

Consolidation of object-discrimination memory is independent of the hippocampus in rats

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Received: 8 November 2006 / Accepted: 31 January 2007 / Published online: 27 February 2007
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Abstract We examined whether retrograde amnesia would be more likely for object discriminations learned an hour before hippocampal damage than object discriminations learned days before. Specifically, rats were trained on two object-discrimination problems 72 h before surgery and another discrimination problem and the reversal of one of the previously learned problems 1 h before surgery. Importantly, novel procedures that minimized overtraining on the object discriminations were used to increase the possibility of the lesions causing amnesia. After either receiving sham or neurotoxic-induced hippocampal damage, rats were tested for retention using an extinction procedure. Control rats and rats with extensive hippocampal damage displayed a strong bias for the rewarded object on each object-discrimination problem and a significant bias for the most recent contingency learned on the reversal problem. These results suggest that, despite the use of very sensitive training and testing procedures, hippocampal damage did not cause retrograde amnesia. The findings imply that the hippocampus is not critical for the consolidation, storage, or retrieval

of object–reward associations, or any other information required for accurate performance of an object discrimination.

Keywords Retrograde amnesia · Temporal gradient · Familiarity · Neurotoxic lesion

Damage to the hippocampus (HPC) in humans and non-human animals can cause temporally graded retrograde amnesia, meaning that information acquired shortly before the brain damage is disrupted, but information acquired at a more distant time is intact (Kim and Fanselow 1992; Scoville and Milner 1957; Zola-Morgan and Squire 1990). One explanation for this time-limited impairment is that learned information undergoes a consolidation process, generally viewed as a time-dependent neural reorganization of learned information from the HPC to neocortical regions (Anagnostaras et al. 2001; Frankland and Bontempi 2005; McClelland et al. 1995; Meeter and Murre 2004; Squire and Alvarez 1995; Squire et al. 2004; Wiltgen et al. 2004).

Despite numerous studies examining the effects of HPC damage on memory, it is unclear as to which types of memories are temporarily dependent on the HPC. It is now strongly believed that spatial memories are permanently dependent on the HPC because recent and remote memories seem to be equally impaired by HPC damage or inactivation (Bolhuis et al. 1994; Broadbent et al. 2006; Clark et al. 2005; Martin et al. 2005; Mumby et al. 1999; Sutherland et al. 2001). In contrast, some non-spatial memories such as memories for episodes in humans (see Squire and Alvarez 1995) and memory for contextual fear conditioning (Anagnostaras et al. 1996; Kim and Fanselow 1992; Maren et al. 1997), and socially transmitted food

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preference (Clark et al. 2002; Ross and Eichenbaum 2006; Winocur et al. 2001) in rats all seem to be temporally dependent on the hippocampus.

Some evidence suggests that object-discrimination memory may also be temporarily dependent on the HPC. Zola-Morgan and Squire (1990) found that aspiration lesions of the HPC in monkeys caused temporally graded retrograde amnesia for object discriminations. Based on this finding, they concluded that the HPC was involved in the long-term consolidation of object discriminations. However, no specific inference could be made on the role of the HPC proper because the lesions that caused temporally graded retrograde amnesia also included the posterior entorhinal cortex and most of the parahippocampal cortex. Ensuing studies that examined the effects of more concise HPC damage in rats found that the HPC did not contribute to long-term consolidation of object-discrimination memories (Mumby et al. 1999; Wible et al. 1992). For instance, Mumby et al. (1999) found that rats with neurotoxic lesions restricted to HPC displayed normal retention of object-discrimination problems learned between 13 weeks and 1 week before surgery. This finding suggests that object discriminations may involve a type of memory that is independent of the HPC and that the amnesia reported in the non-human primate studies was likely due to damage to structures surrounding the HPC or to the combined damage.

