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Research report

Rats with ventral hippocampal damage are impaired at various forms of learning including conditioned inhibition, spatial navigation, and discriminative fear conditioning to similar contexts

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ABSTRACT

The ventral hippocampus (vHPC) has been implicated in learning and memory functions that seem to differ from its dorsal counterpart. The goal of this series of experiments was to provide further insight into the functional contributions of the vHPC. Our previous work implicated the vHPC in spatial learning, inhibitory learning, and fear conditioning to context. However, the specific role of vHPC on these different forms of learning are not clear. Accordingly, we assessed the effects of neurotoxic lesions of the ventral hippocampus on retention of a conditioned inhibitory association, early versus late spatial navigation in the water task, and discriminative fear conditioning to context under high ambiguity conditions. The results showed that the vHPC was necessary for the expression of conditioned inhibition, early spatial learning, and discriminative fear conditioning to context when the paired and unpaired contexts have high cue overlap. We argue that this pattern of effects, combined with previous work, suggests a key role for vHPC in the utilization of broad contextual representations for inhibition and discriminative memory in high ambiguity conditions.

1. Introduction

One fundamental learning and memory function that most organisms possess is the ability to discriminate between the meaning of different cues, places and situations. Discrimination learning is an important process because cues, places and situations predict the presence or absence of biologically significant stimuli (reinforcers). Discriminative behaviour is thought to be supported by excitatory and inhibitory conditioning processes occurring during training [1] whereby the reinforced cue acquires excitatory conditioning and the non-reinforced cue acquires inhibitory conditioning. Excitatory conditioning allow animals to attend to and elicit appropriate behaviour towards signals predictive of reinforcement and inhibitory conditioning reduce attentional/arousal processes towards other cues that do not signal reinforcement.

We have been working toward understanding the neural systems implicated in different forms of discrimination learning [2] and have exploited the interacting memory systems theory perspective to guide this work [3]. This theoretical perspective suggests that there are multiple learning and memory systems in the mammalian brain. These systems are located in different parts of the brain and acquire and store different types of information. In normal circumstances these systems interact either cooperatively or competitively to produce coherent behaviour. These systems are composed of complex neural circuits which have a central structure. These systems include, but are not limited to, the hippocampus, dorso-lateral striatum, amygdala, cerebellum, and perirhinal cortex. Each one of these systems has been implicated in different forms of discriminative learning and memory processes. For example, the hippocampus has been implicated in spatial discriminations in which the subject must learn to approach certain locations and avoid other locations [4–7] and complex non-spatial relational discriminations in which the meaning of cues varies depending on the presence or absence of other cues [8,9]. The other systems have been implicated in other forms of discriminative learning including: instrumental discriminations [dorsal striatum]; pavlovian discriminations [amygdala and cerebellum]; object and picture discriminations [perirhinal cortex] [10–17].

The focus of the present experiments is the learning and memory system centered on the hippocampus. Functional, anatomical, and electrophysiological evidence suggests that the hippocampus is a central structure for encoding and storing relationships among new information making up an experience as well as retrieving these disparate pieces of information from past experience. Several groups have suggested it might perform this function through reinstatement of the

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activity in cortex during the past experience, and thence other effectors of memory-guided behaviour. [18,19].

Interestingly, evidence suggests that the dorsal hippocampus (dHPC) and ventral hippocampus (vHPC) differ in anatomical connectivity and electrophysiological-behavioural correlates [20–23]. Early work provided evidence that the dHPC but not the vHPC was crucial for place learning in the Morris water task [21]. Our previous work also showed that although dHPC is more efficient at spatial processing than vHPC during acquisition of tasks like the Morris Water Task (MWT), the vHPC has spatial processing capabilities [24]. Consistent with this idea, studies investigating the firing properties of dHPC vs. vHPC neurons show that dHPC neurons have more spatial specificity and smaller place fields compared to vHPC [25]. This and other work suggests that the vHPC might have some role in representing contexts in a broader sense whereas the dHPC represents specific spatial locations in a context.

Although we believe that the vHPC is important for forming a broad representation of a context or environment we propose this region also has an important role in conditioned inhibition, which we suggest occurs during discrimination learning and can be context specific [26]. Consistent with this idea, damage to the hippocampus impairs various forms of context conditioning and inhibitory processes like latent inhibition and extinction on pavlovian and instrumental learning tasks [27–32]. We have investigated the role of the dHPC and vHPC in a form of context-specific inhibition. For this paradigm, rats are trained on an 8-arm radial maze version of a visual discrimination task in which the subjects were required to turn and enter reinforced lit arms and not enter darkened arms [33]. The functions of the dorso-lateral striatum are required for the acquisition of this task and the nature of the association supporting this memory-based behaviour was excitatory conditioning to the lit arms. The hippocampus was not necessary for the acquisition of this task but interestingly, using various procedures including context shifts, reversal learning and reinstatement procedures we found that the hippocampus acquired a context-specific inhibitory association to the non-reinforced cue [34]. Further work also showed that this effect was due to impaired vHPC but not dHPC circuitry [35].

Recent work in our laboratory has been directed at understanding the specific role of the HPC in fear conditioning to context. Early work suggested that the hippocampus was crucial for fear conditioning to context [27,28] but more recent research suggest that the hippocampus is important for context discriminations but not non-discriminative single context versions [36,37]. The idea is that context discriminations place a higher demand on the brain to create multiple context representations due to cue overlap in the paired and unpaired context [38]. However, recent work in our laboratory suggests that discriminative fear conditioning in these medium cue overlap conditions is not dependent on the hippocampus in the anterograde direction [39]. It seems likely that HPC function would be necessary in high cue overlap conditions although this remains to be demonstrated clearly in the literature.

The present study was undertaken to assess the specific role of the vHPC in three different forms of learning with potential commonalities including a visual discrimination task developed for the 8-arm radial maze that requires both excitatory and inhibitory conditioning for accurate performance, place learning in the water task, and a high-ambiguity version of the discriminative fear conditioning to context task. The same groups of rats were trained sequentially on the three tasks. All subjects were first trained on a visual discrimination task until reaching asymptotic levels of performance. Half of the subjects were given neurotoxic lesions of the vHPC and then various experimental procedures and transfer tests were performed to assess context-specific conditioned inhibition. Following this task, both groups of rats were trained on the standard spatial version of the Morris water task. A recent study [40] showed that mice with vHPC lesions were impaired at the spatial version of the water task during the early phases of learning. We wanted to replicate this finding in the rat. For the final task, the groups were trained on a new, high-ambiguity version of the discriminative fear conditioning to context task in which both contexts were identical except for one feature.

2. Materials and method

2.1. Animals and handling

Sixteen Long Evans male rats from Charles River colonies were used for the study. Upon arrival, animals were pair housed on a 12:12 light/dark cycle, and had food and water available to them ad libitum. One week following acclimation, animals were placed on a food deprivation schedule to reduce them to 90% of their free-feeding body weight. All rats weighed approximately 350–400 g at the beginning of the experiment. Animals were handled 5 min each by the experimenter for four days prior to experimental training. During this handling phase each rat received 10 Honey Nut Cheerios per day alongside their reduced rat chow diet.

Experiment 1: *Effects of ventral hippocampal lesions on expression of context-specific conditioned inhibition acquired during visual discrimination learning*

2.2. Rationale and hypothesis

Our previous work was the first to implicate the ventral hippocampus in a specific learning and memory process [34,35,38,41], context-specific conditioned inhibition. Briefly, we have shown that during acquisition of a visual discrimination task subjects simultaneously acquired two excitatory associations to the reinforced cue, a Pavlovian association mediated by the basolateral amygdala and related circuits and an instrumental association mediated by the dorsolateral striatum [35,42]. More relevant to the present study, we showed that the same subjects also acquired an inhibitory association to the non-reinforced visual cue and this association became linked to the training context where it is learned. This association indicated that this cue was never reinforced in this context in which reinforcement can be obtained. When reversal learning of this visual discrimination occurs in the same context as training, normal rats show slowed learning compared to rats reversed on the same task in a different context. We have provided evidence that this effect is due to a context-specific inhibitory association accrued to the non-reinforced cue during original training [38] and rats with neurotoxic lesions of the vHPC did not acquire this inhibitory association but rats with dHPC lesions did.

Despite these demonstrations, we have never evaluated the effects of vHPC lesions on conditioned inhibitory processes after the learning has occurred. This would determine if vHPC is part of a neural circuit crucial for the expression of this type of learning. Accordingly, a group of rats were trained on a visual discrimination developed for the 8-arm radial maze until reaching asymptotic levels of performance and were then given neurotoxic lesions of the vHPC. Following recovery, all subjects were given reversal training in the same context as original learning. Our prediction is that rats with vHPC lesions will show accelerated reversal learning compared to intact subjects because the former will have lost the inhibitory association acquired and stored in that region of the hippocampus and those subjects would not have to extinguish that association during reversal learning.

3. Apparatus

An eight-arm radial maze constructed of black metal (Lafayette Instruments) was used as the experimental apparatus. The maze was elevated 60 cm from the floor, and the center platform was 40 cm in diameter. Each arm was 60 cm in length and 9 cm wide with 3 cm high walls along the length and end of the arm. One light bulb was affixed to the end of each arm, and could be turned on/off by a control panel, and a red colored food cup was located at the end of each arm. The maze

was placed in a testing room that was 305 cm long and 216 cm wide. The corner of the north and east walls were covered in black plastic and various shaped cues were affixed on each wall. Other cues in the room included an overhead lamp, a chair, a grey plastic pail, a table with computer, a storage rack, and the experimenter (seated).

