Behavioural Brain Research 417 (2022) 113620

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Ventral hippocampus lesions and allocentric spatial memory in the radial maze: Anterograde and retrograde deficits

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ARTICLE INFO

Keywords:
Spatial memory
Ventral hippocampus
Dorsal hippocampus
Hippocampal longitudinal axis
Radial maze

ABSTRACT

Although the dorsal hippocampus (DHip) has been clearly implicated in spatial learning and memory, there is currently debate as to whether the ventral hippocampus (VHip) is also necessary in allocentric-based navigation tasks. To differentiate between these two subregions of the hippocampal dorsoventral axis, we examined the effect of neurotoxic lesions to the DHip and VHip in different learning situations, using a four-arm plus-shaped maze. In experiment 1 a spatial reference memory task was used, with results showing an acquisition deficit in DHip-lesioned rats but perfect learning in VHip-lesioned rats. However, in experiment 2 an acquisition deficit was found in VHip-lesioned rats using a doubly marked training protocol. In this case the position of the goal arm during training was marked simultaneously by the extramaze constellation of stimuli around the maze and an intramaze cue. The main results indicated that DHip and VHip groups presented significantly more allocentric errors in the probe test than the control rats. In experiments 3 and 4, animals with their brains still intact learned, respectively, a spatial reference memory task or a purely cue-guided navigation task, and DHip and VHip lesions were made 2–3 days after reaching learning criterion. Results indicated a profound retrograde deficit in both lesioned groups but only with regard to allocentric information. So, depending on the training protocol used, our results point to increased integration and cooperation throughout the hippocampal dorsoventral axis when allocentric learning and memory is involved. These data support the existence of a functional continuum from the dorsal to the ventral hippocampus.

1. Introduction

Animals need to have spatial representation of their environment in order to interact with their habitat and organize their experience into episodic memory [1–3]. Although the hippocampus is a key region in this process, it remains unclear how spatial/contextual information is processed within this structure [4–6]. Numerous studies suggest that different subregions of the hippocampus along its dorsoventral axis are involved in functionally distinct processes. Along these lines, one leading proposal is that the dorsal hippocampus of rodents (DHip, also referred to as posterior/septal hippocampus) supports principally spatial memory and other cognitive processes, while the ventral hippocampus (VHip, anterior/temporal) is primarily involved in emotion-related behaviors [7–16].

There is considerable evidence pointing to this functional heterogeneity. First of all, the dorsal and ventral hippocampus differ in gene expression [17–19], plasticity-related proteins [20] and physiological properties of the CA1 neurons [21,22]. Second, the two subregions display distinctive patterns of anatomical connectivity. Specifically, the caudolateral entorhinal cortex projects predominantly to DHip, sending visuospatial information, while the medial band of the entorhinal cortex sends mainly olfactory, visceral and gustatory inputs to VHip [23–26]. With regard to the efferent connections, the DHip projects principally to the retrosplenial and anterior cingulated cortices, two regions involved primarily in visuospatial and memory processing [27–29], and to the medial/lateral mammillary nuclei and the anterior thalamic nuclei, which contain a high number of navigation-related neurons [30–32]. Conversely, the VHip primarily presents massive bidirectional connectivity with amygdalar nuclei [24,25,33] and with periventricular and medial regions of the hypothalamus that are related to motivated behaviors with a strong emotional component [29,34]. Third, studies with single-unit recording techniques have found that dorsal place cells present fields that are small in size and have more stable and spatially selective firing fields than ventral place cells [35–37]. Nonetheless, a recent study showed that individual VHip neurons are sensitive to manipulations in the spatial characteristics of the environment.

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https://doi.org/10.1016/j.bbr.2021.113620
Received 27 May 2021; Received in revised form 17 September 2021; Accepted 3 October 2021
Available online 6 October 2021

Furthermore, the data showed that it is possible to extract high-resolution spatial information from population activity in the VHip [38], suggesting that within the VHip there exists sufficient neural machinery to generate an accurate representation of the environment.

Regarding spatial learning and memory, numerous studies have shown that DHip lesions, but not VHip lesions, impair the acquisition of a reference memory task in the water maze or in appetitively-motivated paradigms [12,39–48]. However, another set of studies, using more sensitive training paradigms, or combining dorsal and ventral lesions in a certain pattern, have shown significant impairment in spatial memory acquisition and/or expression after VHip lesions [[49–59], see also [45] for a deficit in expression]. Thus, there is some controversy as to whether the VHip is necessary in allocentric spatial memory and under what circumstances.

That some studies have observed a certain deficit following VHip lesions while others have not indicates that most likely the type of task and training protocol used are important factors in functionally mobilizing this subregion. In this regard some recent studies that have used tasks requiring a more complex analysis of the environment, for example, navigation in obstacle-rich environments [53] or a context-dependent biconditional discrimination task [52], have observed a clear deficit after VHip lesions. Other studies have detected a deficit in the Morris water maze following VHip lesions in the early stages of training. However, at the end of the training, lesioned and control rats showed similar performances, and it could not be clearly determined whether or not the VHip is essential in spatial learning and memory [54,55]. The foregoing indicates that new tasks and paradigms, ones more sensitive in detecting a deficit in spatial processing, are needed in order to better understand the role of VHip in allocentric learning.

Another set of data supporting a certain VHip function in navigation comes from studies that have combined dorsal and ventral lesions in a certain pattern. Some such studies have shown that rats with DHip lesions presented less of a deficit in spatial reference learning in a water maze than rats with complete hippocampal lesions [60], see also [40]. In line with the foregoing, a recent study, also using the reference version of the water maze, found that small, separate lesions limited to the dorsal or the ventral subregions in mice (affecting, respectively, 18.9% and 28.5% of total hippocampal volume) did not result in any deficit in acquisition of the task. However, the combination of the two subtotal lesions (DHip plus VHip group) did significantly impair acquisition [50]. Finally, further supporting a synergistic integration between dorsal and ventral hippocampus, recent research using the water maze showed that rats with crossed inactivation of the DHip in one hemisphere and the VHip in the contralateral hemisphere, performed the task worse than ipsilateral lesioned or sham-operated animals [51].

The present study is another attempt to explore the contribution of the VHip to spatial cognition. With this aim, and taking into account previous studies showing that the training protocol has a significant influence on hippocampal function [44,53,61–63], in this series of four experiments we modified the type of protocol used and also the time of lesion, pre- or post-learning. In experiment 1 and 2 (pre-learning lesions) the objective was to evaluate the effect of the lesion on acquisition using two different allocentric tasks in a four-arm plus-shaped maze. In experiments 3 and 4 (post-learning lesions) we evaluated whether ventral hippocampus lesions disrupted the retrieval/expression of allocentric (expt. 3) or non-allocentric (expt. 4) spatial information. In order to compare ventral vs. dorsal hippocampal contribution, three groups of rats (dorsal, ventral and sham) were used in all four experiments. Given that in the present series the environmental stimuli, motivational processes and reinforcement aspects of the procedure were identical in all four experiments —the only difference between experiments being the specific training protocol used— it is possible to make direct comparisons of the function of DHip and VHip in different experimental situations. The results indicate that, depending on the training protocol used, the VHip is significantly involved in allocentric memory, acquisition and retrieval/expression, although to a lesser degree than the dorsal subregion.

2. Material and methods

2.1. Experiment 1

Comparison between dorsal and ventral hippocampal lesions in the acquisition of a reference spatial memory task in a four-arm plus-shaped maze.

The main aim of the first experiment was to determine whether this spatial task is hippocampal-dependent and whether its acquisition is differentially affected by lesions to the DHip vs. VHip. The general training protocol followed was essentially the same as the one most often used in the standard version of the water maze, the difference being that this task is appetitively motivated. More specifically, in our case the goal arm occupied the same spatial position at all times and maintained the same relations with the extramaze cues during the entire training. The other three arms of the maze were used as starting arms. In agreement with previous studies using the water maze, we hypothesized that in our task DHip lesions but not VHip lesions would impair learning [39–41,43]. If VHip lesions did in fact impair the learning of the task, they would probably do so in the early stages of the training, as previously shown in the water maze [54,55].

2.1.1. Subjects

The subjects were 33 male Wistar rats from Charles River Laboratories (France), randomly assigned to one of the following four groups: DHip-lesioned (n = 8), VHip-lesioned (n = 8), DHip sham-operated (n = 9) and VHip sham-operated (n = 7). One animal died during surgery. The rats, initially weighing between 270 and 290 g, were individually housed in single polycarbonate cages (480 × 265 × 210 mm, Tecniplast, Italy), maintained at a constant temperature of 22 ± 1 °C and under controlled lighting conditions (light on from 08:00 a.m. to 20:00 p.m.). All experimental procedures were performed during the light phase of the cycle and were in conformity with the relevant European directive (2010/63 EEC) and Spanish legislation (BOE RD 53/2013). The protocol was approved by the Ethics Committee for animal research of the University of Granada (protocol number: 01-CEEA-OH-2013) and by the competent authority of the Regional Government of Andalusia (record number: 31/03/2014/57).