The conclusion, however, that object-discrimination memory in rats does not depend on the HPC may be premature. It is possible that the HPC lesions failed to produce retrograde amnesia for object discriminations in previous studies because the learning-lesion intervals were too long and memories for the discrimination problems had undergone significant consolidation before the lesions were made. The amount of time required for memories to be consolidated may vary on the type of information to be remembered (see Nadel and Moscovitch 1997), and it is not known how long it takes for memory of an object-discrimination problem to become consolidated in long-term memory. In other paradigms, HPC damage made after a very brief learning-lesion intervals caused retrograde amnesia, whereas damage made a few days after learning did not (Kim and Fanselow 1992; Winocur 1990; Winocur et al. 2001). For instance, HPC lesions made 1 day, but not 5 days, after socially transmitted food preference training caused retrograde amnesia (Winocur et al. 2001). These findings suggest that the HPC is critical for a very brief time period after learning and therefore raise the possibility that deficits would have been observed in previous studies with HPC lesions and object discriminations if the lesions had been made

much sooner following training. Given that, in previous studies, the HPC was always damaged at the earliest a few days after learning of the discrimination problems (Mumby et al. 1999), it is necessary to examine the effects of lesions produced within hours of training in order to rule out any involvement of the HPC in retrograde memory for object-discrimination problems. Thus, the primary goal of this study was to determine whether HPC lesions made 1 h after learning would impair retrograde memory for object discriminations.

Another goal of the present study was to address the possibility that the absence of retrograde amnesia for object-discrimination problems in rats is due to aspects of the training procedure previously used. Training rats on object-discrimination problems usually involves several sessions and many trials (Mumby et al. 1995, 1999; Wible et al. 1992). For instance, in Mumby et al.'s (1999) study, the rats received an average of 100–120 trials on each problem, spaced over 5 or 6 daily sessions. Information acquired over many trials or distributed training sessions is often better retained than information acquired during massed training (Fanselow et al. 1993; Fanselow and Tighe 1988; Williams et al. 1991; Yin et al. 1994). Thus, the combination of a relatively large number of trials and the distributed training sessions could potentially obscure the effects of brain damage on memory.

In addition, it is possible that, in previous studies, the spared retention observed in the HPC rats was due to unequal experience with each object involved in the discrimination problems. By the time rats had learned a discrimination problem they had greater experience with the object associated with reward (i.e., S+) than the object that was not associated with the reward (i.e., S-). Consequently, the S+ may have been more familiar than the S- and the familiarity of the S+ may have guided the response selection on the retention test. Indeed, evidence suggests that the HPC is not necessary for memory tasks that assess familiarity (Duzel et al. 2001; King et al. 2004), though contrasting findings have been reported (see Rugg and Yonelinas 2003 for review). Thus, a rat with amnesia for the information about which object is associated with reward may still be able to perform the task correctly if the lesion did not affect the neural substrates that contribute critically to judgments of familiarity. Therefore, prior to inferring that the HPC is not critical for object-reward associations it is necessary to use training procedures that maximize the likelihood of assessing memory for object-reward associations and reduce the influences of familiarity.

In sum, it is possible that the absence of retrograde amnesia for object discriminations in rats in previous

studies is due to several factors that have been overlooked. Given that the HPC may have a rapidly diminishing role in the consolidation of object-discrimination information, it is necessary to examine the effects of HPC damage produced very soon after the learning of discrimination problems. In addition, less extensive training procedures must be used to minimize overtraining on the problems, as well as procedures that equate familiarity of the objects in a problem.

Consequently, in this study, rats were restricted to a single training session to learn a problem and were given substantially fewer trials than in previous studies. We also used much shorter time periods between the time of learning and the time of lesion, than previous studies. Specifically, HPC lesions were made 72 or 1 h after rats learned object discriminations. To eliminate the likelihood that the performance of rats on object-discrimination problems be guided by the greater familiarity of the rewarded object, we trained rats on two discrimination problems using a single pair of objects. At one time point, the rats learned that one object was associated with a food reward, and then, at a later time, they learned that the other object was associated with the reward (i.e., reversal of the initial problem). By having learned two discrimination problems with a single object pair, the rats encountered each object approximately as many times. Therefore, for this object pair, familiarity of the two objects was equated and could no longer confound performance on the retention test. Now, the selection bias, if any, observed during the retention test would necessarily reflect which object–reward association is remembered. A selection bias that followed the object–reward association learned most recently would suggest intact memory. In contrast, a selection bias that followed the object–reward association learned at the remote time point would suggest temporally graded retrograde amnesia, whereas the absence of a selection bias would suggest retrograde amnesia with a flat gradient.

Finally, a subset of the rats was also tested in the Morris water task. This enabled us to verify the behavioral impact of our lesions on a task that is dependent on the integrity of the HPC (Morris et al. 1982; Sutherland et al. 1982) in the event that no retrograde amnesia would be observed for any of the object-discrimination problems.

Method

All procedures were approved by Concordia University's Animal Care Committee and carried out in

accordance with the guidelines of the Canadian Council on Animal Care (CCAC).