4. Procedure

4.1. Pre-exposure

For two consecutive days each rat was placed on the radial maze for five minutes and allowed to freely explore. During this phase no food was present and no lights were lit on the radial maze. After each rat was pre-exposed the maze was wiped down with a soap and warm water solution.

4.2. Discrimination training

Animals received one training trial a day. On each trial four pseudo-randomly selected arms had the lights turned on and were baited with food reward, with the rule that no more than two adjacent arms could be lit and baited. The food reward used for the visual discrimination task was a sweetened cereal (Honey Nut Cheerios). Rats were thereby trained to go to lit arms for reward, and to avoid darkened arms which were not rewarded (L+, D-). Each trial began when a rat was placed on the center platform facing the north wall. The rat was allowed to enter any arm on the maze, and its entries and latency to complete the trial were recorded by the experimenter. Immediately after the rat left a lit arm (indicated by front two paws outside the threshold of the arm), having eaten the food located there the light in that arm was turned off. The trial ended when all of the food was eaten or ten minutes had elapsed. After each rat completed a trial the maze was wiped down with a soap and warm water solution to ensure that scent trails were removed from the maze. Following each training day, a new selection of baited arms was made. A choice accuracy score was calculated by dividing the number of correct choices by the total number of choices for each trial and then multiplying by 100. Mean percent correct scores were calculated for the group, and two trials were averaged for each trial block. The groups were run on the visual discrimination until they reached a criterion of 82% or higher for 2 consecutive trial blocks on their choice accuracy for the light discrimination task. Rats were divided into two groups based on their performance on the visual discrimination over the last 2 trial blocks; ventral hippocampal (vHPC) lesions (n = 9) and sham controls (n = 7).

4.3. Surgery

One hour prior to surgery, all rats were given an intraperitoneal injection of Phenobarbitol (30 mg/kg body weight) as an anticonvulsant. Surgery was conducted while rats were anesthetized with Isofluorane anesthesia (4% with 2 l/min of oxygen for induction and 2% after surgical plane was established) in a standard stereotaxic apparatus. The top of the rat's head was shaved, its head was securely placed into a stereotaxic apparatus and opthamalic ointment was applied to the eyes for protection. The scalp was cleaned with stanhexodine and alcohol (thrice). A subcutaneous injection of Metacam (5 mg/ml) was given as an analgesic prior to a midline incision. Neurotoxic lesions of the vHPC were induced by injecting a 7.5 mg/ml solution of NMDA in pH balanced phosphate buffer through 30-gauge stainless steel cannulae attached to a Harvard mini-pump. All coordinates are in millimeters relative to bregma and skull surface [43]. The coordinates for the first injection site were: AP: -5; L: ± 5.2; V: -5, -7.3. The coordinates for the second injection site were: AP: -5.8; L ± : 4.4; V: -4.4. The coordinates for the third injection site were: AP: -5.8; L ± 5.1; V: -6.2, -7.5. The infusion rate for the first two injection sites were 0.1 µl/min for 3 min, and the cannula were left in the brain tissue for an additional 3 min. The infusion rate for the last injection site was 0.1 µl/min for 5 min, and the diffusion time was 5 min. Diazepam (5 mg/kg) was also injected intraperitoneally to each animal, although its main purpose was to reduce seizure activity in the lesioned rats. The sham animals were treated the same as the lesion group except that no stereotaxic surgery occurred. Following surgery each animal was given 3 ml of saline subcutaneously and monitored until it was awake. The animals were returned to their home cages 24 h after surgery. Following surgery the animals were allowed one week to recover before reversal training began.

4.4. Reversal learning

The same procedures were followed as in acquisition, except that food was placed into food cups of dark arms instead of lit arms (D+, L-). This reversal learning occurred in the same room as training. Animals were run until their accuracy levels reached 82% for 2 consecutive trial blocks.

5. Results

5.1. Discrimination training

As can be seen in Fig. 1, all the rats learned to go to lit arms for food reward and avoid dark arms. After reaching criterion the rats were grouped, based on their percent correct accuracy over the last two trial blocks of training, and underwent Sham or ventral hippocampal lesion surgery.

5.2. Reversal learning

Fig. 2 (top panel) illustrates the reversal learning curves for the sham and vHPC groups on the visual discrimination. Initially, both groups incrementally learn the reversed contingencies similarly, but the vHPC group perform better than the sham group half way through and reach criterion more quickly (Fig. 2-bottom panel). In fact, the vHPC group required 23 trial blocks to reach criterion whereas the Sham group needed 33 trial blocks. A Two-way ANOVA with repeated measures reported significant effects of Trial Block $F_{(22,308)} = 26.58$, $p < 0.0001$, and interaction $F_{(22,308)} = 1.657$, $p = 0.0338$. Although no Group difference was indicated $F(1,14) = 1.762$, $p = 0.2056$, post-hoc comparisons revealed significant differences at the middle and end of training. A *t*-test analysis on trial blocks to criterion also revealed that the vHPC group needed significantly fewer training blocks to learn the reversal contingencies than the sham group $T(1,14) = 4.56$, $p = 0.004$.

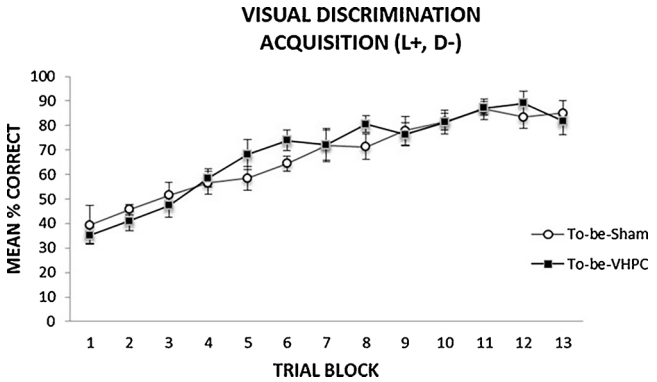

Fig. 1. Acquisition curves for two groups of normal rats that were subsequently divided into two sub-groups with one receiving neurotoxic lesions of the ventral hippocampus and the other no lesions. The data is depicted as the mean percentage correct arm choices over 13 trial blocks. Both groups acquired the visual discrimination task and reached asymptotic levels of performance by the end of training.

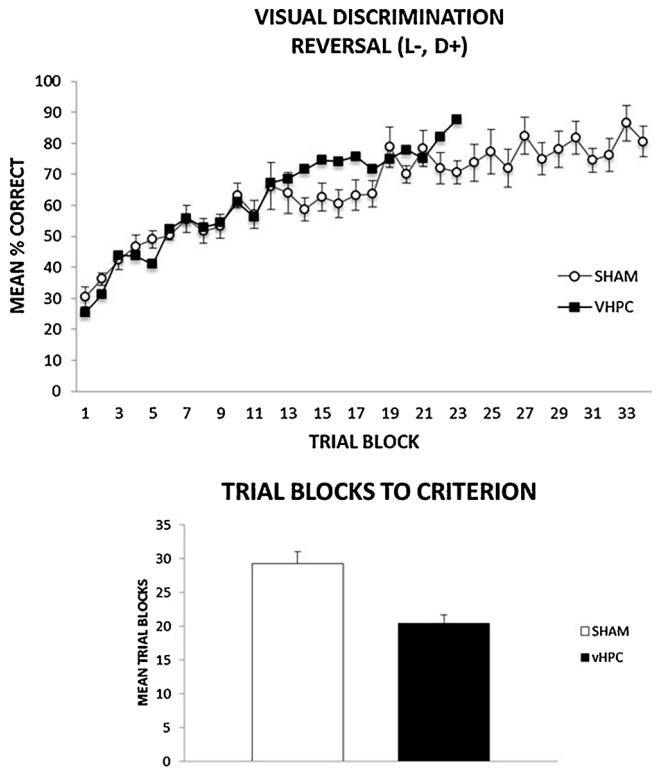

Fig. 2. (Top panel) Reversal learning curves for sham control and rats with ventral hippocampal lesions. The data is depicted as the mean percentage correct arm choices over 33 trial blocks. Rats with ventral hippocampal damage acquired the reversal task more efficiently compared to sham control subjects. (Bottom panel) The mean trial blocks it took to reach criterion during reversal learning of the visual discrimination task in the sham control and ventral hippocampal lesion groups. The data clearly indicates that the lesion group acquired the reversal learning task faster than sham group taking 10 more trial blocks of training to reach criterion.

5.3. Summary

Consistent with our prediction, rats with neurotoxic lesions of the vHPC showed enhanced reversal learning on the visual discrimination task. These results provide evidence that the vHPC is not only involved in the acquisition of context-specific inhibition but it is also necessary for the expression of this conditioned behaviour.

Experiment 2: *Effects of ventral hippocampal lesions on acquisition of the standard spatial version of the Morris Water Task (MWT).*

5.4. Rationale and hypothesis

The role of the vHPC in place learning in the MWT is not clear. Early classic work showed the vHPC was not necessary for this form of place learning [21] unless the lesions were very large. Our lab also investigated the role of dHPC and vHPC in spatial learning in the water task ([24]; Ferbinteanu et al., 2003) and the pattern of effects reported in this work was suggestive of some role of vHPC in place learning but that the dHPC was more efficient at these processes. One strength of the latter findings was that neurotoxic lesions were employed versus aspiration lesions in the former. Recent work, using mice, suggests that the vHPC is important in the early stages of place learning in the water task but that the dHPC becomes important later in training. This pattern of effects was interpreted as evidence that the vHPC is involved in getting the subject to the general area of escape and the dHPC is involved in more precise spatial navigation [40]. We sought to replicate this effect in rats and further clarify the role of vHPC in early stages of place learning. The same subjects from Experiment 1 were used and trained on the standard spatial version of the water task until reaching asymptotic levels of performance. Based on the findings of Ruediger et al. [40] in mice, we predicted that rats with vHPC lesions would be impaired at the early stages of training but eventually show precise spatial navigational behaviours later in training.