2.1.2. Surgery

Under the effects of sodium pentobarbital anesthesia (60 mg/kg, i.p., Sigma Chemical, St. Louis, Missouri) and buprenorphine (0.1 mg/kg, i.p., Bupaq®, Richter Pharma AG, Austria), the rats were placed in a David Kopf stereotaxic apparatus (mod. 900, David Kopf Instruments, Tujunga, California) with the incisor bar adjusted so that lambda and bregma were level. When necessary the animals were reinjected with a small amount of pentobarbital to maintain the anesthesia until the end of surgery. The lesioned subjects received bilateral injections of N-methyl-D-aspartic acid (NMDA, Sigma Chemical, PBS, pH 7.4, 0.07 M) through the insertion of a 30-gauge stainless steel cannula in eight sites

Table 1
Stereotaxic coordinates for the hippocampal excitotoxic lesions.

Dorsal Hippocampus			Ventral Hippocampus		
AP	ML	DV	AP	ML	DV
+5.9	±1.6	+6.5	+4.5	±4.5	+3.1
+4.8	±2.5	+6.5	+5.7	±5.7	+3.1
+3.8	±3.2	+6.5	+4.5	±4.5	+3.1
+3.0	±4.0	+5.4	+5.7	±5.7	+3.1

Anteroposterior (AP), midline (ML) and dorsoventral (DV) coordinates are in millimeters from the interaural zero point according to the Paxinos and Watson atlas [64].

of the DHip and the VHip in relation to the interaural zero point [64]. Table 1 shows the stereotaxic coordinates used to lesion DHip and VHip. The neurotoxin was administered in a 0.25 μl volume at each site through the cannula attached to a 5 μl Hamilton microsyringe (Teknokroma, Barcelona, Spain). The solution was delivered by a Harvard Apparatus pump set (model 22, Panlab-Harvard Apparatus, Barcelona, Spain), at an infusion rate of 0.1 μl/min. The cannula was left in situ for an additional 5 min before being withdrawn. The control groups underwent identical surgical procedures with one exception, that equivalent volumes of phosphate-buffered saline (PBS) were infused into the dorsal or ventral hippocampus. After surgery, each rat was injected with buprenorphine to reduce post-operative pain (0.2 mg/kg, i.p., Bupaq®, Richter Pharma AG, Austria).

2.1.3. Apparatus

The apparatus used was a black Plexiglas four-arm plus-shaped maze built by the University of Granada Technical Services Department. Each arm of the maze measured 60 cm in length x 10 cm in width and was

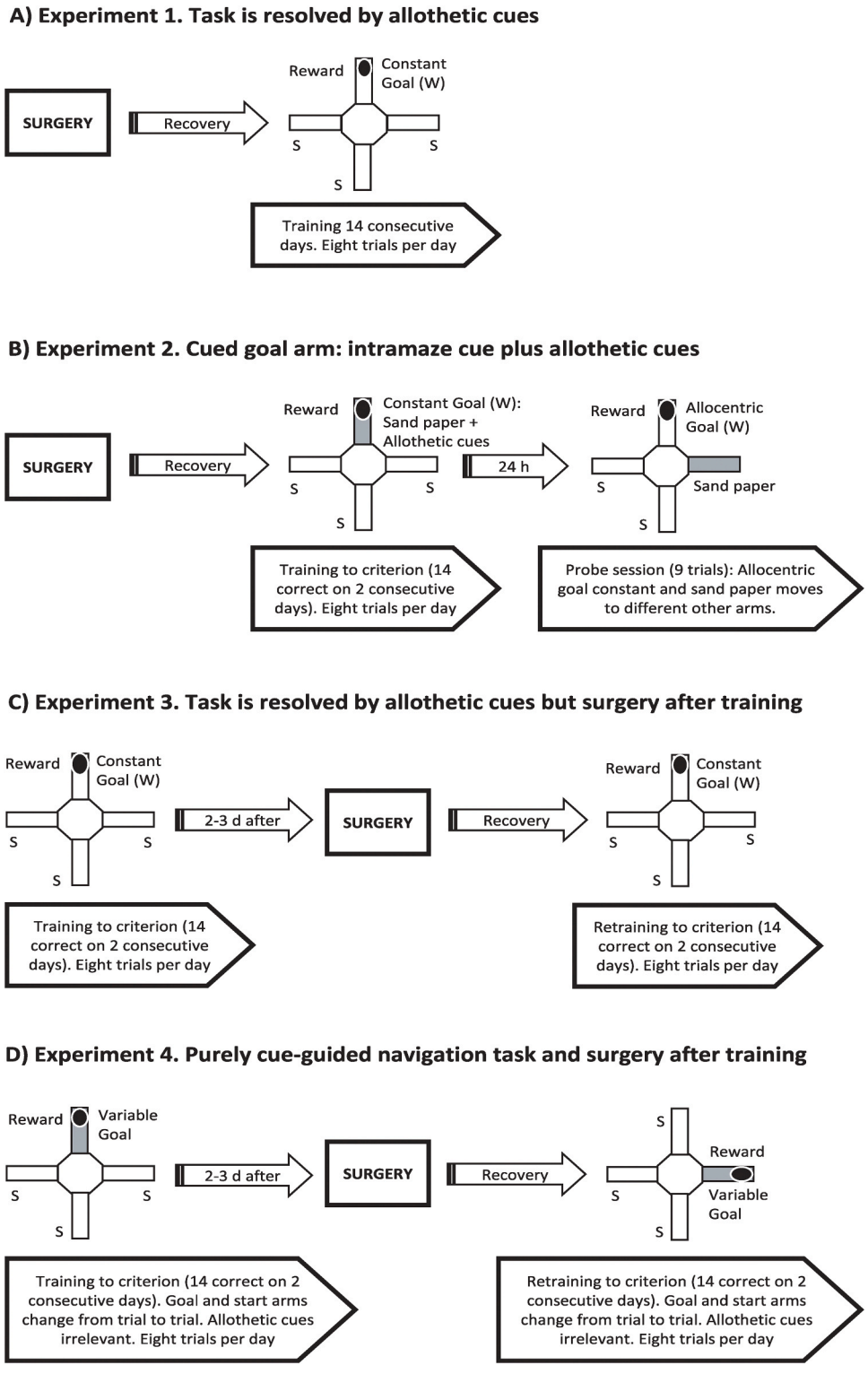

Fig. 1. Overview of experimental procedures. A) Experiment 1. Surgery was performed before training. Rats had to learn a spatial reference memory task in a four-arm plus-shaped maze. The west arm (W) was used as a constant goal arm and the other three arms alternated as starting arms (S). The task is solved by using allothetic cues. B) Experiment 2. Surgery before training. As in expt. 1, the west arm was used as the goal arm and the other three arms alternated as starting arms. However, the goal arm was doubly marked (by sand paper on floor –gray– and the allothetic cues of the experimental room). Twenty-four hours after reaching the learning criterion, rats were given a probe session with 9 trials. During this probe the allocentric goal arm was always baited and the sand paper was moved to the other arms, so place and S-R memories competed. C) Experiment 3. The experimental procedure was the same as for expt. 1 but surgery took place 2–3 d after training. Following recovery, animals were retrained in the same task. D) Experiment 4. Rats learned a purely cue-guided navigation task. In this case the goal arm was indicated by sand paper on floor (gray) and the goal changed from trial to trial. Surgery took place 2–3 d after the learning criterion was reached. Following recovery, animals were retrained in the same task.

connected to an octagonal central platform 35 cm in diameter. The walls of the central platform were 15 cm in height and the walls of each arm measured 5 cm in height. The maze was 80 cm from the floor and was located in the middle of an experimental room measuring 3 × 2.8 m. The distance between the various extramaze cues and the maze itself was therefore quite small. Posters on the wall, a window covered in black adhesive plastic, metal shelves and a cabinet were the main extramaze cues used by the rats to learn the task. A schematic diagram of the maze and cues in the testing room has been presented elsewhere [see [65], Fig. 3]. The maze and the cues in the experimental room were illuminated by two tubes, of 100-W each, placed symetrically on the ceiling and also by one 200-W light bulb hanging from the ceiling 1.2 m above the center of the maze. This provided a high level of illumination (459.8 lux).