Subjects

Thirteen male Long–Evans hooded rats (Charles-River, St-Constant, QC, Canada) that weighed between 350 and 450 g served as subjects. They were housed individually in standard laboratory cages on a 12:12 light–dark cycle, and all testing was conducted during the light phase. They had free access to water in their home cages and were fed approximately 25 g of rat chow per day throughout the experiment, except for the first week of postsurgery recovery, when they received food *ad libitum*.

Apparatus

The apparatus for the object-discrimination task has been described in detail elsewhere (Mumby et al. 1990). Briefly, it consisted of an elevated runway, separated from identical goal areas at each end by opaque guillotine doors. Each goal area contained two food wells into which food pellets (45 mg Bio-Serv, Inc., Frenchtown, NJ, USA) could be delivered by hand through plastic tubes that were mounted on the outside of the apparatus. A short divider wall protruded from the center of the end wall and separated the two food wells.

The stimuli for the object-discrimination problems were objects of various shapes, sizes, textures, and colors, each made of a similar plastic material. Each object was large enough to cover a food well but small enough and light enough to be easily displaced by a rat. The objects were washed after every session with a solution of diluted chlorine bleach to remove any extraneous scents they might have acquired during displacement by the rats or handling by the experimenter.

The place-learning task was conducted in a circular pool, 137 cm in diameter and 46 cm high, and filled with water (23°C) to a depth of approximately 30 cm. The water was made opaque by adding instant skim milk powder. A movable Plexiglas platform (10 cm × 10 cm × 28 cm) was hidden approximately 2 cm below the surface of the water. The rats could not see the platform, but several extramaze cues (e.g., posters, shelves, a computer, ventilation duct, etc.) were visible or audible from within the pool, and the rats could learn the location of the platform relative to these distal cues. Swim paths and latencies were recorded using a VP118 Super Tracker with HVSWater software (HVS Image Ltd., Hampton, UK) and these

raw data were stored on computer (IBM compatible, 486 DX) for later analysis.

Procedure

Preoperative object-discrimination training

The rats were habituated to the apparatus and their behavior was shaped to retrieve food pellets from the food wells (see Mumby et al. 1990). The principal phase of the experiment began a few days later. Six objects were divided into three pairs, each pair serving as the discriminanda for an object-discrimination problem. One of the objects in each pair was designated S+ (rewarded) and the other one was designated S− (not rewarded), counterbalanced within groups.

The rats learned two object-discrimination problems concurrently approximately 72 h before surgery and another two problems concurrently approximately 1 h before surgery. Specifically, 72 h before surgery, each rat was given ten consecutive trials on one object-discrimination problem (−72HR) followed by ten consecutive trials on a second problem (REVERSAL). This was repeated twice for a total of 40 trials and corrections were given on these trials, meaning that if the rat displaced S−, it was allowed to correct its choice by displacing S+ in order to receive a food pellet. After the correction trials, each problem was presented in blocks of two trials until the rat chose S+ seven consecutive times for each problem.

The same procedure was followed for the learning of the two new object-discrimination problems 1 h prior to surgery. However, for one object-discrimination problem a new object pair was introduced (−1HR), whereas for the other problem the reward contingency of problem REVERSAL was switched. Hence, the rats learned a reversed contingency of a previously learned problem and a new contingency with novel objects.

The object pairs were counterbalanced between rats. Subsequent to the end of the second training session, rats were matched according to the number of trials to criterion for the two object-discrimination training sessions and assigned to either the SHAM or HPC group.

Surgery

All rats were anesthetized with sodium pentobarbital (Somnotol, 65 mg/kg). The rats in the HPC group received a bilateral lesion of the hippocampal formation ($n = 6$) made by intrahippocampal injections of a 5.1 M solution of *N*-methyl-D-aspartate (NMDA) dissolved in 0.1 M phosphate buffered saline. Injections

were made at ten sites bilaterally (see Mumby et al. 1999 for coordinates) using 10 μ l Hamilton syringes mounted on an infusion pump (KD Scientific) and connected to 30-gauge cannulae by polyethylene tubing. The NMDA solution was infused at a flow rate of .15 μ l/min until a total of .4 μ l had been injected at each site. The cannulae were left in place for an additional 2.5 min before being retracted. The scalp incision was closed with wound clips and an antibiotic powder was applied to the wound. As a rat began awakening from the anesthetic, it was given diazepam (approximately 10 mg/kg) as a prophylaxis against seizures. Rats in the SHAM group ($n = 7$) received the same anesthetic dose, scalp incision, and postsurgery diazepam injection, but no damage was done to the skull or brain.