5.5. Training room and pool

A white plastic pool 1.5 m in diameter and 0.5 m deep was filled with water (20–22 °C.) to a level of 0.31 m. The water was made opaque by adding non-toxic white paint (Tempra). The water level was kept approximately 2 cm above the platform surface, to render the platform invisible. The 12 cm x 12 cm clear Plexiglas platform had small holes drilled into the top of it to provide grip for the animals. The test room was 3.1 x 6.1 m, with the pool raised 48 cm above the floor in the center of the room. The walls of the pool room had multiple black and white as well as colored posters, which served as distal cues. Other cues in the room included the computer rack, the animal holding cages, a sink, a door, and the experimenter.

5.6. Data collection

A computer-based rat tracker, Noldus^{TM} was used to collect and analyze data obtained from an overhead video camera. The measures of performance were latency to escape onto the hidden platform, path length, heading angle and quadrant preference.

5.7. Hidden platform training

All rats were treated the same way during training. The hidden platform was located at the center of the south-east quadrant of the pool. Each rat was given two 4-trial blocks per day for 5 days, for a total of 40 trials. Each trial within a block started at one of the 4 points, N, E, S and W. The start order was randomized for each rat on each day, but was the same for the two blocks for each rat on a given day. For a trial, the rat was put in the water facing the wall at the starting point and allowed to swim until it located the platform or until 60 s had elapsed. If a rat did not find the platform by the end of the 60 s termination interval the experimenter placed it on the platform. Following escape or aided placement onto the platform, the animal was left there for 10 s and then put into a holding cage while other animals were trained.

5.8. Retention probe

24 h after the last training trial, the hidden platform was removed from the pool and each rat was given a 30 s swim. After the probe trial time had elapsed the rat was removed from the pool and the water was gently stirred to eliminate the possibility that the next rat was following scent trails left by the previous animal's probe test.

5.9. Navigation strategy analysis

The navigation strategies used by the rats during acquisition training were assessed and analysed. Each swim path was replayed and categorized by an experimenter blind to the grouping of each rat. The categories were inspired by early work describing the different strategies used by rats in the MWT [44,6] and a recent automated analysis [40]. Each swim path was replayed in real time and watched by the experimenter, each track was evaluated in combination with data readouts of our tracking package (HVS image). Each swim path was categorized as indicating the use of one of six potential strategies. 1) Thigmotoxis: this strategy was assigned if the rat spent 80% (+/-10) of time in the zone closest to the wall (HVS zone A); 2) Searching: this strategy was assigned if the rat spent 80% of time in zones A and B with at least 20% of time in zone B; 3) Circumnavigation: 60% (+/- 10) of time in annulus zone (zone B); 4) Constrained search: this strategy was

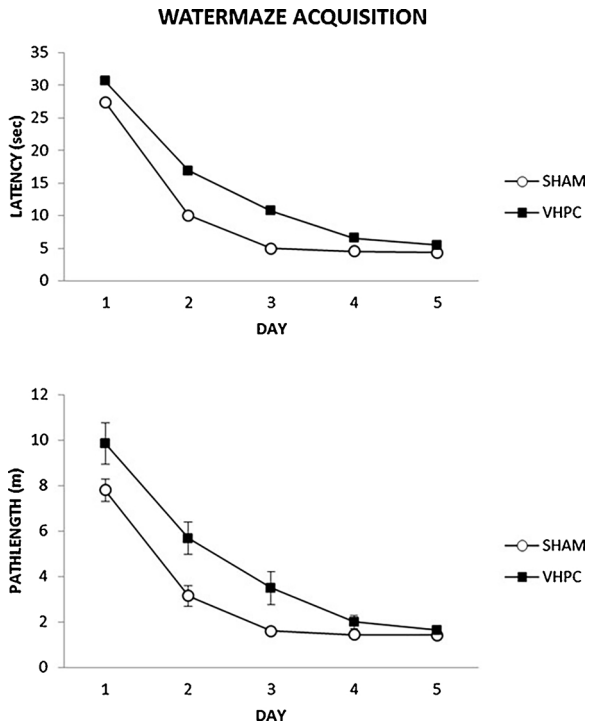

Fig. 3. (Top panel) Mean latency in seconds to find a fixed,hidden escape platform in the spatial version of the Morris water task for control and rats with ventral hippocampal lesions. The data clearly show that rats with ventral hippocampal lesions are impaired at the early phases of learning but eventually acquire the task by the end of training. (Bottom panel) Mean path length in meters to find a fixed, hidden escape platform in thespatial version of the Morris water task for control and rats with ventral hippocampal damage. Consistent with the latency data, the rats with ventral hippocampal lesions swam longer distances, early in training, to find the hidden platform but eventually find the platform efficiently by the end of training.

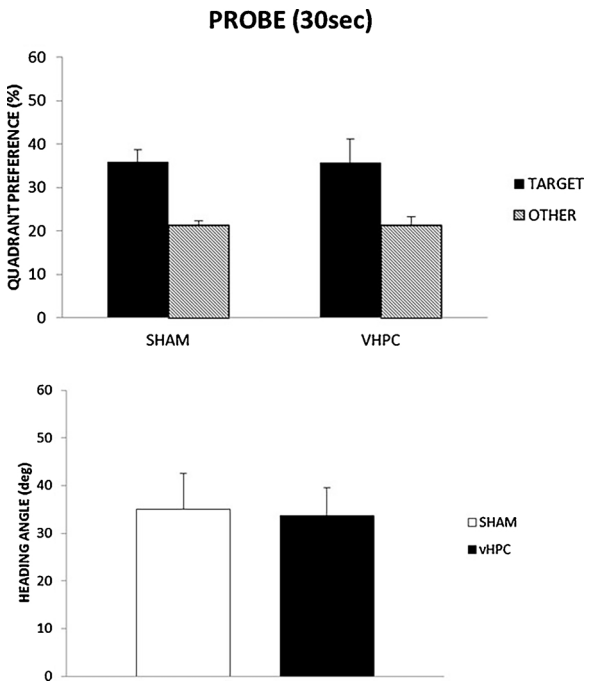

Fig. 4. The results from a 30 second probe trial in which the escape platform was removed from the pool and each subject was allowed to swim freely. A mean quadrant preference for the target versus an average of the other quadrants as well as heading direction towards the precise location of the platform was calculated for the control and lesion group. Clearly, both groups by the end of training show a preference for the target location and head directly to the correct spatial location.

assigned if the rat spent 60% (+/-10) or more time in the goal corridor; 5) Focused search: this strategy was assigned if the rat exhibited a heading angle of 30% (+/-5) or less and spent 40% (+/-5) in the target quadrant; 6) Direct swim to target: this response strategy was assigned if the rat spent 100% (+/-10) in the goal corridor and the heading angle was 20 (+/-10) or less. Approximately 7% of the swim paths could not be slotted into one of these categories. In these cases, the swim was placed in the category closest to the swim characteristics.

6. Results

6.1. Hidden platform acquisition

Fig. 3 represents the learning curves for the Sham and vHPC groups over the 5 days of hidden platform training. The top panel of Fig. 3 depicts the latency to find the hidden platform over training and the bottom panel shows the path length. As can be seen in this figure, the sham rats learned the location of the escape platform more quickly than the vHPC group. However, this impairment does not persist as the vHPC do learn the platform location by the 4th training day. A repeated measures Two-way ANOVA analysis performed on latency revealed significant effects of Group $F_(1,14_)$ = 8.772, p = 0.0103, and Day $F_(4,56_)$ = 73.43, $p < 0.0001$, but no interaction $F_(4,56_)$ = 1.092, p = 0.3696. The same pattern of results were obtained with the path length measure of learning indicating significant effects of Group $F_(1,14_)$ = 15.01, p = 0.001, and Day $F_(4,56_)$ = 75.63, $p < 0.0001$, but no interaction $F_(4,56_)$ = 2.116, p = 0.09. Post hoc comparisons further revealed significant group differences on day 2 (p = 0.007) and day 3 (p = 0.02) for latency and on day 1 (p = 0.006), day 2 (p = 0.001), and day 3 (p = 0.01) for path length.

6.2. Retention probe

As can be seen in the top panel of Fig. 4, both groups spent more time in the target quadrant than the other quadrants during the 30 s probe swim. This was verified by an analysis indicating a significant effect of Quadrant $F_(1,14_)$ = 11.15, p = 0.005, but no effects of Group $F_(1,14_)$ = 0.00182, p = 0.966, or interaction $F_(1,14_)$ = 0.00182, p = 0.966. A t-test performed on heading angle (Fig. 4, bottom panel) indicated no significant Group differences $T_(1,14_)$ = 0.451, p = 0.659. Taken together, these data suggest that rats with vHPC lesions are slower at learning the spatial location of the hidden platform but with continued training they are comparable to the sham controls.

6.3. Navigation strategy analysis

Strategy transitions within and between training days is depicted in the navigation strategy plot in Fig. 5. The plot depicts the mean strategy recruitment values for the first and eighth trials of each day. The sham controls show a clear transition from early utilization of random searching strategies like thigmotoxis and circumnavigation to later use of more goal directed strategies like focused searches and extensive use of direct swims to the target. The vHPC rats appeared to use the general searching and circumnavigation strategies longer than the controls across days 2 and 3 and later utilization of direct swims to the target.

