivational conflict is detected. The present results also indicate that the ventral HPC is not invariably involved in all forms of impulse control, since the ability to avoid an aversive cue remained intact in ventral HPC lesioned animals. Thus, the activation of the ventral HPC-mediated behavioral inhibition system is specific to circumstances when approach-avoidance, or cost-benefit decision making is required (McHugh et al., 2008; Abela et al., 2013). One potential limitation to our interpretation, however, is that the altered pattern of behavior observed in the ventral HPC-lesioned rats may have been a consequence of differences in the cue conditioning levels achieved in the prior training phase (between the lesioned and sham control rats), as opposed to an altered decision making process. While we would argue that the direction of the observed effects in the conflict test (in the ventral HPC-lesioned group) is opposite to what we would have expected on the basis of the conditioned cue preference/avoidance tests (potentiated conditioned cue aversion should lead to avoidance of conflict cues), we would need to conduct a post-training lesion study to fully resolve this issue.

Ventral HPC and Novelty Processing

It is also possible that alterations in novelty/familiarity processing in the ventral HPC-lesioned animals could have contributed to the observed preference for the mixed valenced cue

over the neutral cue. Despite the fact that all rats were pre-exposed to the combined cue during one of the habituation sessions, ventral HPC lesions may have rendered the rats insensitive to the habituation process, or alternatively, more sensitive to novelty. However, this account has difficulty explaining why we did not observe any other novelty-related effects in the early stages of conditioning. A disruption in familiarity/novelty processing may have manifested as increased exploratory behavior of all cues during the first conditioned cue preference and aversion test, but we did not observe such effects in the ventral HPC-lesioned rats. Such an account would also be difficult to reconcile with substantial data, which implicate the dorsal HPC preferentially in novelty processing (Lisman and Grace 2005; Moncada and Viola 2007; Honey et al., 2008; Hunsaker et al., 2008; Ballarini et al., 2009; Wells et al., 2013). It is worth noting that most of these studies did not specifically aim to assess the role of the ventral HPC in novelty processing, and as such, the exact role of ventral HPC in novelty processing remains uncertain, and warrants further investigation.

Ventral HPC and Anxiety

The display of enhanced approach tendency in the face of motivational conflict in the ventral HPC-lesioned rats is consistent with previous data showing ventral HPC lesions to reduce approach-avoidance conflict (by reducing avoidance of open arms) in unlearned tests of anxiety such as the elevated plus/T-maze (Bannerman et al., 2002; Kjelstrup et al., 2002; Bannerman et al., 2003; Trivedi and Coover, 2004). Ventral HPC lesions also attenuate conditioned defensive behavior (freezing and risk assessment) during exposure to potential threat stimuli (cat-odor: Pentkowski et al., 2006). In both the elevated plus/T-maze and the predatory odour exposure task, an animal experiences a motivational conflict in choosing between two goal alternatives; to explore a potentially "dangerous" environment ("open arms" or runway), or to freeze and/or remain in safe, "dark" compartments (Bannerman et al., 2014). Ventral HPC lesions potentiate approach tendencies in these circumstances, enhancing exploratory (risk analysis) behavior. Our data extend these findings to implicate the ventral HPC in the resolution of conflicts between two opposing associative memory, in other words, for stimuli that have no inherent incentive value, but have acquired incentive value through prior learning. Importantly, the approach-avoidance conflict scenario that we have attempted to capture in the present study is arguably more reflective of the type of conflict we may encounter in real-world situations.

While it may be tempting to attribute the present effect of ventral HPC lesions upon the conflict test simply to a reduction in anxiety, it is important to note that our measure of learned approach avoidance conflict does not invariably correlate with measures of unlearned anxiety such as the elevated plus maze. For instance, we have found that rats that had undergone repeated administration of cocaine exhibit marked preference for the mixed valence conflict cue compared to the neutral cue, in the absence of any changes in anxiety levels, as measured in the elevated plus maze (Nguyen et al., 2015). Furthermore, we would argue, that an aberrant state of anxiety is a product of deficient approach-avoidance conflict resolution, and not the underlying source of aberrant approach-avoidance conflict resolution.

Role of Ventral HPC in Conditioned Cue Avoidance Vs. Approach-Avoidance Conflict Resolution

The absence of an effect of ventral HPC lesions on conditioned cue avoidance in the present study may at first sight be surprising, given previous evidence showing a reduction in passive avoidance behavior on the elevated T-maze following electrolytic lesions of the ventral HPC (Trivedi and Coover, 2004). However, this effect is most likely a result of damage to fibers of passage, and in particular, damage that may have extended to the lateral amygdala (Strauss et al., 2003). Furthermore, according to Gray and McNaughton's (2000) classification of defensive behaviors, conditioned cue avoidance may be conceptualized as an expression of "defensive avoidance" (fear), which involves the avoidance/escape of aversive situations, and is dependent on the activation of the Fight-Flight-Freeze system comprising the medial hypothalamus, amygdala, periaqueductal gray, and anterior cingulate cortex. Indeed, the amygdala (central, lateral and basolateral nuclei) plays a key role in the acquisition and expression of conditioned fear [freezing - (Davis, 1992; LeDoux, 2000; Maren and Quirk, 2004)]. Furthermore, pre-training reversible inactivations of the BLA selectively impairs the acquisition of conditioned cue preference and avoidance, in the absence of any alterations in approach-avoidance decision making (unpublished). The "defensive avoidance system" exists alongside the "defensive approach" system in which the HPC plays a central part, serving to facilitate exploratory behaviors in the face of a motivational conflict, while inhibiting prepotent responses. The present data supports the notion that the neural substrates underlying the acquisition and expression of conditioned cue preference and avoidance are dissociable from those involved in approach-avoidance conflict resolution. The ventral HPC plays a critical role in the resolution of approach-avoidance conflict decisions, but not in the acquisition and expression of conditioned cue preference or avoidance.

In summary, this study places the ventral HPC on the map as a key brain region governing the control of approach-avoidance decisions. It is proposed that the ventral HPC is uniquely activated in situations in which a motivational conflict is detected, to decrease positive affective bias and/or increase negative affective bias of available cues, and inhibit prepotent (approach) responses. In contrast, we did not find evidence to suggest that the dorsal HPC subserves a regulatory role in approach-avoidance decisions, despite the fact that the dorsal HPC-lesioned rats showed a greater degree of overall cell loss (compared to ventral HPC lesions), and also exhibited an expected performance deficit in the delayed rewarded alternation T-maze task (Bannerman et al., 2002). Furthering our

understanding of the neural mechanisms of approach-avoidance decision making has wide implications for the neuropathology of mental health disorders such as addiction, depression and anxiety, which are characterized by disruptions in approach-avoidance conflict resolution.

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