Postoperative object-discrimination testing

Fourteen days after surgery all the rats were trained on a new object-discrimination problem (NEW) to eliminate possible positional selection biases that may have occurred following surgery. The rats received 40 trial sessions once a day until they reached an 80% criterion (32/40 correct trials), with a minimum of two sessions.

The rats' retention for the object-discrimination problems learned preoperatively was assessed in two sessions of 60 trials each on successive days. Each rat received blocks of ten consecutive trials for each of the preoperatively acquired discriminations (i.e., −72HR, −1HR, and REVERSAL). However, following each block of ten trials, the rat received a block of ten trials on the postoperatively acquired discrimination (i.e., NEW). The order of presentation of the three preoperatively acquired problems was counterbalanced. The rats were subjected to an extinction procedure for the three preoperatively acquired discrimination problems, such that when these problems were presented rats received no food reward after displacing an object. Thus, the selection bias, if any, during the retention test sessions could be confidently attributed to the rats' memory of the preoperatively acquired object–reward association; their choices would not be influenced by additional reinforcement. However, if the rats displaced S+ for the postoperatively acquired discrimination (NEW), they did receive a food pellet. This procedure served to maintain the rats' motivation to displace objects.

Water task testing

Five rats of the SHAM group and four from the HPC group were randomly selected for testing in the Morris water task. Each rat was trained on a place-memory problem in a single session. The session consisted of 16

hidden-platform trials in which the platform was submerged below the surface of the water and positioned in the center of the NW quadrant of the pool. To begin a trial, the rat was placed into the pool facing the wall at one of the four compass points (N, S, E, W), which varied in a pseudorandom sequence over trials. They had 60 s to escape onto the submerged platform located in the center of the NW quadrant. If they did not do so within that time, then they were placed on the platform by the experimenter. All rats were left on the platform for 10 s before being removed from the pool. The rats had a rest period of about 5 min between successive trials. The main performance measure on the hidden-platform trials was the latency to escape onto the platform.

Histology

Upon completion of behavioral testing, all rats received an overdose of sodium pentobarbital (1 cc; 65 mg/ml, i.p.), and were perfused intracardially with 0.9% saline followed by 10% formalin. Their brains were excised and stored in a 10% formalin–30% sucrose solution for at least 48 h and then sectioned (40 μ m), mounted on gelatin-coated slides, and stained with cresyl violet. The stained sections were examined through a light microscope (Zeiss, Germany) to determine and quantify the extent of the lesions.

The amount of HPC damage in each lesion rat was estimated according to principles of the Cavalieri method (Schmitz and Hof 2005). Briefly, five images were captured under 1 \times magnification using an Axio-Cam camera (Zeiss, Germany) connected to the light microscope. The captured sections corresponded approximately to -2.3 , -3.3 , -4.3 , -5.3 , and -6.3 mm relative to Bregma. A systematic sampling grid with an area per point of 20,000² pixels was then randomly laid over each image and the number of points hitting intact HPC tissue was counted. Grids were generated using ImageJ software (<http://www.rsb.info.nih.gov/ij/>). The total number of hits in each rat was divided over the average number of hits obtained by three control rats. This resulted in the proportion of remaining HPC. The inverse proportion was used as the percent HPC damage estimate.

Results

Histological findings

Figure 1 illustrates the extent of the hippocampal lesions. The NMDA injections produced extensive cell