2.1.4. Behavioral procedure

The rats were given 10–12 days to recover from the surgery. Following this period, all subjects were put on a food schedule to maintain them at 85–90% of their free-feeding body weight. Beginning on the same day as the food scheduling, all rats were handled on 7 succesive days for 10 min each. On the following day the behavioral training began in the four-arm plus-shaped maze (Fig. 1A). Rats received eight trials per session, one session per day. All rats were trained on 14 consecutive days in order to obtain a learning curve. At the beginning of a trial, the rat was placed at the end of one of the arms used for starting (S, N and E), with its back to the central platform. The order in which the different arms were used for starting was randomized in each daily session. In addition, the frequency with which each animal started from each arm during the training period was the same, and therefore turning left, turning right or going straight was not predictive of reaching the goal arm. During each training trial, two 45-mg food pellets (P.J Noyes Lancaster, NH, USA) were placed in the food cup at the end of the west arm. Identification of the goal arm by smell was prevented by placing five inaccessible 45-mg food pellets under each of the four arms. The pellets were placed at the end of each arm, under the food cup, using adhesive tape and were replaced by fresh ones every 2 days. After a choice was made and the rat passed the mid-way point of the chosen arm with all four of its limbs, the experimenter placed a wooden cube measuring 10 cm × 10 cm x 10 cm just behind the rat. This way the animal was made to stay at the end of the chosen arm for 8–10 s. Then the rat was picked up and confined in a box for an intertrial interval of 30 s. Between trials the maze was rotated 90° in a clockwise direction in order to prevent the animals from using olfactory signals to reach the goal arm. For this reason, the floor of the testing room was marked to assure that the position of the maze remained constant in relation to the room cues. Performance was assessed by percentage of correct responses recorded during each daily session.

2.1.5. Histology

When the behavioral testing was completed, the rats were deeply anesthetized with sodium pentobarbital (180 mg/kg, i.p.) and perfused intercardially with 0.9% saline, followed by 10% formalin. After extraction from the skull, the brains were post-fixed in 10% formalin for several days and subsequently in 10% formalin-30% sucrose until sectioning. Coronal sections (40 μm) were cut on a cryostat (Leica CM 1850, Leica Microsystems, Germany) and stained with cresyl violet, a Nissl stain.

In order to quantify the extension of the damage in each lesioned rat, regions of cell loss and gliosis identified microscopically were plotted on drawings of coronal sections from the Paxinos and Watson atlas [64]. For each DHip-lesioned rat, the reconstruction of the lesion was based on five coronal sections (anteroposterior levels from interaural zero point: +6.4, +5.7, +4.8, +3.8 and +2.9 mm). Each coronal section was digitized and the lesioned area was calculated by a computer program (ImageJ, http://imagej.nih.gov/ij/). The volume of damage was expressed as a percentage, reflecting the amount of lesioned tissue in proportion to the total volume of the hippocampus measured in 3 normal non-lesioned rats. Similarly, for each VHip-lesioned rat, the reconstruction of the lesion was made based on five coronal sections (anteroposterior levels from interaural zero point: +4.7, +4.2, +3.7, +3.2 and +2.7 mm).

2.1.6. Data analysis

The performance of the spatial task over days was analysed using a 2-way mixed design analysis of variance (ANOVA) with group as the between-subject variable and day as the within-subject variable. Post-hoc Tukey tests for the analysis of simple main effects were used where appropriate. All analyses were conducted with the Statistica software 8.0 (StatSoft, Tulsa, Oklahoma).

2.2. Experiment 2

Effect of dorsal and ventral hippocampal lesions on allocentric spatial learning using a doubly marked task in the four-arm plus-shaped maze.

Since the VHip-lesioned rats of experiment 1 did not display an acquisition deficit in the spatial reference memory task in the four-arm plus-shaped maze, in this experiment we modified the training protocol. We made it more sensitive and suited for detecting a possible allocentric impairment [66]. So, in this experiment the animals could choose between two different acquisition strategies to adequately solve the learning situation. Specifically, they could opt for an allocentric/place strategy or an S-R/habit strategy, each of which depends on a different memory system, hippocampus vs. dorsal striatum, respectively [67–70]. Thus, if a learning system has been affected by the lesions, compensation within this system is not necessary because the animal can choose the co-existing parallel strategy during the learning situation. Based on the foregoing, we hypothesized that if VHip lesions produce a deficit in allocentric spatial processing, lesioned rats will select the allocentric strategy to a lesser degree than the control animals. Likewise, VHip-lesioned rats will select the S-R strategy to a greater degree than the control animals.

2.2.1. Subjects

The subjects were 25 male Wistar rats from Charles River Laboratories (France), randomly assigned to one of the following three groups: DHip-lesioned (n = 9), VHip-lesioned (n = 9) sham-operated rats (n = 7). The rest of the characteristics were described above in experiment 1.

2.2.2. Surgery, apparatus and histology

As described experiment 1.

2.2.3. Behavioral procedure

The procedure of experiment 2 was identical to that of experiment 1 except in three aspects (Fig. 1B). First, in experiment 2 two landmarks of different types were present simultaneously during the training and they consistently indicated the location of the goal arm. One landmark was the extramaze constellation of stimuli which allowed the animals to use an allocentric strategy. The other landmark was an intramaze stimulus placed in the goal arm that allowed the animals to opt for a '*guidance*' or S-R/habit strategy [for similar procedures see, [69,71–75]]. The intramaze cue consisted of a piece of sandpaper (roughness reference P50) completely covering the floor of the goal arm. The sandpaper was located in the goal arm (west) during the entire training period. The second difference was that the training of each rat ended when the animal reached a learning criterion of at least 14 correct trials on 2 consecutive days (87%). Third, the day after reaching criterion, in order to determine which learning strategy had been used by each rat, animals underwent a probe test that consisted of 9 trials. During this test, the allocentric goal arm (west) was always baited with two pellets and the animals could choose between the allocentric goal arm or the intramaze

cue that was now placed in one of the remaining arms. In the 9 trials comprising the test, the animals left 3 times from each of the starting arms, in such a way that during 3 trials the allocentric goal (west) was situated to the right of, left of or opposite the starting arm. Likewise, in 3 out of the 9 trials in the test, the intramaze cue was situated to the right of, left of or opposite the starting arm. The order in which the different starting arms were used was randomized and was the same for each rat.

2.2.4. Data analysis

The mean number of errors to criterion were analyzed using one-way analysis of variance. In order to analyze the percentage of correct responses to the allocentric cues and to the intramaze stimulus during the probe test, a 2-way mixed design analysis of variance (ANOVA) was used, with group as the between-subject variable and allocentric and intramaze correct responses as the within-subject variable. Last of all, a 2-way mixed ANOVA with group as the between-subject variable and trials as the within-subject variable was used to analyze the percentage of allocentric correct responses during the nine trials comprising the probe test. Post-hoc Tukey tests for the analysis of simple main effects were used where appropriate.

2.3. Experiment 3

Effect of post-training DHip and VHip lesions on retrieval/expression of allocentric spatial memory.

Few studies have examined the effect of post-training VHip lesions on the retrieval/expression of spatial information. To the best of our knowledge only two studies have addressed this question using neurotoxic lesions [41,45]. Both studies observed a profound impairment after lesions to the two subregions and found that the magnitude of the deficit in VHip-lesioned or DHip-lesioned rats was practically the same, thus showing a clear contribution by the VHip to retrieval/expression. These results are surprising given that the same authors did not find an anterograde deficit following pre-training VHip lesions, even though the same training protocol was used and the lesions were of a similar extension [39,40,45]. So, these data initially suggest that, in neurologically intact rats, spatial memory is retrieved by a widely distributed hippocampal network, with the VHip acting as an essential region.

Despite the foregoing data obtained in the standard version of the water maze, as far as we know no study has looked into the effect of permanent lesions to the VHip in retrieval of spatial memory using an appetitively-motivated task. Therefore, using a spatial reference memory task in the four-arm plus-shaped maze like the one used in expt. 1, the aim of this experiment was to compare the effect of neurotoxic lesions to the DHip and the VHip on the retrieval of allocentric spatial information learned before surgery.

2.3.1. Subjects

The subjects were 32 male Wistar rats from Charles River Laboratories (France), randomly assigned to one of the following four groups: DHip-lesioned (n = 8), VHip-lesioned (n = 8), DHip sham-operated (n = 8) and VHip sham-operated (n = 7). One animal died during surgery. The rest of the characteristics are described above in experiment 1.

2.3.2. Surgery, apparatus and histology

As described in experiment 1.

2.3.3. Behavioral procedure

The procedure of experiment 3 was identical to that of experiment 1 except in three aspects (Fig. 1C). First, during the pre-surgery acquisition period, training ended when each rat reached a learning criterion of at least 14 correct trials (87%) on two consecutive days. Second, 2–3 days after reaching the criterion, the rats underwent stereotaxic surgery (DHip lesions, VHip lesions or sham-operated) following the same surgical procedure as in expt. 1. Third, the animals were given a 10–12 day period to recover from the surgery. To determine if there was a deficit in retrieval/expression, the animals underwent a retraining of the task they had learned prior to surgery. The procedure followed during the retraining phase was identical to that followed during the acquisition period. The retraining ended when each rat attained the criterion of at least 14 correct trials on 2 consecutive days.