Consistent with this qualitative assessment of the strategy transition data, Fig. 6 and 7 shows the use of each strategy over the entire experiment for each group of rats. The only clear differences between strategies used for the two groups seem to be for the thigmotoxis strategy (strategy 1) and the directed swim strategy (strategy 4). As can be seen in Fig. 6 (top left panel), the sham rats used the thigmotaxis strategy to a lesser extent than the rats with vHPC lesions. This

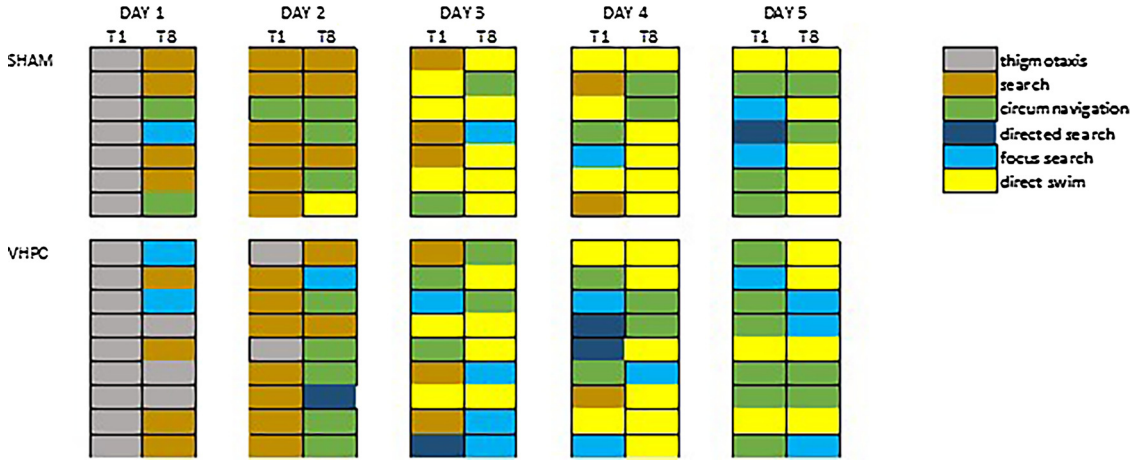

Fig. 5. Strategy transitions within and between training days is depicted in this navigation strategy plot. The mean strategy recruitment values for the first and eighth trials of each day are shown. The controls show a clear transition from early utilization of random searching strategies to later use of more goal directed strategies like focused searches and extensive use of direct swims to the target. The rats with vHPC damage appeared to use the random searching and circumnavigation strategies longer than the controls across days 2 and 3 and later utilization of direct swims to the target compared to intact controls.

impression was confirmed with a one-way ANOVA indicating a significant Group difference $F_{(}1,14_{)} = 5.945$, $p < 0.03$. Fig. 6 (bottom left panel), shows that the vHPC group used a directed swim strategy to find the hidden platform more than the sham rats throughout training. A one-way ANOVA on this data set was consistent with this interpretation of the data indicating a significant Group effect $F_{(}1,14_{)} = 4.645$, $p < 0.05$.

A final analysis was completed on the swim paths from this study. A calculation of the length of strategy blocks was completed for both groups based on total block lengths for each strategy used by each rat averaged over all rats from each group (sham and vHPC).

Strategy blocks were defined as a sequence of at least three trials with the same strategy with one-trial interruptions tolerated. Total block lengths were the sum of all blocks for one strategy and one rat. As depicted in Fig. 8, the results of this analysis showed interesting patterns in the two groups. As can be seen, we focused our analysis on the searching (strategy 2) and the direct swim to target (strategy 6) strategies as these are good representatives of early versus late strategies utilized by the different groups of rats and they were the most commonly deployed strategies throughout training. Fig. 8 (left panel) shows the mean block length (trials) for strategy 2 (searching strategy) and strategy 6 (direct swim to targets) on day 2. As can be seen, the rats

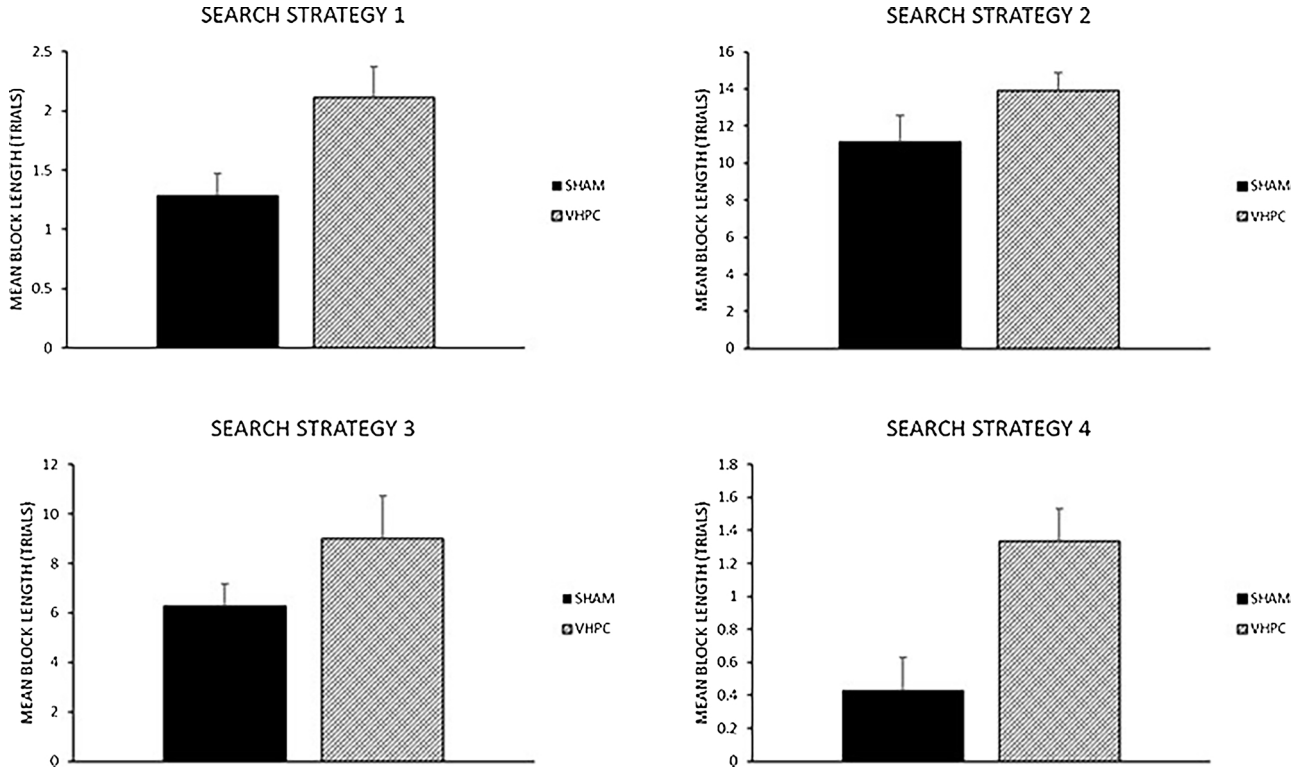

Fig. 6. The use of the first four navigational strategies over the entire experiment for each group of rats. The only clear differences between strategies used for the two groups seem to be for the thigmotoxis strategy (strategy 1) and the directed swim strategy (strategy 4). As can be seen in the top left panel, the control rats used the thigmotaxis strategy to a lesser extent than the rats with vHPC lesions. In the bottom right panel of this graph, the ventral hippocampal group seem to use a directed swim strategy (strategy 4) to find the hidden platform more than the sham rats throughout training.

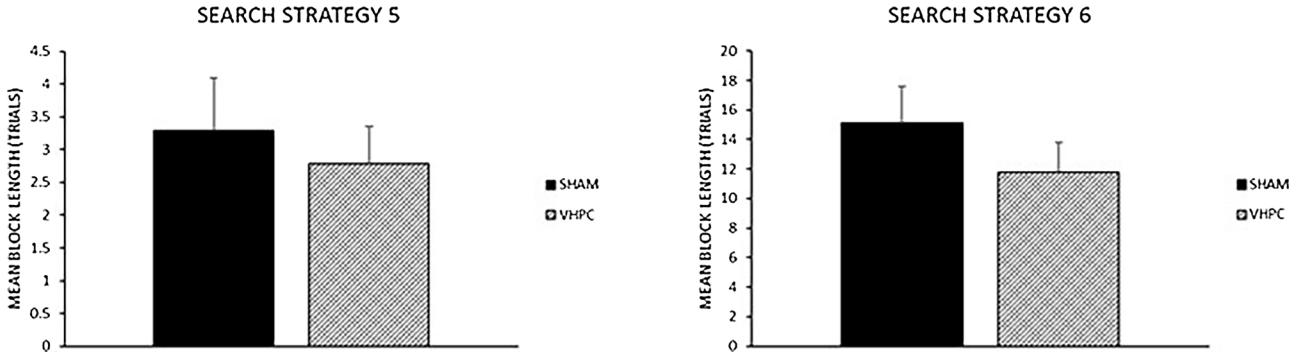

Fig. 7. The use of the last two navigational strategies over the entire experiment for each group of rats. These graphs clearly indicate no differences between the groups on the use of strategies 5 and 6 over the course of the experiment. Statistical analysis indicated no difference between the groups using these strategies.