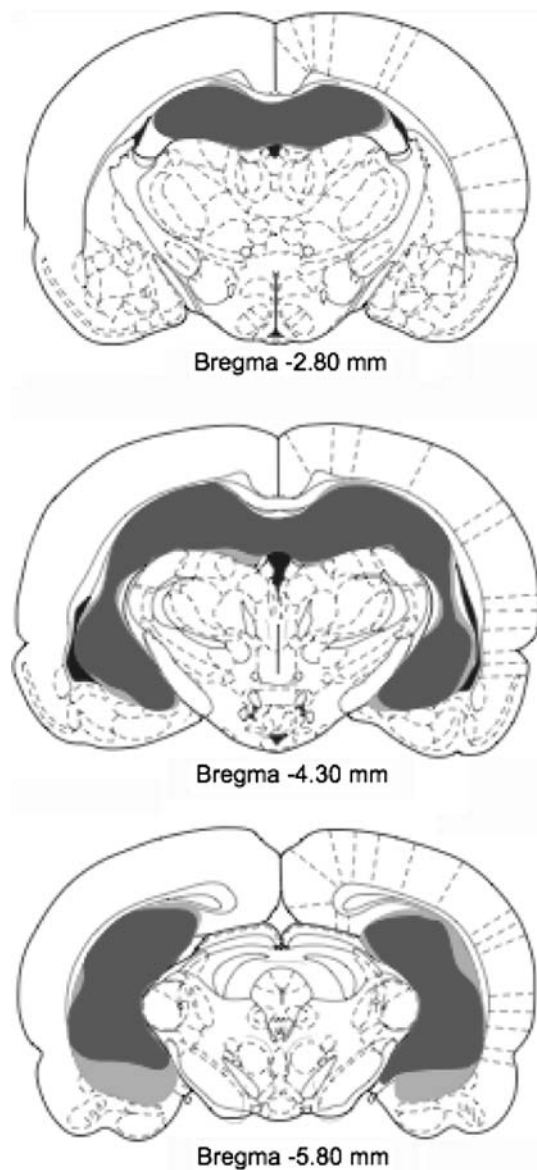


Fig. 1 Illustrations of the smallest (*dark grey*) and largest (*light grey*) lesion observed bilaterally through the rostral and caudal extent of the HPC. Atlas plates from Paxinos and Watson (1997)

loss in the dentate gyrus and all principle subfields of the hippocampus. It was estimated that the amount of HPC damage ranged between 64 and 93% across lesion rats and that 75% of the HPC was damaged on average for the group. There was sparing of cells in the most ventral CA1 subfield unilaterally in two rats and bilaterally in three rats. Cells in the dorsal lateral CA2 and CA3 fields were spared unilaterally in one rat. The extent of damage to the subiculum was variable, but there was bilateral loss of cells in the subiculum in each rat. The fimbria/fornix was mostly spared in all rats. No damage was found in the thalamus or the rhinal cortex. There was some thinning of the parietal and occipital cortex where the injection cannulae were inserted.

Object discriminations

The SHAM and HPC rats learned the preoperative object-discrimination problems at a similar rate. The mean number of trials needed to reach the criterion of seven consecutive trials in a row on each problem varied between 56 and 67 trials for the SHAM rats and between 46 and 68 trials for the HPC rats. Both groups took more trials to learn the reversal problem at the -1 h time point, but an ANOVA, with Lesion as a between-subjects factor and Problem as a within-subjects factor, revealed no significant main effects or interaction (all p s > 0.05). However, given that the two problems were learned concurrently, rats typically reached criterion on one problem before they reached criterion on the other. Although they had reached criterion on one problem they continued trials for that problem until they also reached criterion on the other. Consequently, the number of trials to reach criterion does not accurately reflect the amount of experience the rats received on each problem. On average, the rats received a total of 65–79 trials on each problem. Again the rats received the most trials on the reversal problem at the -1 h time point, where the SHAM rats received 79 trials and the HPC rats 76 trials. An ANOVA, with Lesion as a between-groups factor and Problem as within-groups factor did not reveal significant main effects or interaction (all p s > 0.05).

After surgery, all the rats of group SHAM and HPC reached the 80% correct learning criterion on the post-operative object discrimination during the first two sessions. Therefore, none of the rats needed additional sessions for this new problem. Specifically, SHAM and HPC rats selected the S+ on 92 and 90% of trials, respectively, and a t -test revealed no difference in correct responses between the two groups (t [11] = 1.177, p = 0.264). Similarly, when analyzing performance on the first 20 trials of the first day no group difference was found (t [11] = 1.042, p = 0.320) or when analyzing group differences on the first four trials (t [11] = 0.610, p = 0.554).