2.3.4. Data analyses

In both the acquisition phase and the retraining phase, the dependent variable was the number of errors before reaching criterion. To compare the performance of the different groups a one-way analysis of variance was used. Post-hoc Tukey tests for the analysis of simple main effects were used where appropriate.

2.4. Experiment 4

Effect of post-training DHip and VHip lesions on retrieval/expression of non-allocentric spatial memory.

In the fourth experiment the goal was to clarify whether the retrograde deficit observed in experiment 3 was in allocentric information only or in any type of spatial information. With this objective, intact rats learned a spatial task in the four-arm plus-shaped maze based exclusively on a '*guidance*' or S-R/habit strategy and ventral or dorsal hippocampal lesions were made 2–3 days after learning. Also, this experiment served as a control experiment in which the locomotion behavior and underlying motivation to learn the task was the same as in previous experiments. Based on numerous studies that have suggested that the hippocampal memory system is necessary for the performance of allocentric tasks, but not simple associative tasks [46,63,75,76], we hypothesized that no retrograde deficit would be observed in this experiment following dorsal or ventral hippocampal lesions.

2.4.1. Subjects

The subjects were 32 male Wistar rats from Charles River Laboratories (France), randomly assigned to one of the following four groups: DHip-lesioned (n = 7), VHip-lesioned (n = 8) DHip sham-operated (n = 7) and VHip sham-operated rats (n = 8). Two animals died during surgery. The rest of the characteristics were described above in experiment 1.

2.4.2. Surgery, apparatus and histology

As described experiment 1.

2.4.3. Behavioral procedure

Unlike the earlier experiments, in this one the animals had to learn to navigate to the goal arm using as a guide an intramaze cue whose spatial position changed from trial to trial (Fig. 1D). Therefore, to solve the task successfully the rats had to make use of an S-R association between the intramaze cue and the approach response [69,71,73,75]. In its general aspects the procedure was the same as the one used in expt. 1, except in three points. First, throughout the training during the pre-surgery period, a piece of sandpaper measuring 10 × 60 cm (roughness reference P50) was placed on the floor of the goal arm. In two of the eight daily training trials the goal arm was positioned in the west, in two trials it was in the east, in two it was in the south and in two it was in the north. At the beginning of each trial the animal was placed in one of the three arms that did not contain the sandpaper. The order in which the different goal arms were used was randomized and it was the same for all animals. Also, the relation between the starting arm and the goal arm was controlled in such a way that at the end of the training period (and the post-surgery retraining period) the number of trials in which the goal arm was located to the right, left or opposite the starting arm was the same. This created a situation in which the extramaze information was not relevant and in which it was necessary for the animal to use a '*guidance*' strategy versus an allocentric strategy to effectively solve the spatial problem [68,71,75]. Training ended when each animal reached a learning criterion of at least 14 correct trials on two consecutive days. A

second difference was that 2–3 days after reaching criterion, the rats underwent stereotaxic surgery (DHip lesions, VHip lesions or sham-operated). Third, after a recovery period like the one in previous experiments, the rats received retraining on the '*guidance*' task learned during the acquisition phase, in order to evaluate the retrieval of non-allocentric information. The procedure used during the retraining phase of testing was identical to that of the acquisition phase.

2.4.4. Data analyses

As described in experiment 3.

3. Results

3.1. Histological findings

Dorsal hippocampal lesions. A schematic representation of the hippocampal lesions appears in Fig. 2. The extent of the DHip damage was similar in the four lesioned groups of the present series. The lesion began in the rostral pole of the DHip at the most caudal level of the paraventricular nucleus of the hypothalamus. At this level practically the totality of the hippocampus was lesioned, with the dentate gyrus and all

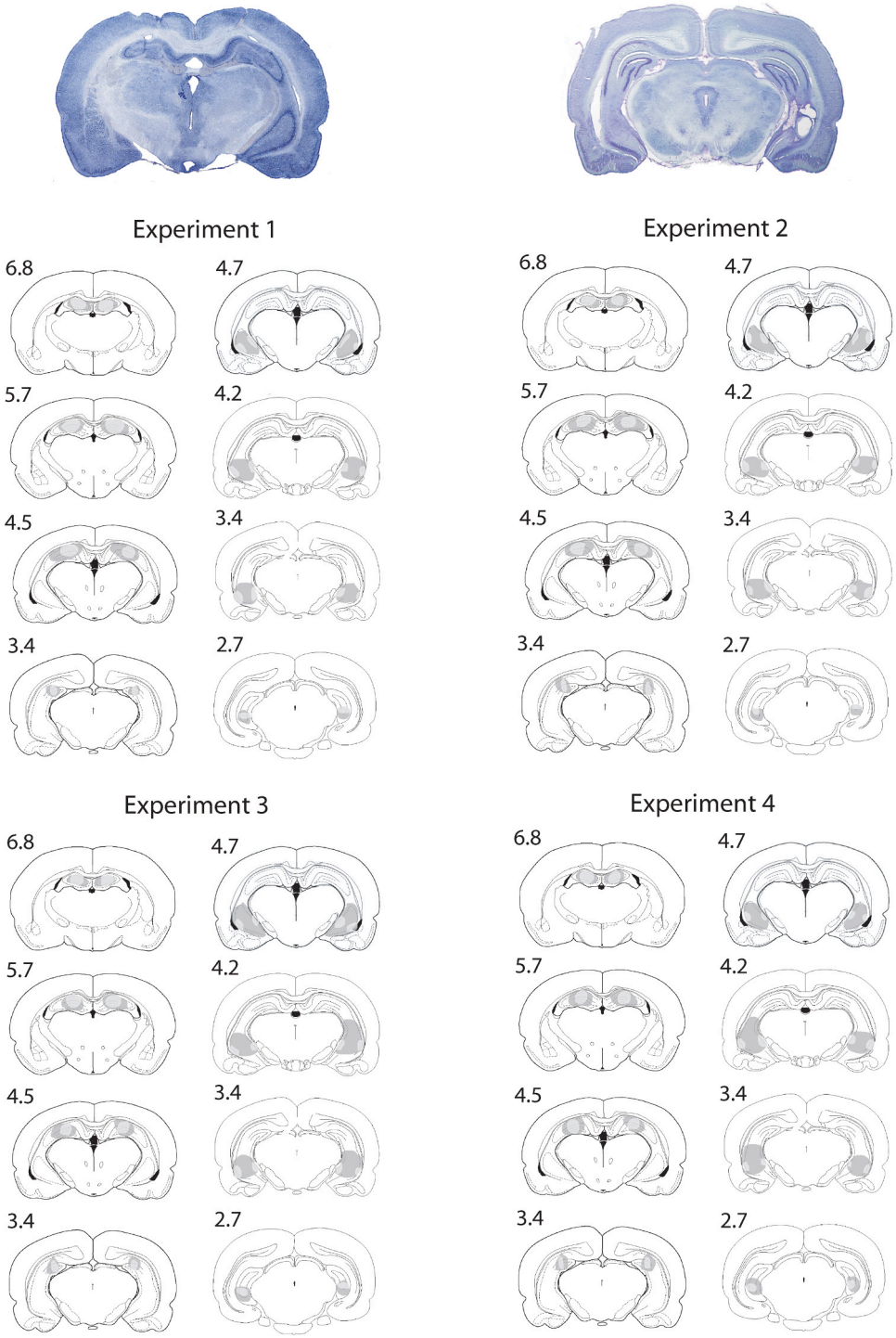

Fig. 2. Photomicrographs showing representative lesions and serial reconstruction of the smallest (central white area) and largest (gray) excitotoxic lesions of the dorsal (left) and ventral (right) hippocampus. AP coordinates are shown in relation to the interaural zero point according to the Paxinos and Watson atlas [64].

CA fields being affected. At more posterior levels, specifically at the level of the mammillary nuclei of the hypothalamus, the lesion had a similar configuration, with extensive zones showing necrosis or missing tissue in the hippocampal CA1-CA3 fields. The most lateral zone of the CA3 field, however, appeared intact in all the rats. At this level, in most cases the dentate gyrus was affected but its most medial region appeared intact to varying degrees in most of the rats. Lesions ended between +3.2 mm and +2.9 mm anterior to the interaural zero point [64], at the beginning of the Sylvius aqueduct. At this level the hippocampal CA1 and CA3 dorsal fields and the dentate gyrus were affected to varying degrees in all the animals. In all the rats the intermediate and the ventral regions of the hippocampus remained completely intact. The amount of lesioned tissue, starting from the dorsal pole, in relation to the total volume of the hippocampus, was 42.8 ± 0.9% (mean ± SEM).