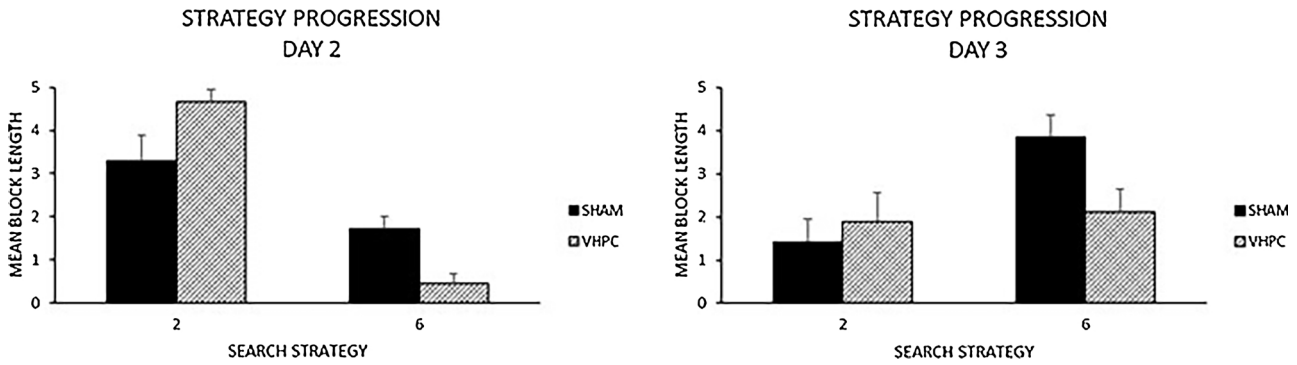

Fig. 8. The length of strategy blocks based on total block lengths for strategy 2 and 6 used by each rat averaged over all rats from each group (sham control and vHPC). Strategy blocks were defined as a sequence of at least three trials with the same strategy. The left panel of this figure shows the mean block length (trials) for strategy 2 (random searching) and strategy 6 (direct swim to targets) on day 2. As can be seen, the rats with vHPC damage deployed strategy 2 more consistently on day 2 than control animals. The left panel also shows the mean block length (trials) for strategy 6 on day2. The control rats deployed strategy 6 more consistently on day 2 than the rats with vHPC lesions. An identical analysis was completed for day 3 and can be seen in Figure 8 (right panel) indicating that both groups used strategy2 (random search) less and strategy 6 (direct swim to target) more often.

with vHPC damage deployed strategy 2 more consistently on day 2 than sham control animals. A one-way ANOVA performed on the block lengths of strategy 2 for the two groups on day 2 for the two groups showed that they were statistically different. Fig. 8 (left panel) also shows the mean block length (trials) for strategy 6 (direct swim to targets) on day 2. The control rats deployed strategy 6 more consistently on day 2 than the rats with vHPC lesions. A one-way ANOVA was consistent with this impression showing that the sham rats used the random search strategy significantly less than the rats with vhpc lesions $F_(1,14_) = 4.906$, $p < 0.05$.

An identical analysis was completed for day 3. As can be seen in Fig. 8 (right panel),

both groups used strategy 2 (random search strategy) less and swam directly to the platform (strategy 6) more often. The deployment of strategy 2 was not statistically different between the groups on day 3 but it appeared that the sham control rats were more likely to deploy strategy 6 (direct swim to target) than the vHPC group. A one-way ANOVA indicated a statistical difference between the groups in the usage of strategy 6 suggesting that indeed the sham control rats used the direct swim to the platform strategy more than the lesion group $F_(1,14_) = 11.618$, $p < 0.004$.

6.4. Summary

The results clearly showed a role of vHPC in the early stages of spatial learning in the water task which is consistent with earlier reports of a role of vHPC in place learning (Ferbinteanu et al., 2003) and specifically the early stages of learning on this complex navigational task [40]. The implications of this finding as it relates to what the functional role of vHPC will be explored in the discussion.

Experiment 3: *Effects of ventral hippocampal lesions on acquisition of a high-cue overlap version of a discriminative fear conditioning to context task.*

6.5. Rationale and hypothesis

The important role of environmental contexts in learning is well documented [38,45–47] and this important form of learning and memory is thought to depend on hippocampal circuitry although its specific role is still controversial [27,48,49]. Part of this controversy has probably emerged because environmental context can be represented as a collection of individual features or cues, or as a conjunction of elements making up a learning experience [8,50–52] with only the latter commonly thought to require the hippocampus. A further problem is that there can be many different sources of information available in a context, including the physical layout and position of visible objects, scent cues, ambient sound, and lighting to name a few. Thus, it is difficult to determine which elements of an environment are part of the representations formed during learning.

We have completed a significant amount of research developing fear conditioning paradigms that attempted to improve on earlier approaches. In the past, fear conditioning tasks used a non-discriminative procedure consisting of a single context chamber was used [48]. We developed a discriminative version of a fear conditioning paradigm using multiple measures of fear [53]. This work also investigated the role of hippocampus and amygdala on this improved fear conditioning paradigm. It was found that the hippocampus and amygdala both participate in the conditioning of freezing, preference, locomotion and

ultrasonic vocalizations; in addition to the amygdala mediating heart rate and the hippocampus mediating defecation and body temperature [36]. Importantly, the design of this paradigm avoided certain confounds found in non-discriminative paradigms such as acquiring a fear response to unrelated static stimuli and sensitization. In our discriminative task all paired sessions occurred in a separate room (shock room), while all unpaired sessions and testing sessions (freezing and preference) occurred in a safe room. This allowed us to show that any learned association demonstrated in testing is to the context itself and not the room *per se*.

In earlier work, we hypothesized that the rats with hippocampal damage were impaired on this task because the task had a greater level of ambiguity than the non-discriminative version. The hippocampus is known for its participation in the rapid acquisition, storage, and retrieval of complex relational and contextual representations linked with events, cues, and actions experienced [54,55]. This version of the task appears to present a medium level of ambiguity for the subject as the contexts each have unique elements but they also share some common cues. However, recent work has shown that rats with large neurotoxic lesions to the hippocampus are not impaired at this task [39].

Even with the improvements associated with the discriminative design described above and the recording of multiple measures of fear, a persistent problem is that little is known about what contextual features are guiding associative learning in these paradigms. Furthermore, it is not clear what the nature of the representations acquired during training might be and what role if any does the hippocampus play in this form of learning? Related to this issue, an advantage of the discriminative fear conditioning paradigm is that it can be explicitly designed to evaluate what types of cues and related representations may be influencing learned behaviour by manipulating the amount of cue overlap between the paired and unpaired contexts. Accordingly, we modified the paradigm into a high ambiguity version, by increasing cue overlap, and thereby possibly necessitating hippocampal involvement [7,18].

To do this, we designed a discriminative fear conditioning to context task in which the cues defining the contexts were identical except for one. For this paradigm, the paired and unpaired contexts were identical on the visual, tactile, and geometric shape dimensions. The only difference between the contexts was the olfactory scent. Here, we investigated the effects of neurotoxic lesions of the vHPC completed prior to training. We hypothesized that because of the high level of ambiguity associated with a discrimination task in which the contexts have many overlapping features the computational power of the entire expanse of the hippocampus would be required to solve this task so vHPC damage would be sufficient to produce an impairment.

7. Apparatus and procedure

7.1. Context chambers

Two identical context chambers were used with the only difference being the olfactory scent cue associated with each chamber. The context chambers were white squares (41 cm X 41 cm X 20 cm) with floors made of metal bars spaced 1.5 cm apart. A small plastic cylinder containing a distinct odorant was mounted on one wall of each chamber. Daily, each odorant, serving as an olfactory cue, was placed on a cotton ball that was inserted into the cylinder container. One chamber contained a eucalyptus scent and the other chamber had an amylacetate scent. During pre-exposure and preference the two chambers were connected by a grey alley (16.5 cm long × 11 cm wide × 11 cm high). The entire structure was placed on a clear Plexiglas table with a height of 100 cm A mirror (91 cm long × 61 cm wide), inclined by 45°, was placed on the floor under the clear table, and allowed the experimenter to see the interior of the chambers. A video camera was placed in front of the mirror to record the testing and preference phases of the experiment. Pre-exposure, unpaired training days, and test days (freezing and preference) occurred in room A, and the paired training days occurred in room B. The entire apparatus was cleaned with a dilute, unscented soap solution after each rat.

7.2. Pre-exposure

To allow animals to acclimate to the testing apparatus, each rat was placed into the middle alley and allowed to freely explore the entire apparatus for 10 min. The experimenter recorded dwell time for each context chamber. A rat was considered in a chamber when both forepaws were past the threshold of the doorway into the chamber and considered out of the chamber when both forepaws were back in the alleyway.

7.3. Training

Training began approximately 24 h following pre-exposure. The rats' training was counterbalanced such that half the animals from each group were assigned to the white square with eucalyptus scent as their paired context and the other half was paired with the white square containing amylacetate scent. The animals were further counterbalanced so that half the animals would begin training in their paired context and the other half would start in their unpaired training context. Plexiglas panels were inserted into the doors of the chambers to block access to the middle alley. In the unpaired condition, each animal was placed individually in its assigned context and remained there for 5 min. For the paired (foot-shock) condition, 0.6 mA of current (scrambled shock) was delivered for 2 s through the grid flooring at the 2-, 3-, and 4-minute marks. Animals experienced their contexts on alternating days, such that animals that were assigned to begin training in their paired context on training day one would then experience their unpaired context on training day two, whereas, those assigned to begin in the unpaired context, would be placed in the paired context on the second day. This alternating training sequence was repeated over 8 training days so that all animals received four training sessions in their paired (shock) context and four training days in their unpaired (neutral) context.