The HPC rats performed as well as the SHAM rats on the retention test for the problems that were learned before surgery. Figure 2 shows the mean percent of correct choices (object that was S+ during training) for -72 HR and -1 HR. One sample t -tests revealed that SHAM and HPC rats had a significantly greater than chance bias for the S+ on the -72 HR problem (SHAM t [6] = 5.851, p < 0.05 ; HPC t [5] = 3.206, p < 0.05) and -1 HR problem (SHAM t [6] = 23.497, p < 0.05 ; HPC t [5] = 5.838, p < 0.05). In addition, a repeated measures ANOVA, using Lesion

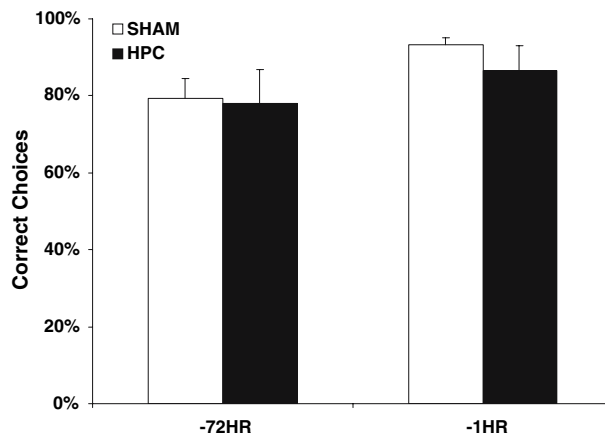


Fig. 2 Mean (+SEM) percent correct choices on the retention trials for the -72 HR and -1 HR object-discrimination problems. SHAM and HPC rats' selection of the correct object was significantly greater than chance (50%) for each problem (p < 0.05)

and Time as factors, revealed a significant main effect of Time (F [1, 11] = 9.696, p < 0.05), indicating that the rats performed better on the -1 HR problem than the -72 HR problem. No significant effect of Lesion (F [1, 11] = 0.292, p = 0.6) or interaction (F [1, 11] = 0.514, p = 0.489) was found.

Figure 3 illustrates the results of the retention test for the REVERSAL problem. One sample t -tests revealed that rats in both groups selected the object that was S+ immediately before surgery (-1 h) significantly more than expected by chance (SHAM t [6] = 3.596, p < 0.05 ; HPC t [5] = 2.879, p < 0.035), suggesting that they remembered the last learned object-reward contingency. In addition, a t -test on the mean percent selection of the object that was S+ 1 h prior to surgery showed no significant difference between the HPC and SHAM rats for the reversal (t [11] = -0.24 , p = 0.981).

Water task

The HPC rats did not learn the location of the hidden platform in the Morris water task as well as the SHAM rats. Figure 4 depicts the mean escape latencies of the SHAM and HPC groups for the 16 swim trials in blocks of 4. A mixed design ANOVA revealed a significant main effect of Group (F [1, 7] = 21.125, p < 0.05), a significant main effect of Trial (F [15, 105] = 2.233, p < 0.05), and a significant Group \times Trial interaction (F [15, 105] = 2.564, p < 0.05). Further analyses indicated that the SHAM rats showed a significant decrease in latencies across trials (p < 0.05), whereas the latencies of the HPC rats tended to increase (p = 0.068).

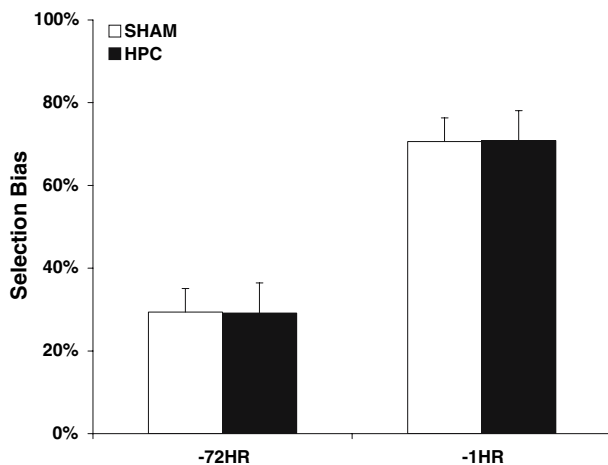


Fig. 3 Mean (\pm SEM) percent selection of the object that was rewarded (S+) 72 and 1 h before surgery on the retention trials of the REVERSAL problem. Both groups showed a significant bias for the object associated with the reward 1 h before surgery ($p < 0.05$), suggesting that they remembered the last reward contingency