Ventral hippocampal lesions. In relation to the Paxinos and Watson atlas [64], the lesions began +4.8/+4.6 mm anterior to the interaural zero point and ended +2.4/+2.2 mm anterior to this point. In the most rostral region of the lesion, specifically, that coinciding with the most posterior area of the mammillary bodies of the hypothalamus, extensive cell loss and intense gliosis were observed in CA1-CA3 subfields of the VHip in all the animals, with little variation in the extent of the damage (see Fig. 2). The most ventral area of the gyrus dentate, however, was spared. This configuration was maintained throughout all the anteroposterior extension of the lesions. Thus, the amount of lesioned tissue, starting from the ventral pole and in relation to the total volume of the hippocampus, was 35.5 ± 0.8% (mean ± SEM).

Since some studies using a genomic-neuroanatomic approach have divided fields CA1 and CA3 into three distinct domains along the longitudinal axis (dorsal, intermediate and ventral), we attempted to determine the degree to which the ventral lesions had affected the intermediate hippocampus [17,19]. To determine the extent of this area of the lesion, we selected three coronal sections (+4.2, +3.7 and +3.2) of the Paxinos and Watson atlas [64], in which the anteroposterior extension of the intermediate hippocampus is represented. At these levels the intermediate-ventral border is approximately level with the dorsal edge of the rhinal fissure [17, 19; see also 10]. Additionally, although the genetic-anatomic analyses performed by Thompson and associates [17] and by Dong and associates [19] were carried out in C57BL/6 J mice, the three domains correspond approximately to the septal (dorsal), caudal (intermediate) and temporal (ventral) poles defined in the rat based on connectivity data [77]. Histological results indicated that in our four experiments ventral hippocampus damage ranged from 61% to 69% of the total extension as measured in 3 normal non-lesioned rats. Also, the ventral region of the intermediate hippocampus was affected in all the experiments. Specifically, intermediate hippocampus damage ranged from 23% to 29% of its total extension. In one animal from expt. 3 and another from expt. 4 unilateral damage was observed, affecting 59% and 63%, respectively, of the total extension of the intermediate hippocampus. In these rats, however, ventral hippocampus damage presented practically the same extension as observed in the rest of the animals. Finally, no rat showed damage in the adjacent perirhinal or entorhinal cortices, nor in the piriform cortex. Likewise, no damage was apparent in the caudal part of the basolateral and central nuclei of the amygdala. In all rats the septal region of the hippocampus remained completely intact.

3.2. Experiment 1

Fig. 3 depicts the performance of the hippocampal and control groups during the fourteen days of training. An initial 2-way mixed ANOVA showed no significant differences between the two control groups (VHip-sham vs. DHip-sham) over the 14 days the training lasted, except for the factor day ($F_{1, 14}$ group = 2.20, p = 0.16; $F_{13, 182}$ day = 33.04, $p < 0.0001$; $F_{13, 182}$ interaction = 0.70, p = 0.75). These data were therefore pooled to form a single sham group. A 2-way mixed ANOVA to compare the two experimental groups with the pooled control group (3 group x 14 day) revealed a significant effect of group ($F_{2, 29} = 14.67$, $p < 0.0001$, $\eta^2_p = 0.50$), day ($F_{13, 377} = 32.33$, $p < 0.0001$, $\eta^2_p = 0.52$) and interaction ($F_{26, 377} = 3.72$, $p < 0.0001$, $\eta^2_p = 0.20$). Post-hoc Tukey tests to analyze the interaction indicated that the VHip-lesioned group did not differ significantly from the control group on any of the 14 days (p between 0.96 and 1.0). In contrast, DHip-lesioned rats performed the task significantly worse than the controls from day 9 until day 14 of training ($p < 0.00004$, $p < 0.002$, $p < 0.001$, $p < 0.00004$, $p < 0.00004$ and $p < 0.005$, respectively). Importantly, upon comparing VHip vs. DHip groups, Tukey tests showed that dorsal rats performed the task significantly worse than the VHip-lesioned group on day 9 ($p < 0.02$), day 12 ($p < 0.006$) and day 13 of the training ($p < 0.02$). Tukey tests to analyze the group factor also indicated a learning deficit only in the DHip group, but not in the VHip group (DHip vs. sham, $p < 0.0001$; VHip vs. sham, p = 0.25; DHip vs. VHip, $p < 0.007$).

The results of this first experiment are essentially the same as those observed previously by other authors in the water maze task [39,40,45] or using a four-baited/four-unbaited version of the eight-arm radial maze task [43]. For this reason, in the following experiment we modified the training protocol with the aim of isolating a possible deficit in

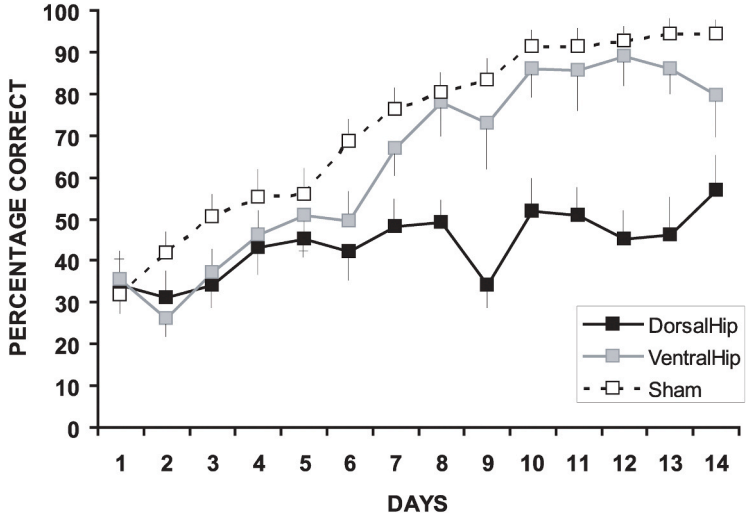

Fig. 3. Experiment 1: Acquisition of a spatial reference memory task in a four-arm plus-shaped maze. Mean (±SEM) percentage of correct responses observed in dorsal hippocampus, ventral hippocampus and sham groups during the 14 days of training.

VHip-lesioned rats.

3.3. Experiment 2

Fig. 4A-C shows the main results of experiment 2 in which an allocentric/place or an S-R/habit strategy could be used to reach the goal arm during the acquisition period. During the training phase, one-way ANOVA did not detect significant differences between the three groups in the number of errors before reaching criterion ($F_{2, 22} = 2.65$, $p = 0.09$; Fig. 4A). To determine which strategy the animals had used to learn the task, during the 9 trials making up the probe test we computed in each rat the mean percentage of correct allocentric responses and the mean percentage of correct responses based on an S-R/habit strategy. Correct responses based on an allocentric strategy were those in which the animals went to the arm that had been used during the acquisition period as goal arm (west), as indicated by the extramaze constellation of stimuli around the maze. The correct responses based on an S-R strategy were those in which the rats went to the arm that contained the intramaze cue. A 2-way mixed ANOVA (3 group x 2 type of strategy) showed a significant effect for group factor ($F_{2, 22} = 5.34$, $p < 0.01$, $\eta^2_p = 0.32$), type of strategy ($F_{1, 22} = 24.63$, $p < 0.0001$, $\eta^2_p = 0.52$) and group x type of strategy interaction ($F_{2, 22} = 15.13$, $p < 0.0001$, $\eta^2_p = 0.57$). These results are depicted in Fig. 4B. To analyze the interaction Tukey tests were conducted, with results indicating that the control rats had a significantly higher percentage of allocentric correct responses than the DHip-lesioned ($p < 0.0001$) and VHip-lesioned rats ($p < 0.03$). Also, upon comparing the two experimental groups, Tukey tests indicated marginally significant differences between DHip vs. VHip groups ($p = 0.06$). Additionally, during the probe test, the percentage of allocentric correct responses in the control group was significantly higher than chance level ($t_6 = 30.88$, $p < 0.0001$), but no significant differences were found when comparing the DHip-lesioned group with chance level ($t_8 = 0.72$, $p = 0.48$). As for the VHip-lesioned group, it occupied an intermediate position between the two preceding groups, showing a performance significantly higher than chance level ($t_8 = 3.69$, $p < 0.006$). Thus, taken together, these data suggest an allocentric deficit in both experimental groups, although DHip-lesioned presented greater impairment than the VHip group.

With respect to the percentage of correct responses to the intramaze cue during the probe test, the pattern of results was just the opposite, as a significantly higher percentage of responses was observed in DHip as compared to sham rats ($p < 0.0009$). Likewise, the data indicated a higher percentage of correct responses in VHip-lesioned vs. sham rats, but with only marginally significant differences ($p = 0.06$). The performance of the VHip vs. DHip groups did not differ significantly as regards the percentage of correct responses to the intramaze cue ($p = 0.36$). Upon comparing the performance of each group to chance level during the probe test, it was found that the percentage of correct responses to the intramaze cue in the control group was significantly lower than chance level ($t_6 = 14.57$, $p < 0.0001$). On the other hand, the DHip group presented a percentage of correct responses to the intramaze cue that was significantly higher than chance level ($t_8 = 2.98$, $p < 0.01$), with the VHip rats occupying an intermediate position, as no significant differences with chance level were observed ($t_8 = 0.11$, $p = 0.90$).

