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Two strategies used to solve a navigation task: A different use of the hippocampus by males and females? A preliminary study in rats

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There is abundant research (both in rodents and in humans) showing that males and females often use different types of information in spatial navigation. Males prefer geometry as a source of information, whereas females tend to focus on landmarks (which are often near to a goal objects). However, when considering the role of the hippocampus, the research focuses primarily on males only. In the present study, based on Rodríguez, Torres, Mackintosh, and Chamizo's (2010, Experiment 2) navigation protocol, we conducted two experiments, one with males and another with females, in order to tentatively evaluate the role of the dorsal hippocampus in the acquisition of two tasks: one based on landmark learning and the alternate one on local pool-geometry learning. Both when landmark learning and when geometry learning, Sham male rats learned significantly faster than Lesion male animals. This was not the case with female rats in geometry learning. These results suggest that the dorsal hippocampus could play an important role in males only.

The hippocampus seems to play a critical role in many spatial tasks (Eichenbaum, 2017; Good, 2002; O'Keefe & Nadel, 1978; Pearce, 2009; Martin & Clark, 2007). However, there have been few studies that have used

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both sexes to understand the functional role of the hippocampal system (for a review see Koss & Frick, 2017). The present study addresses the involvement of the hippocampus in the acquisition of two strategies used for spatial navigation. Specifically, the two tasks, local pool-geometry learning and single landmark learning, of the Rodríguez, Torres, Mackintosh, and Chamizo (2010, Experiment 2) protocol were used. To this end, male (Experiment 1) and female (Experiment 2) rats were employed.

Experiments on spatial learning and memory have shown that males and females of many mammalian species, including humans, differ in their use of cues for spatial navigation (Chamizo & Rodríguez, 2012; Coluccia & Louse, 2004; Jones, Braithwaite, & Healy, 2003; Mackintosh, 2011). It is not just that males often learn to solve a spatial problem faster than females, but that males and females can use different strategies to solve the same problem. In both rats and humans, males seem more likely to rely on geometrical information to reach a goal, while females are more likely to use landmarks (Choi & Silverman, 2003; Galea & Kimura, 1993; Williams, Barnett, & Meck, 1990; Williams & Meck, 1991). For example, in the pioneer study by Williams et al. (1990) rats were trained in a radial maze and, after they had reached asymptotic performance, they were tested following various manipulations to the geometry of the room or to the landmarks. Provided the geometry of the room was unchanged, males' performance was unaffected by any change to the landmarks, but alteration of the geometry of the testing room disrupted their performance, even when the landmarks were still available for navigation. In contrast, females' performance was disrupted by rearrangement of the landmarks whether the geometry of the room was changed or not, although they were unaffected by the removal of the landmarks provided the geometry of the room was unchanged. Based on this work, a more recent study by Rodríguez et al. (2010 –see also Chamizo, Rodríguez, Sánchez, & Mármol, 2016; Keeley, Tyndall, Scott, & Saucier, 2013; Rodríguez, Mackintosh, & Chamizo, 2013) in which rats were trained in a water maze (i.e., using an aversive procedure instead of an appetitive one like Williams et al., 1990) has shown rather similar effects. These results seem to imply that geometry is more salient for males and landmarks somewhat more salient for females (as subsequent demonstrated by Rodríguez, Chamizo, & Mackintosh, 2011, in a study where cue competition designs were used).

Employing the same apparatus and general procedure as Rodríguez et al. (2010), this new study by Rodríguez et al. (2011) showed an asymmetrical overshadowing effect in both sexes. In males, geometry overshadowed landmark learning, but landmark learning did not overshadow learning about geometry; while in females, landmark learning overshadowed learning about

geometry, but geometry learning did not overshadow landmark learning. It was the more discriminable, salient, or preferred source of information (pool-geometry for males and landmark for females, as shown by Rodríguez et al., 2010) that overshadowed the less discriminable, salient, or preferred cue. The study by Rodríguez et al. (2011) helps to explain, at least partly, a frequently reported failure in the literature of landmarks to overshadow or block learning about geometry in male rats (for a review see Pearce, 2009). Taken together, the clear implication is that the rules that govern learning about the shape of the environment (Cheng, 1986; Gallistel, 1990), or its boundaries (Doeller & Burgess, 2008), are not necessarily different to those that govern learning about landmarks.