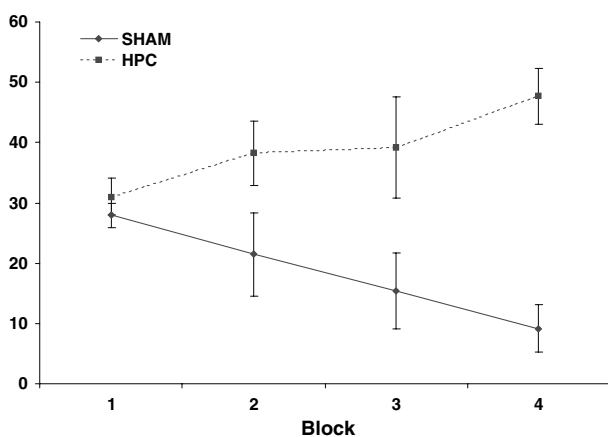


Fig. 4 Mean (\pm SEM) latency to find the hidden platform for each block of four trials in the Morris water task. SHAM rats showed a significant decrease in latencies across trials ($p < 0.05$), but not HPC rats ($p > 0.05$)

Discussion

The results of this study suggest that HPC lesions do not produce retrograde amnesia for object-discrimination problems. Rats with widespread, if not complete, lesions of the HPC performed as well as SHAM rats on the retention test for problems learned 72 and 1 h prior to surgery. These results are similar to findings from other studies suggesting that the HPC is not critical for retrograde memory for object discriminations (Mumby et al. 1999; Wible et al. 1992). These prior studies, however, used long intervals between acquisition of the object-discrimination problem and the time of insult to

the HPC and these intervals may have been long enough for the information to be consolidated. The findings of the current study clearly demonstrate that even for very short intervals (i.e., 1 h) the HPC is not necessary. Thus, the HPC does not appear to be critical for the consolidation, storage, and/or retrieval of information pertaining to object discriminations.

The findings from the REVERSAL problem further confirm that HPC lesions do not disrupt retrograde memory for object–reward associations. It is possible that on the discrimination retention test, a rat may select the object that was associated with the reward not because they remembered the object–reward association, but because it was more familiar. However, the differential familiarity of the S+ and S– does not account for successful performance of HPC rats on the object-discrimination problems. Despite equating the familiarity of the two objects within a discrimination problem, the HPC rats performed just like the SHAM rats on the retention test. When tested on the REVERSAL which required the rats to learn two discrimination problems with a single object pair at two different time points before surgery, the rats in both groups showed a marked tendency to select the object that was associated with the reward immediately before surgery. These findings suggest that the HPC rats remembered the most recently learned object–reward association.

The HPC damage also did not cause anterograde amnesia for object-discrimination memory. After surgery, all the rats of group SHAM and HPC learned the postoperative object discrimination (NEW) during the first two sessions. Even when considering the initial learning trials, the phase during which the HPC damage is most likely to cause deficits (Teng et al. 2000), SHAM and HPC rats did not significantly differ, suggesting that the object-discrimination learning rate was not affected by the HPC damage. The latter findings are consistent with previous studies (Mumby et al. 1999; Wible et al. 1992; Wood et al. 1993), though mild deficits have been reported (Mumby et al. 1995). However, the lesions in that study were made by aspiration and included damage to the posterior parietal cortex and fibers comprising the corpus callosum overlying the dorsal HPC and the alveus, which all were largely spared by the present NMDA lesions. The extrahippocampal damage most likely contributed to the mild deficits that were observed.

The absence of amnesia for the discrimination problems is not likely due to incomplete lesions of the HPC. In all HPC rats there was extensive damage to both the dorsal and ventral regions of the HPC and on average approximately 75% of the HPC was damaged. Even

the rat with the most damage (93%) did not show evidence of a deficit on any of the discrimination problems. In addition, a subset of rats was tested on a HPC-dependent task in the Morris water task to test the effectiveness of the lesions and a spatial learning impairment was found. In fact, the HPC rats had a tendency to do worse on the later trials. This peculiar finding may be the result of the HPC rats swimming faster or more erratically in the early phase of the test, which could have increased the chances of accidentally finding the hidden platform. Regardless, the HPC lesions seemed effective in disrupting behavior that is HPC-dependent and further suggests that the structure is not required for normal performance on object-discrimination problems.

The training on the discrimination problems in the current study was substantially less than that involved in previous studies. Contrary to previous studies of retrograde amnesia for object discriminations (Mumby et al. 1999; Wible et al. 1992), training on each preoperative discrimination problem in the present study was limited to a single session that could be as short as 40 min. The rats in the present study also received considerably fewer training trials than the rats in previous studies (Mumby et al. 1999; Wible et al. 1992). By training rats in this manner, we reduced the chances of overtraining the rats on the discrimination problems, yet the HPC rats performed equally well as SHAM rats on the retention test. It remains possible that HPC damage would impair memory for a discrimination learned in a single trial. However, it is unknown whether rats could remember for several days an object discrimination acquired in a single trial.