To analyze the allocentric correct responses, trial to trial, during the 9 trials comprising the probe test, a 2-way mixed ANOVA (3 group x 9 trial) revealed that only the group factor was significant ($F_{2, 22}$ group $= 15.73$, $p < 0.0001$, $\eta^2_p = 0.58$; $F_{8, 176}$ trial $= 1.06$, $p = 0.39$; $F_{16, 176}$ interaction $= 1.02$, $p = 0.43$). Interestingly, the fact that neither of the two lesioned groups was able to improve its performance over the 9 trials of the probe session, as the trial factor indicates, reinforces the idea of a profound deficit in allocentric learning in both groups. In addition, upon analyzing the group factor, Tukey tests showed that control rats presented significantly more allocentric correct responses, trial to trial, than the DHip-lesioned ($p < 0.0001$) and VHip-lesioned groups ($p < 0.01$). Similarly, the VHip group showed a significantly better performance than the DHip group ($p < 0.02$) during the probe test (Fig. 4C). Thus, the deficit is greater in the dorsal group than in VHip-lesioned animals.

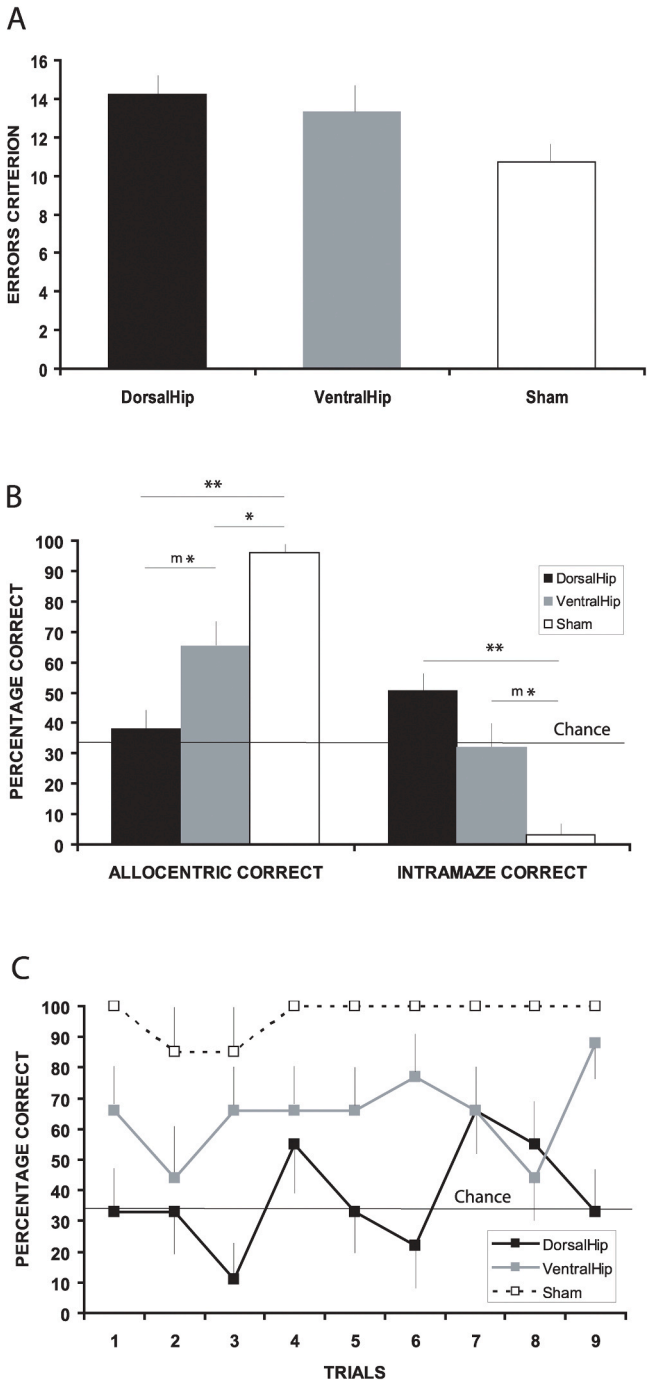

Fig. 4. Experiment 2: Acquisition using a doubly marked task (allocentric simultaneous to S-R/habit strategy) in a four-arm plus-shaped maze. A) Mean (±SEM) number of errors to criterion for dorsal, ventral and control groups during the learning period. B) Mean (±SEM) percentage of allocentric correct responses and of intramaze cue correct responses obtained during the probe test 1 day after criterion. C) Mean (±SEM) percentage of allocentric correct responses in dorsal, ventral and control groups during the nine trials comprising the probe test. Horizontal line in B and C represents chance level. ** from $p < 0.01$ to $p < 0.0001$; * from $p < 0.05$ to $p < 0.01$; m* p marginal ($p = 0.06$).

3.4. Experiment 3

Fig. 5A illustrates the results obtained during the training phase (acquisition) in neurologically intact rats. A one-way ANOVA found no significant differences between the two control groups (VHip-sham vs. DHip-sham, $F < 1$) in the number of errors before reaching criterion. These data were therefore pooled to form a single sham group. Upon comparing the single sham group with the two experimental groups with a one-way ANOVA, no differences were detected in the acquisition of the allocentric task ($F_{2, 28} = 0.01$, $p = 0.98$).

Fig. 5B represents the data obtained during the post-surgical period, when the animals were subjected to retraining. The two control groups were pooled during this phase of testing as well because no significant differences between them were found ($F < 1$). Upon comparing the single sham group with the two lesioned groups, a one-way ANOVA found significant differences between groups in the number of errors to criterion ($F_{2, 28} = 86.41$, $p < 0.0001$, $\eta^2_p = 0.86$). Simple main effects analyses revealed that both the DHip ($p < 0.0001$) and the VHip group ($p < 0.0001$) committed more errors than the controls. Also, Tukey tests showed that DHip-lesioned committed more errors before reaching criterion than VHip-lesioned animals ($p < 0.0001$).

These findings suggest that both the dorsal and the ventral hippocampus are necessary to retrieve allocentric spatial memory when the spatial information was acquired by rats with an intact brain. Such results contrast with those of experiment 1 of our series, in which it was found that only dorsal lesions produced a deficit in acquisition. This pattern of results agrees with the findings of other authors using the water maze [40,41,45]. Taken together, this data suggests that to retrieve/express allocentric spatial memory an extensive neural network is needed, one that encompasses all the hippocampus along the dorsoventral axis. However, the present results indicate that the involvement of the DHip is greater than that of the VHip.

3.5. Experiment 4

Fig. 6A shows the performance of hippocampal and control groups during the pre-surgical acquisition phase. First, a one-way ANOVA did not detect significant differences between the two control groups in the number of errors before reaching criterion, and they were thus pooled in the subsequent analysis (DHip-sham vs. VHip-sham, $F_{1, 13} = 2.12$, $p = 0.16$). Next, a one-way ANOVA found no significant differences between the groups in the number of errors before reaching criterion ($F_{2,}$