7.4. Freezing

The amount of time rats spent freezing within each chamber was recorded as a measure of whether the animals learned to associate the context with the aversive foot-shock and whether they were able to discriminate the aversive associated context from the neutral context. Normal animals exhibit discriminative freezing evidenced as spending more time freezing within their paired than unpaired context. Testing began approximately 24 h following the final training session. No shocks were administered throughout testing. According to their counterbalanced groups rats were placed within either the paired or unpaired context on the first testing day, then were placed in the opposite context on the second testing day. A testing block consisted of one test day within the paired and another in the unpaired context. During testing rats were placed into one of the enclosed contexts for 5 min and a trained observer recorded the time spent freezing. Freezing constituted total immobility of the rat's body and whiskers, other than the movement required for breathing. All testing sessions were filmed so that freezing scores could be later verified from the recording.

7.5. Preference

Preference testing was conducted to establish if the rats would show an aversion to the context previously paired with shock as expressed by avoidance. Normal rats easily learn to avoid the paired (foot-shock) context as exhibited by spending more time within the unpaired context. Preference testing began approximately 24 h after test day 2. The same procedure and dwell time scoring criterion were used as in pre-

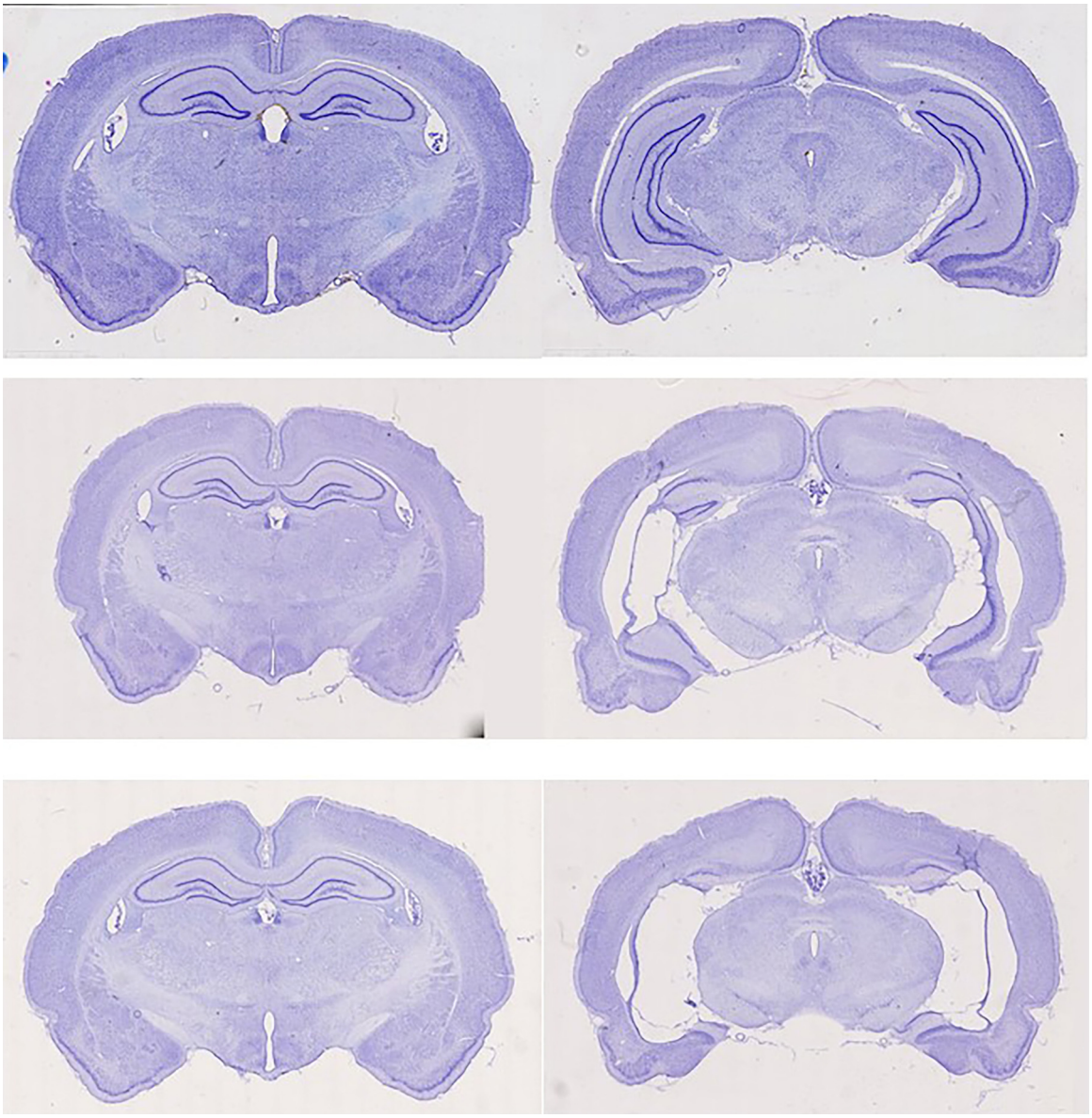

Fig. 9. Photomicrographs indicating the extent of hippocampal damage incurred following our lesion procedure for the current experiments. The specimens in the top panel are from a sham control subject from the behavioural experiments. The specimens in the middle panel are from a rat with ventral hippocampal damage with the smallest lesion in this study. The specimens in the bottom panel are from a rat with ventral hippocampal damage with the largest lesion.

exposure.

8. Histology

After completion of the behavioural experiments animals were administered an overdose of sodium pentobarbital and perfused intracardially with 1X phosphate-buffered saline solution and then 4% paraformaldehyde (PFA) in 1X PBS. Brains were extracted and stored overnight in 4% PFA, and transferred to 0.02% sodium azide in 30% sucrose PBS solution for at least 48 h prior to cryosectioning at -20 °C. Sections were sliced at 40 u m thickness and allowed to dry at room temperature before staining with cresyl violet. Fig. 9 contains photomicrographs indicating the extent of hippocampal damage incurred following our lesion procedure. The specimens include: a sham control subject (top panel); a rat with vHPC damage sustaining the smallest lesion; a rat with vHPC damage sustaining the largest lesion. The volume of spared HPC was quantified using the Cavalieri estimator method [56]. Total HPC volume estimates in vHPC-lesioned rats were then compared against three control HPC volumes to determine the percentage of HPC damage. Histological verification revealed that vHPC lesions began in the ventral portion of CA3 at -3.79 mm relative to Bregma (SD = 0.259; max + 0.491 mm / - 0.379 mm; [43]) and extended throughout the remaining extent of the HPC to include all subregions. Cavelieri volume estimation showed an average 51.22% total HPC lesion in the vHPC group (SD = 10.02; max +12.44; min – 14.48). Qualitative observations showed no signs of cell degeneration in the dorsal extent of the HPC, suggesting that behavioural deficits observed in the present study are due specifically to damage extending throughout the ventral half of the structure.

9. Results

9.1. Pre-exposure

The data from the pre-exposure day are shown in Fig. 10 (top panel). T-tests on the dwell time in the to-be-paired and to-be-unpaired contexts showed no bias for the sham

$T_{(1,6)} = 0.05$, $p = 0.96$, or the vHPC group $T_{(1,8)} = 1.08$, $p = 0.3$.

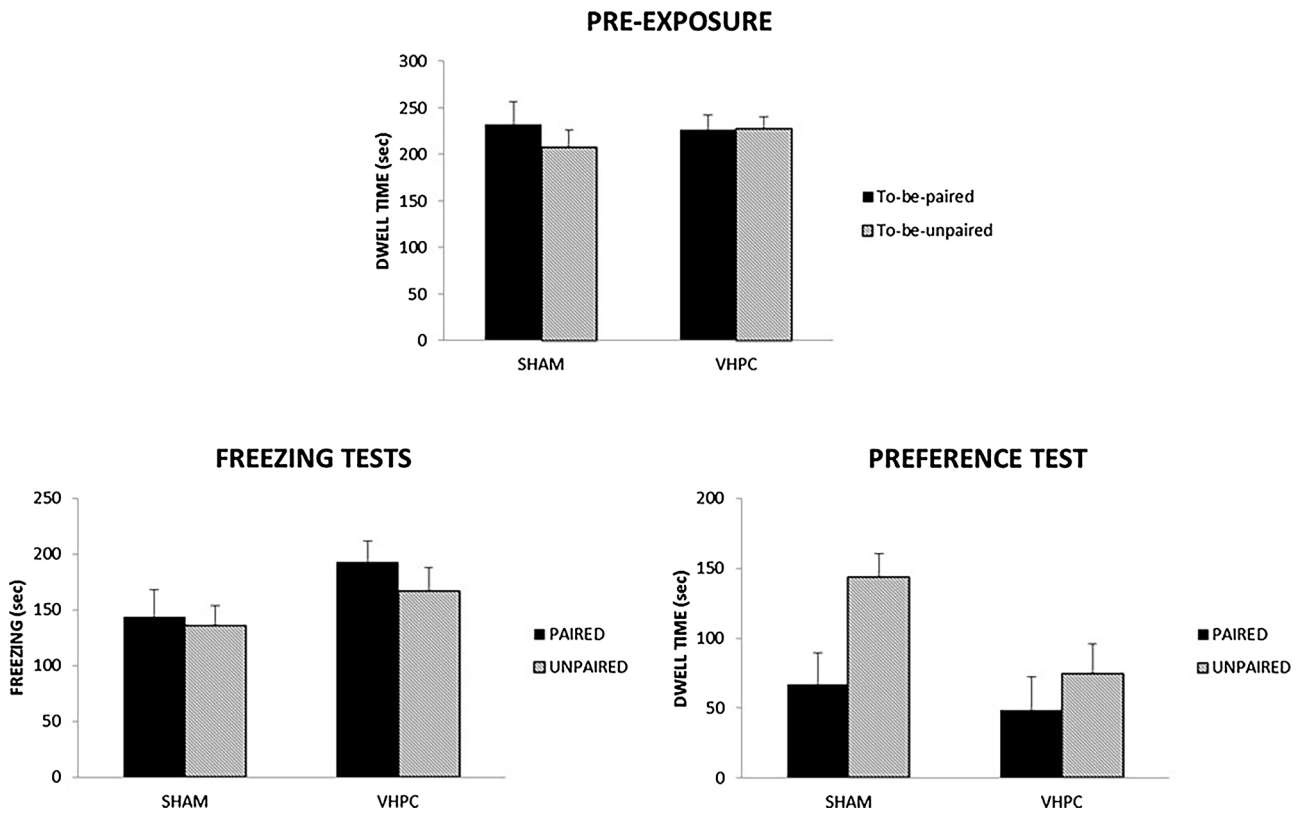

Fig. 10. (Top panel) Results from the pre-exposure phase of the discriminative fear conditioning to context task. Neither group of rats showed an initial preference for the "to be" paired and unpaired contexts.(Bottom left panel) Mean amount of freezing behaviour in the paired and unpaired contexts during the test day. Neither groups of rats showed discriminative fear conditioning to context on this measure of conditioned fear. (Bottom right panel) Mean amount of time spent in the previously paired and unpaired contexts during final preference test. The control rats show a clear preference for the previously unpaired (safe) context but the rats with ventral hippocampal damage did not.