The extinction procedure, during which the rats are not rewarded for their selections on the retention test, also did not reveal deficits in HPC rats for the preoperatively learned problems. An extinction procedure is arguably a more sensitive procedure to detect memory impairments for the problems if there are any (Mumby et al. 2002). This procedure disentangles anterograde and retrograde memory contributions to the performance on the retention test. The delivery of a reward on the retention test enables reacquisition of the object–reward association that can undermine the assessment of the retrograde amnesia. In contrast, if the selection bias on the retention test is not rewarded, then the persistence in selecting the object that was previously paired with a reward necessarily reflects memory and not strengthening of the response from ongoing reinforcement. When using this procedure, HPC rats still remembered the object-discrimination problems as well as SHAM rats. This finding suggests that not only memory for object discrimination in HPC

rats is spared, but that this memory is robust enough to persist despite several extinction trials.

The pattern of findings in the current study is similar to that found following HPC damage in another type of discrimination task. Specifically, Jonasson et al. (2004) reported that HPC lesions produced soon after rapidly acquired olfactory discriminations did not cause retrograde amnesia. Although both studies suggest that the HPC is not involved in consolidation and storage of these memories, they cannot be equated. The stimuli involved in object discriminations are arguably more complex and likely rely on more information than that involved in olfactory discriminations. Nevertheless, future studies should examine whether object-discrimination memory survives HPC damage because of the olfactory properties of the objects.

It also cannot be concluded that the HPC is generally not involved in discrimination memory. Even though object and olfactory discriminations are independent of the HPC, there is evidence that the HPC is involved in visual discrimination memory (Driscoll et al. 2005; Sutherland et al. 2001). For instance, lesions of the hippocampus caused retrograde amnesia in a task that required rats to remember that one of two cues predicted escape from water (Sutherland et al. 2001). Thus, there must be properties particular to olfactory and object discriminations that make them HPC-independent. Possibly, the role of the HPC in discrimination memory is specific to the visual modality. Moreover, it cannot be assumed that object discriminations do not require the HPC because they are a form of habit learning. Many trials are needed to acquire habits and this form of memory does not require the HPC (Squire 1987). One may thus be tempted to argue that object discriminations must be a kind of habit and that would account for the absence of retrograde amnesia following HPC damage. Accordingly, all discriminations acquired over repeated trials must also be habits and should equally be spared after extensive HPC damage. Yet, there are examples of visual discriminations that are acquired over many trials and multiple days that depend on the HPC (Driscoll et al. 2005; Sutherland et al. 2001). Thus, the habit learning argument cannot readily account for the current findings and some other properties must make object discriminations independent of the HPC.

Considering the studies that examined the effects of HPC damage on retrograde amnesia in rats, little evidence suggests that complete HPC damage causes temporally graded retrograde amnesia. The vast majority of studies supporting the view that memories are temporarily dependent on the HPC until consolidated in neocortical regions in rats only examined the effects of

dorsal HPC damage (Anagnostaras et al. 1996; Debiec et al. 2002; Kim and Fanselow 1992; Maren et al. 1997; Ramos 1998; Winocur 1990), which spares most of the structure. Thus, the conclusions from these studies may be misleading because the intact remote memories may have become dependent on the spared HPC tissue rather than on other structures. Temporally graded retrograde amnesia following large HPC lesions in rats has only been consistently reported for socially transmitted food preference (Clark et al. 2002; Ross and Eichenbaum 2006; Winocur et al. 2001). The consolidation period for this type of memory is very short, less than 5 days according to Winocur (2001). This short consolidation period raised the possibility that previous studies examining the effects of HPC damage on memory for object discriminations failed to find amnesia because the interval between training and surgery was sufficiently long for the information to be consolidated (Mumby et al. 1995, 1999; Wible et al. 1992). However, the current findings suggest that the consolidation of object-discrimination memory is independent of the HPC because damage induced as soon as 1 h after learning did not cause amnesia. Consequently, object discriminations appear to be amongst the type of memories that are reliably independent of the HPC.

Acknowledgments This research was funded by grants from Natural Sciences and Engineering Research Council of Canada, and FCAR, Quebec.

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