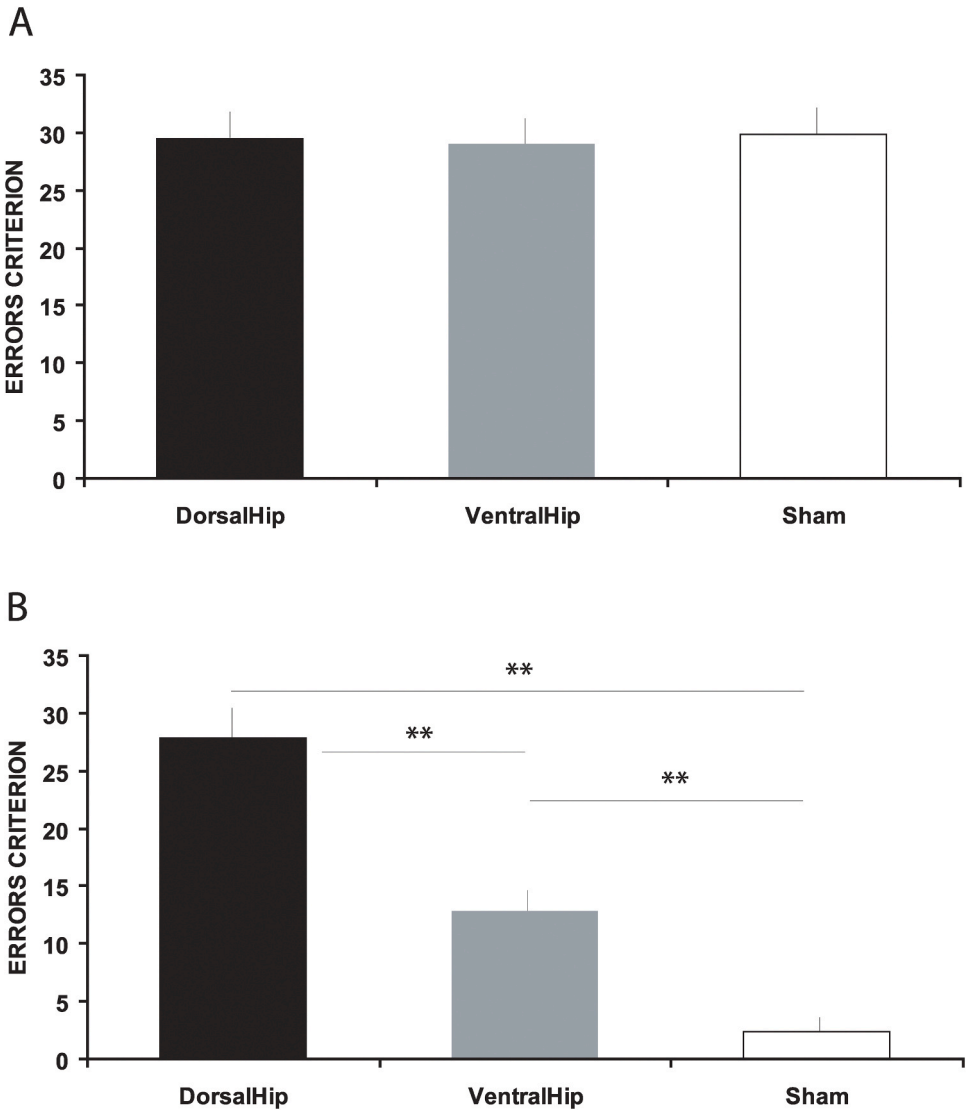

Fig. 5. Experiment 3: Retrieval/expression of presurgically acquired allocentric information in a spatial reference memory task similar to that used in experiment 1. A) Mean (±SEM) number of errors to criterion for dorsal, ventral and control groups during the training phase of testing before surgery. B) Mean (±SEM) number of errors to criterion for dorsal, ventral and control groups during the retraining phase (retrieval/expression) of testing, after recovery from surgery. ** $p < 0.0001$.

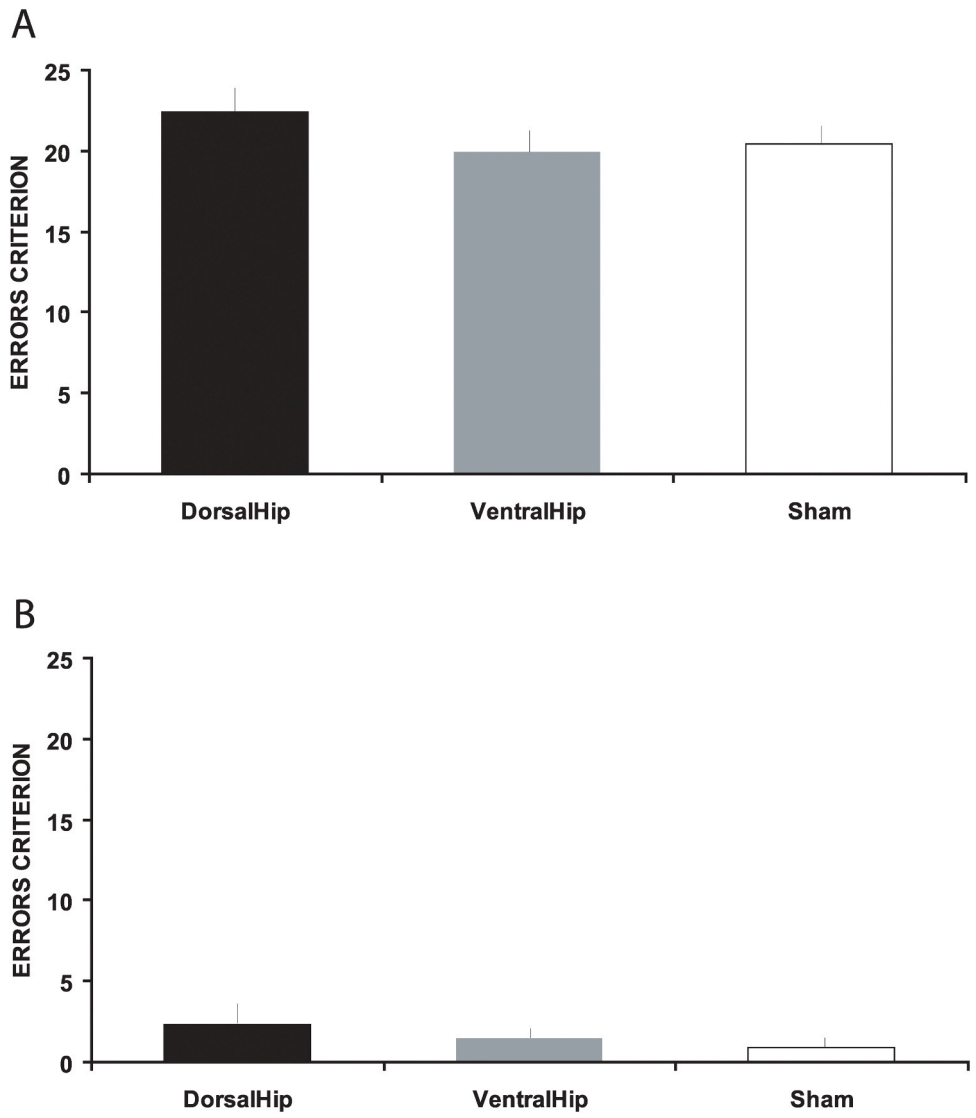

Fig. 6. Experiment 4: Retrieval/expression of presurgically acquired non-allocentric information. A) Mean (±SEM) number of errors to criterion for dorsal, ventral and control groups during the training phase of testing before surgery. B) Mean (±SEM) number of errors to criterion for dorsal, ventral and control groups during the retraining phase (retrieval/expression) of testing, after recovery from surgery.

$_{27} = 0.55$, $p = 0.55$).

Fig. 6B depicts the performance of hippocampal and control rats in the post-surgical retraining phase. A one-way ANOVA revealed that lesioned and control rats did not differ significantly in the number of errors to criterion ($F_{2, 27} = 2.06$, $p = 0.14$). Thus, overall, these results agree with previous studies indicating that neither the DHip nor the VHip is involved in the learning or retrieval of simple associative tasks, and in this regard they support the traditional view of multiple memory systems with different neurobiological bases [70,71,78–80].

4. Discussion

To compare the involvement of DHip vs. VHip in the learning of allocentric spatial information, in two experiments we examined the effect of the hippocampal lesions in two different learning situations, using a four-arm plus-shaped maze in both. In the first experiment, to correctly navigate to the goal arm, animals could only be guided by the extramaze constellation of stimuli around the maze. Results indicated that only DHip lesions disrupted the learning, while the VHip-lesioned and control groups learned the task perfectly and at the same speed. A deficit in VHip-lesioned rats, however, was observed in experiment 2, using a slightly different training protocol. In this case, during the training, the goal arm was doubly marked in such a way that to correctly navigate to the goal the animals could guide themselves by using an allocentric or a *guidance*/S-R strategy. A probe test after acquisition indicated that in the two lesioned groups the percentage of allocentric correct responses was significantly lower than in the controls, although DHip showed a worse performance than VHip animals. In the other two experiments we examined the effect of the hippocampal lesions on the expression of allocentric (expt. 3) and non-allocentric (expt. 4) information learned pre-surgically. The main results showed that both dorsal and ventral lesions produced a clear deficit in expression when the spatial information had been learned using an allocentric strategy, but not when it had been learned using an S-R strategy.

As for the first experiment of our series, the results agree with previous studies that used the water maze [39,40,45] or a four-baited/four-unbaited version of the eight-arm radial-maze task [43]. All of them, unanimously, observed a profound deficit in the acquisition of spatial reference memory in rats with excitotoxic DHip lesions but the absence of deficit in rats with VHip lesions. However, some recent studies in the water maze disagree with the earlier ones, observing certain deficit in ventral lesioned animals in the early stages of

learning, although the deficit disappears after a few days of training [54,55]. One possible interpretation of this transient deficit is that to compensate for a moderate deficit in allocentric processing ventral lesioned rats alternate between different types of strategies at the beginning of the training and took longer to select the most effective strategy.