9.2. Freezing

The results of the freezing test are shown in Fig. 10 (bottom left panel). T-tests indicated no significant difference in the amount of freezing in the paired vs. unpaired context for the sham $T_{(1,6)} = 0.32$, $p = 0.76$ and lesion group $T_{(1,8)} = 1.72$, $p = 0.12$.

9.3. Preference

As can be seen in Fig. 10 (bottom right panel), the sham group spent more time in the unpaired vs. paired context during the preference test, whereas the vHPC group did not show this pattern of behaviour. T-tests performed on this dataset confirmed these impressions as a significant context effect was found in the sham group $T_{(1,6)} = 3.6$, $p = 0.01$, and no difference was found in the vHPC group $T_{(1,8)} = 0.88$, $p = 0.40$.

9.4. Summary

Rats with damage to the vHPC were impaired at discriminative fear conditioning to context in high cue overlap conditions in which the paired and unpaired contexts were identical on all cue dimensions except for smell (olfactory).

10. Discussion

The role of the rodent vHPC was investigated using three different learning and memory paradigms in the rat including: 1) a visual discrimination task that allowed the assessment of context-specific conditioned inhibitory associative learning; 2) a spatial navigation task assessing memory-based goal directed behavior; 3) a discriminative fear conditioning to context paradigm with high cue overlap. The results show an interesting pattern of effects on the three different tasks. Rats with neurotoxic lesions of the vHPC showed impaired context-specific conditioned inhibition, difficulties in early spatial navigational abilities in the water task, and an inability to discriminate between highly similar contexts when one was associated with an aversive stimulus and the other with safety. The implications of this pattern of functional effects following vHPC damage will be discussed below.

10.1. Context-specific inhibition

In experiment 1, rats were trained on a visual discrimination task until reaching asymptotic performance levels. Immediately after training, approximately half of the rats received NMDA lesions of the vHPC and the other half received sham procedures. Following a one-week recovery period, both groups of rats were given reversal training in the same context as original learning. We have previously shown that reversal learning in intact rats is slower in the same context as original training versus another context and provided evidence that this was because a context-specific inhibitory association was accrued to the non-reinforced cue [38]. Reversal learning was hypothesized to be slow in the same context because in these conditions, extinction of both the excitatory and inhibitory associations would have to occur as well as new excitatory conditioning to the new reinforced cue. Subjects given reversal training in a different context would only have to extinguish the excitatory association and acquire a new one as the excitatory was shown to transfer to new contexts but the inhibitory association did not [33]. We also demonstrated that rats with vHPC lesions do not acquire this context-specific inhibitory association [35,41], but rats with dHPC lesions did.

In the present study, we wanted to know if the vHPC was necessary for the expression of the context-specific inhibitory association. Rats with neurotoxic lesions of the vHPC acquired the reversal of the visual discrimination faster and more efficiently than sham controls. This is consistent with our previous work suggesting a key role for vHPC in context-specific conditioned inhibition, and further implicates this subregion of the hippocampus in the expression of this conditioned behaviour. This also implies that the vHPC and related neural circuits are not only involved in the encoding of this type of inhibitory association but that circuitry is necessary for the inhibition to be expressed in behavior.

We have developed a theoretical framework predicting what systems and mechanisms are engaged when a subject is in a particular learning scenario [57]. One of these learning scenarios focused on neural circuits and mechanisms engaged during discrimination learning with a particular focus on the non-reinforced cue and the activation of the "indirect" inhibitory pathway [58]. The kind of learning situation facing the rats in the present study when they were trained and then given reversal training in the same context as original training on the visual discrimination task.

In this example, we presented the visual discrimination learning scenario indicating the type of cortico-limbic processing that is likely engaged. For the reinforced cue, plasticity processes supporting excitatory classical and instrumental conditioning occurring in neural circuits centered on the amygdala and dorsal striatum respectively. These associative processes have been hypothesized to occur via training that eventually lead to the expression of various types of non-specific and specific conditioned responses via dopamine (D1) activation of the indirect pathway. Simultaneously, because the subject is being reinforced in the same context and on the same apparatus, early in training the subject expects to be rewarded in the context, regardless of the context they encounter (paired versus unpaired). When they are not rewarded during the unpaired cue presentation in an excitatory context, we argued this causes the vHPC to activate context-specific inhibitory processes in the nucleus accumbens so as to prevent orientation and approach responses to that cue. Under these training conditions, the vHPC interacts with portions of the ventral striatum, namely the core region of the nucleus accumbens (NAc) to activate context-specific inhibition via a dopamine (D2) mechanism. According to this view of simple visual discrimination learning, there are multiple parallel representations that are acquired during training, one that engages the direct striatal output pathway to elicit responding to the reinforced cue, and another that engages the indirect pathway to inhibit responding to the non-reinforced cue. Both of these are thought to act synergistically, at a functional level, to support asymptotic levels of discrimination learning.

Interestingly, these dual representations can lose their influence or come into conflict in which case they may compete for behavioral control. The context-specific conditioned inhibition representation to the non-reinforced cue can lose influence on behaviour by a simple switch in context. Competitive interactions can occur between the representations acquired during discrimination learning when the reinforced and non-reinforced cues are reversed in the same context. In this scenario, it is likely that both the excitatory and inhibitory associations must be extinguished and new ones acquired. If the subjects are reversed in a different context only the excitatory association would need to be extinguished because it transfers to new contexts whereas the inhibitory association does not [33]. We have argued that the demonstrations of context-independent excitatory conditioning and context-dependent inhibitory learning on this task is why reversal learning in a different context from original training is faster in normal rats.

Consistent with these ideas, studies investigating the firing properties of dHPC vs. vHPC neurons show that dHPC neurons have more spatial specificity and smaller place fields compared to vHPC [25,59–61]. This and other work suggests that the vHPC might have some role in forming broad contextual representations that could be used for general recognition processes to identify where you are (e.g. I have been in this town square before), whereas the dHPC represents specific spatial locations in a context (e.g. there is an amazing Tapas restaurant in the south west corner of the square behind that Conquistador fountain). The vHPC would allow the organism to associate cues and objects with different reinforcement histories to a place, but the representation supports general identification of the context not specific locations. The context would imbue meaning on different cues and objects depending on reinforcement contingencies presented there.

A recent single unit study [62] extends this idea providing evidence that dHPC neurons rapidly acquire the identity of reinforced objects with specific locations while vHPC neurons gradually accumulate information that generalize across events within a context and distinguish events across contexts. The idea that the vHPC associates context with certain cues and events [61] is compatible with our work and the present findings reported in Experiment 1.

10.2. Place learning in the water task

Previous work suggested that vHPC had little or no role in place learning in the water task [21,23,63]. Our earlier work showed that although dHPC was more efficient at place learning in the water maze, the vHPC did make a contribution and could in fact compensate for the dHPC in the right training conditions ([24]; Ferbinteanu et al., 2003). More recent work in mice has implicated the vHPC in the early and the dHPC in the later stages of place learning [40]. In the rest of this section we would like to review our earlier work and place it in the context of these new findings.

Interestingly, in both of our early studies we used somewhat non-traditional versions of the water task. One experiment assessed the effects of neurotoxic lesions of the dPHC or vHPC on a task sometimes referred to as the cue-place task [64]. For this task, subjects are trained to swim to a visible platform in the same spatial position for 3 days (4 trials per day) followed by one day of invisible platform training to the same spatial position. This sequence was repeated 3 times for a total of 12 days of training. On day 13, a competition test was employed in which subjects could swim to the visible platform placed in a new spatial position or to the previously correct spatial position. We have previously shown that in a group of normal subjects half of them go to the place while the other half go to the cue (reference). Rats with neurotoxic lesions of the hippocampus all go directly to the visible cue. This result has been interpreted as evidence that a non-hippocampal stimulus-response habit learning and memory system gained control of voluntary behavior [64–66] during the competition test. Interestingly, in this case we did not observe a deficit on place learning in the water task in rats with vHPC lesions, however, the behavioural paradigm utilized a visual platform component that encouraged the rats to swim away from the pool wall early and indicated where in the maze the platform would be located. The visible platform training days were interspersed between an invisible training day during the early, middle, and late stages of training. This visible platform training might have helped rats with vHPC damage on place days by encouraging search behaviours away from the pool wall, and direct searches to general regions of the pool associated with the escape platform, etc. The visible platform would be used by the rats with vHPC in lieu of early unsupervised trial and error learning thought to be mediated by this system. In other words, the presence of the visual platform during these training trials might have compensated for vHPC function. However, the spatial representation that was acquired without the vHPC, in these training conditions, appeared to be weaker than in rats with an intact hippocampus because when the rats with vHPC lesions were placed in a probe test situation in which there was a competition between representations, the stimulus-response representation gained control over behaviour. Clearly the vHPC is not just involved in the early stages of spatial learning, this result suggests that the vHPC is also important for the overall integrity and strength of the spatial representation formed

and is stored across the septal/temporal poles of the hippocampus.

The other water maze paradigm we used to assess the different contributions of dHPC and vHPC was one-trial place learning in which the hidden platform stays in one spatial position during the day of training (8 trials) but switches each day [24]. This requires the subject to search for the platform on the first trial of each day and then continue to swim there for the rest of the trials on that session. After extensive training, normal rats show one-trial place learning in which they search for the platform position on the first swim and then swim directly to the new position on the next swim. This version of the task places a high demand on hippocampal processing and is more sensitive to subtle alterations of hippocampal function [67,68]. Our analysis of this task led us to suspect that the early place learning functions mediated by the vHPC would be needed on each day of training alongside with the precise place navigational processes mediated by the dHPC. Consistent with this idea, rats with damage to either sub-regions of the hippocampus were impaired on this task [69].

The demonstration that rats with vHPC lesions are impaired in the early stages of place learning in the water task is consistent with predictions made in a recent review/theoretical perspective [57]. Relevant to the place learning findings reported here, we reviewed data suggesting that the vHPC, amygdala, ventromedial prefrontal cortex, and ventral striatum combined with dopamine input from the ventral tegmental area form a functional neural network thought to triage responses to stimuli based on their associated affective value in a context. Early in learning in situations where specific stimuli are associated with positive rewards, amygdala and vHPC networks rapidly acquire responses to these conditioned stimuli and their outputs activate portions of the ventral striatum and dopamine neurons as to promote orientation and preferences for general regions of the training context These general attentional and approach behaviours allows the goal-oriented system to acquire contingencies and discover appropriate operant responses. In the case of the standard spatial version of the water task, early in training the vHPC and related network would invigorate exploratory behaviours and attentional processes towards particular regions and salient cues in the training room. This set of conditioned responses would get the rats in the general region of the escape platform resulting in regular reinforcement and allow the dHPC and related circuits to acquire more specific navigational behaviours to the exact region of the platform.

There is an alternative explanation for the impairment exhibited by rodents with vHPC damage on place learning in the water task. It is possible that the conditioned inhibition functions of the vHPC supports early learning in this scenario as well. According to this view, learning to inhibit approach responses to non-reinforced regions and cues of the training context is a key part of spatial behaviours and it is possible that the vHPC contributes to the early phases of spatial training in the water task in this way. Interestingly, we have previously shown that context-specific conditioned inhibition acquired during visual discrimination learning on the radial maze task is also acquired during the early stages of training and not the middle or late stages [38]. Further research is required to assess the early triaging versus conditioned inhibition accounts of this vHPC effect.

Ruediger et al. [40] findings in mice are consistent with the results reported in the current experiments, although their view of the specific role of the vHPC is somewhat different than our view. They suggest that the vHPC "mediates early task-specific goal-oriented searching" and is "tuned to the detection and consolidation of consistent associations between goal and local task-specific features" ([40], pp. 1570). This view is similar to our early triaging view [57] although less specific about the processes and mechanisms involved and very different from the alternative conditioned inhibition view also offered in the present study.

Finally, we did a navigation response strategy analysis of every swim by all of the subjects in the MWT experiment based on early classic descriptions of response strategies [44,6] and a recent extensive response analysis by Ruediger et al [40]. Our analysis showed that early in training rats with vHPC lesions used general random searching strategies more than sham control animals and were also slow to deploy direct searches towards the goal. In general this is consistent with the results reported by Ruediger et al. [40], and colleagues although there were also some clear differences. First, the impairment in acquisition of the MWT we observed in our rats with vHPC lesions was not as large as the deficit they reported in mice despite the fact that our lesions were larger (included portions of intermediate hippocampal regions). Second, the rats with vHPC damage in our present experiment showed extended use of general random search strategies in the early and middle stages of training while the sham control rats switched to direct search strategies towards the goal in the middle stages of training, quite a bit earlier than control mice in the [40]) experiments. One explanation for this difference comes from the fact that we did not see many instances of strategy 4, for example, like the mice exhibited. One thing we noticed about strategy 4 (directed search) was that when our rats deployed something like this strategy they made large loops towards the platform location but the size of these loops was large enough to take them out of the goal corridor region. It is possible that mice, with their smaller bodies, make smaller loops when using this strategy and they stay in the corridor region. Further research is required to assess these kinds of potential species differences in navigational strategy usage.

Finally, we want to raise a general caveat about these kinds of analyses of navigational strategies in the MWT. In the present experiment, our data collection consisted of viewing each swim path combined with analysis of those paths by our tracking system. We found that in some cases the criterion we set out for a particular strategy did not capture the rat's behaviour very well (strategy 4) or some swims might have been categorized incorrectly, or no categorization fit a particular swim pattern. For example, later in training some of the navigational strategies swims were what we would call hybrids. These strategies would include thigmotaxis and then a quick swim to the platform when close to the correct position in the pool. Other examples we noted also include: circumnavigation but in zones A and B (HVS image); looping but with most time in zone A (found mostly on day 4); single short loop to platform. Also, although anecdotal, we have also found that depending on the characteristics of the testing room, strain of rat, and age of the rat some navigational strategies do not appear.

The Ruediger et al. [40] study was slightly different from ours in that they used mice as subjects and they used automated algorithms to categorize the navigational strategy. Although we understand the advantages of using automated algorithms in this situation it also has some disadvantages as based on our experimental results and analysis it is likely that there are cases in which strategies are missed or categorized incorrectly. The point is that, although revealing, one has to interpret these kinds of navigational strategy assessments with caution.

10.3. Hippocampus and fear conditioning to context

Context conditioning has long been associated with mammalian hippocampal function [27,48,49,70]. However, evidence has emerged recently that rats with hippocampal damage can show normal acquisition of fear conditioning to context tasks even when discriminative procedures are implemented [39]. A clear explanation of the inability to replicate these earlier reports is beyond the scope of the present paper but probably is due to particular task parameters like shock intensity [27] and the use of multiple and long extinction trials throughout training [53].

Of interest to us, while investigating the functions of the vHPC, was whether the hippocampus was necessary for acquisition of discriminative fear conditioning to context when the paired and unpaired contexts were highly similar. It was hypothesized that highly similar contexts with overlapping features can result in the disruption of appropriate behaviour, in rats with hippocampal damage, because of a

failure to differentiate which threatening and unthreatening contexts. When experiencing highly ambiguous situations, detailed representations formed in the hippocampus could be used to discriminate between overlapping cues or contexts [18]. Evidence suggests that the hippocampus is involved in decreasing interference by separating events, cues, and contexts into distinct non-overlapping or orthogonalized representations [18,71,72]. Highly ambiguous contexts or situations become increasingly difficult to interpret when cue overlap produces representational interference and can disrupt subsequent behavioural patterns. HPC contributions are probably required in order to separate or orthogonalize context and cue representations during encoding, as a means to decrease interference between potentially conflicting representations.

In previous work using non-discriminative procedures there is only one context so there is little ambiguity about where the animal currently finds itself. More recent work using discriminative procedures, based on our analysis and the results of other groups, might be considered discriminations with medium levels of cue ambiguity. The two contexts used in these experiments had several common elements (Plexi-glas walls and roof, steel rod floors, location in the larger training room, opaque white roof) but also had unique features (olfactory, visual, shape). Accordingly, we modified the discriminative context paradigm we have been using extensively into a high ambiguity version by increasing the feature overlap and by doing so we hypothesized this would necessitate hippocampal involvement and engaging the entire septal/temporal poles of the structure to amplify computational power. According to this analysis, the high ambiguity version of the context task should make this task sensitive to damage to either dHPC or vHPC damage on their own. Our results clearly show that normal rats can solve this high ambiguity version of the discriminative fear conditioning to context task and that rats with vHPC are impaired.

Other work has implicated the hippocampus in using context to disambiguate olfactory representations [73] specifically. In these studies, using single-unit electrophysiology and behavioural analysis, the results suggested that background context is thought to help retrieve the correct representation and reduce interference from other conflicting representations.

10.4. Summary

Ideas put forth here on the functions of different sub-regions of the hippocampus, based on previous work and the present findings, indicate several interesting things about the functions of this area. First, the vHPC seems to be involved in context-specific inhibitory associative processes by forming and utilizing a more general context representation. It might seem odd to have a general, broad representation of a context (vHPC) as well as a representation of precise locations within that context (dHPC) but the former would allow general context recognition as well as recognition linked to more specific associations to objects and cues found in that context. Second, the vHPC is important early in spatial training in the water task. We have argued that the vHPC contributes to early learning in this navigational task by virtue of its role in a set of neural circuits involved in early triaging functions that activates general locomotor activity, attentional and approach responses to relevant cues and cue constellations associated with the goal. The vHPC by virtue of its role in conditioned inhibition processes, might alternatively contribute to the early stages of spatial learning in the water task by reducing attention and responses towards non-reinforced cues in the training environment. The results from the final experiment suggests that the entire septal/temporal poles of the hippocampus are required for discriminative fear conditioning to context tasks with high feature overlap, presumably placing a high demand on pattern separation/pattern completion processes.

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