The idea of a possible deficit in allocentric processing in VHip-lesioned rats is clearly supported by the data of our experiment 2. Numerous studies have demonstrated that during a learning situation, several specialized parallel learning systems compete to gain control over the behavior [68–71,80]. So, in experiment 2 of our series, unlike experiment 1, the training procedure used created circumstances that allowed the navigation task to be learned either by an allocentric or a S-R strategy. Therefore, in these circumstances, an allocentric deficit weakens the competition between these two strategies, reducing the use of the allocentric strategy and increasing that of the S-R strategy [68,70,73]. In effect, in the control animals of expt. 2 results indicate that the allocentric system wins out over the non-allocentric system and, as shown by the data obtained in the probe test, this leads to this system taking over completely during the acquisition and rats acquiring the task using a place strategy. However, in DHip and VHip-lesioned animals such competition is presumably affected by a deficit in allocentric processing. In effect, when the hippocampal lesioned rats were subjected to the probe test in which there was a competition between two types of response, allocentric vs. S-R strategy, the allocentric system loses control over behavior and lesioned rats use the allocentrically-based strategy less frequently than the control rats. In contrast, lesioned animals use the S-R strategy more frequently than the control group during the probe test. One difference between the DHip and VHip-lesioned groups is the degree to which each subregion of the longitudinal axis is involved in allocentric processing. Our data indicate that during the probe test dorsal lesions affect the competition between systems significantly more than ventral lesions, which leads to the dorsal-lesioned animals more frequently using a non-allocentric system to learn the task than the ventral-lesioned group. In addition, the fact that in the probe test VHip-lesioned rats present a significantly lower percentage of correct allocentric responses than the control group indicates that the ventral hippocampus contributes importantly to the building of an integrated and complete spatial representation of the environment.

Recently, some studies have suggested that in tasks with dual place/S-R solution, similar to the ones used in our expt. 2, cooperative interactions between the hippocampus and dorsolateral striatum memory systems occur [79,81–83]. Thus, each type of memory is constructed by its own memory system but, in addition, each memory system seems to be linked to the other in such a way that it is possible to produce a deficit in spatial memory by lesioning the dorsal striatum or a deficit in S-R memory by lesioning the hippocampus [79,84]. Such interaction was not the aim of the present study, as our intervention was limited to the hippocampal system. However, two points must be considered here. First, under the specific training circumstances used in expt. 2, control rats select an allocentric strategy to solve the spatial problem, as shown by the behavior of the animals during the probe session. Second, in the lesioned animals the use of the allocentric strategy decreases and the use of the alternative strategy, that is, the S-R memory system, increases. This suggests that under the training protocol of expt. 2, allocentric processing still depends on the hippocampus and not on the dorsal striatum. In fact, in the DHip group the allocentric performance during the probe session is similar to chance level.

The results and conclusions of expt. 2 concur with previous reports that have suggested a certain contribution by VHip in spatial processing. First, several studies measuring the expression of Arc mRNA or c-Fos along the dorsoventral axis have observed in rats a greater expression of these immediate early genes in DHip than in VHip, in goal-directed navigation tasks or simply in spatial sampling tasks [85–89]. Importantly, although dorsal expression is higher than ventral, ventral pyramidal cells did show behaviorally-induced Arc expression above that of control rats, suggesting an active role of the ventral subregion in spatial processing [see, for example, 85]. Based on the above results some authors have suggested that the granularity of spatial memory representation is greater in DHip vs. VHip [86]. This would imply a progressive spatial scaling gradient in the dorsoventral axis, with the DHip being essential for fine-grained/detailed spatial representations and the VHip being essential for a coarse/global representation of the environment, although, importantly, both subregions would be necessary for a complete and integrated representation of the environment [35–37,85,90]. Second, some studies have presented direct evidence that VHip lesions cause a deficit in the performance of navigation/contextual tasks. However, in those cases, as in our expt. 2, a modification was made in the training method, or in the complexity of the task or the environment [44,52,53,57]. For example, in the study by Wang and Cai [57] an acquisition deficit in the Morris water maze was found after inactivation of ventral hippocampus with muscimol, injected 30 min prior to the beginning of the daily training sessions. But this study used a massive training procedure that lasted only three days, with two daily training sessions consisting of four trials each. Therefore, it may be that under these conditions, in which the animal is asked to learn a task in a short time, the ventral hippocampus makes a significant contribution. These data agree with the results of our expt. 2, suggesting that under certain training circumstances the spatial processing performed by the VHip becomes more necessary than others for solving the task required. Third, other studies have manipulated the extension or the combination of dorsal and ventral lesions, with results indicating a significant contribution of the ventral hippocampus. For example, in one recent study, small separate subtotal lesions to either the dorsal or ventral hippocampus did not impair the acquisition of reference memory in the water maze. In contrast, combining the two subtotal lesions significantly affected learning [50]. In another study, the authors contralaterally inactivated the dorsal and ventral hippocampus, observing a greater performance deficit than when the two subregions were ipsilaterally inactivated [51]. In agreement with our results, the two aforementioned studies suggest that VHip plays a certain role during spatial learning.

Regarding experiment 3, here the results also support the participation of VHip in spatial processing. It is important to recall that the training protocol used in expt. 3 was identical to that used in expt 1, but the two experiments produced opposite results, due to the moment in which the lesions were made, before or after training. This suggests that the VHip is normally not necessary for the learning, storage and retrieval of allocentric information when the training takes place with only the DHip functionally intact, except when special circumstances are present, such as those indicated in expt. 2. However, VHip does play an essential role when animals learn the task with a fully functional hippocampus. Thus, the results of expt. 3 suggest that allocentric processing in an intact brain takes place over a distributed hippocampal network involving dorsal and ventral subregions and in consequence both areas are necessary for retrieval. Retrieval probably involves a hippocampus-prefrontal cortex circuit in order to organize the most appropriate search strategy [91–93]. Specifically, the fact that the principal input from the hippocampus to medial prefrontal cortex has its origin in the VHip suggests that the ventral region has a key function in retrieval/expression [94,95]. Some studies supporting this idea show that the unilateral inactivation of ventral hippocampus in one hemisphere and the inactivation of the medial prefrontal cortex in the contralateral hemisphere impaired the performance and retrieval of spatial discrimination in rats [56,57].

A comparison of the results of expt. 3 and those of expt. 2, obtained during the acquisition period, deserves some extra attention. In effect, as the reader will recall, the control animals of expt. 2 learn the task allocentrically more quickly than the non-operated control animals of expt. 3. This acceleration of the learning can be explained by the intramaze cue used in expt. 2 promoting greater flexibility and variability in the responses of the animals during the training. So, the constant presence of the intramaze cue in the allocentric goal arm during

the whole acquisition process could have discouraged the use of perseverative choices, thus allowing a direct orientation of the animals to the allocentric goal and favoring the quick development of an allocentric style of processing [96,97].

Results of expt. 3 are consistent with previous findings. First, 2-deoxyglucose or immediate early genes studies in rodents have observed increased activity in the dorsal and ventral hippocampus during retrieval of recently learned spatial reference memory [88,91,92]. Second, earlier studies in the water maze have obtained results similar to ours, observing a profound deficit in retrieval when excitotoxic lesions of dorsal or ventral lesions were made following learning [41,45]. Interestingly, our results also agree with a previous study that used reversible inactivation of DHip and VHip in rats performing a spatial reference memory task in the water maze [59]. In that study the authors observed that lidocaine infusions into dorsal or ventral hippocampus before a probe trial impaired retrieval performance; however, pre-training inactivation of the VHip did not prevent task acquisition, while pre-training inactivation of DHip did prevent it. So it is unlikely that the deficits observed in our experiments can be attributed to a possible reorganization of the underlying circuit triggered by our permanent lesions. However, one difference between our study and previous research is that while in the aforementioned studies the retrieval deficit was of similar magnitude in dorsal vs. ventral lesioned rats, in our study a significantly greater impairment was observed in DHip-lesioned, which suggests a greater involvement of this subregion.

In summary, the present results join a growing number of studies that support a significant role for the VHip in the building of a complete representation of both the environment and allocentric navigation. Our results also suggest that the involvement of this subregion probably depends on the characteristics of the task and the complexity of the environment [53,98]. So, the present research agrees with the idea of a progressive functional gradient from the dorsal to the ventral hippocampus as opposed to a dichotomic model based on a strict functional differentiation along the hippocampal longitudinal axis [99–101]. Finally, although our ventral lesions affect predominantly the ventral pole of the hippocampus, the intermediate hippocampus was also damaged, albeit to a lesser degree. For this reason, in future studies it would be interesting to examine the effect of lesions centered mainly in the intermediate hippocampus on navigation tasks similar to the ones used in this study.

CRediT authorship contribution statement

Ignacio Morón: Investigation; Methodology; Funding acquisition (investigator); Revision of the original draft and the revised version. **Juan M. J. Ramos**: Conceptualization; Formal analysis; Funding acquisition (principal investigator); Investigation; Methodology; Project administration; Supervision; Visualization; Writing – original draft; Writing – review & editing.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgements

This work was supported by a grant from the Spanish Subdirección General de Proyectos de Investigación, Ministerio de Economía y Competitividad (Madrid, Spain) and the European Regional Development Fund – ERDF (PSI2013–41098-P). We thank Alexia Weninger for editing the language of the manuscript.

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