The hippocampus is one of the key cerebral structures involved in spatial learning (O'Keefe & Nadel, 1978; Whishaw, 1998; McGregor, Hayward, Pearce, & Good, 2004; Pearce, Good, Jones, & McGregor, 2004; Jones, Pearce, Davies, Good, & McGregor, 2007), particularly the dorsal hippocampus (Bannerman et al., 2014; Oler, Penley, Sava, & Markus, 2008). Although hippocampal lesions do not impair rats' ability to swim to a visible platform in a Morris pool, such lesions have a drastic effect on their ability to swim to an invisible platform (Morris, Garrud, Rawlins, & O'Keefe, 1982; Pearce, Roberts, & Good, 1998; Sutherland, Whishaw, & Kolb, 1983). Unfortunately, when considering the role of the hippocampus in spatial tasks, research has focused primarily on males only. Male predominance in basic research with rodents is generalized, and lately openly criticized (Clayton & Collins, 2014; Collins & Tabak, 2014).

The present study addresses the involvement of the dorsal hippocampus in the acquisition of two learning strategies, two tasks, used for spatial navigation (Rodríguez et al., 2010, Experiment 2) in male and female rats. In the two tasks (landmark, geometry) an escape procedure was used and the critical cue (a specific object in the landmark task, and a particular corner of the pool in the geometry task) along with the platform were rotated from trial to trial. The aim of the landmark learning task was to establish that, when trained in a circular pool to find a hidden platform whose location was defined in terms of a single landmark the rats would learn to locate the platform—the test of such learning being that they would spend more time in a target area than in a control area on a test trial without the platform. The aim of the geometry task was to establish that, when trained in a triangular-shaped pool to find a hidden platform whose location was defined in terms of a particular corner of the pool, the rats would learn to locate the platform—the test of such learning being that they would spend more time in a target area than in a control area on a test trial without the platform. In order to make the hippocampal lesions ibotenic acid was used, following Izaki, Takita and

Akema (2008) procedure [i.e., therefore, the standard method used by Jarrard, (1989) was not employed]. Following surgery, Lesion (hippocampus lesioned) and Sham (surgery without lesion) rats were randomly assigned to one or another task (landmark and local pool geometry −from now on we will refer to this source of information only as geometry) for subsequent training and a final test trial in a water maze (i.e., a escape procedure). Is the integrity of the dorsal hippocampus needed to solve the landmark task and the geometry task? Is it necessary in both males and females? Our aim in the present study was to tentatively answer these questions.

Importantly, we should mention that in previous studies (Rodríguez et al., 2010, Experiment 1; Rodríguez et al., 2011, Experiments 1 and 2a) where the procedure, experimental room, and triangular-shaped pool were the same as those used in the present set of experiments, we examined the possibility that the estrus cycle of females could influence their performance. Before the experiments began, the rats were examined for 8 days to establish the estrus cycle by a daily collection of vaginal smear. During the experiments, they continued to be examined every day, and on test days, they were examined both before and after the experimental session to ensure that they did not change over to the next estrus cycle phase during testing. An ANOVA conducted on the female test data that included the variables of estrus cycle (i.e., high and low level of estradiol) and landmark versus shape revealed no significant effect of estrus cycle on preference for landmark or geometry in any of the experiments (for the same results with a related task see Rodríguez, Aguilar & Chamizo, 2011). Given these null results, we did not measure the rats' estrus cycle in Experiment 2 in order to avoid unnecessarily stressing them.

EXPERIMENTS 1 AND 2

METHODS AND MATERIALS

Subjects. In Experiments 1 and 2 the animals were Long Evans rats (*Rattus norvegicus*) from our own colony, approximately two months old at the beginning of the surgery and three months old at the beginning of the behavioural protocol. The rats were housed in pairs in standard plastic cages (25 x 15 x 50 cm), under a 12:12 h light-dark cycle, with *ad libitum* access to food and water. Working sessions were conducted within the first 8 hr of the light cycle and all rats were always habituated to handling before surgery. The animals were randomly assigned into two groups (i.e., Lesion −hippocampus lesioned− and Sham −surgery without lesion), in the two

learning strategies, geometry and landmark (i.e., Lesion group and Sham group when geometry learning, and Lesion group and Sham group when landmark learning), both in males (Experiment 1) and in females (Experiment 2). Initially, the animals were 20 males (Experiment 1) and 22 females (Experiment 2). However, after the histology analysis to check the validation of the lesions (see the Results section for more details), the number of rats that could be taken into account in each experiment varied.

Experimental procedures were approved by the Local Ethical Committee of the University of Barcelona, following European (2010/63/UE) and Spanish (RD 53/2013) regulations for the care and use of laboratory animals.

Surgery. Following the Izaki et al., (2008) surgery protocol, male rats (Experiment 1) were anesthetized with sodium pentobarbital (60 mg/kg i.p.), in phosphate-buffered saline (PBS) and bilaterally injected in the ventral hippocampus with 5 µg ibotenic acid (Sigma-Aldrich, Spain) in 0.5 µL PBS (Lesion group) or 5 µL PBS for control (Sham group), using a stereotaxic apparatus. However, female rats (Experiment 2) were anesthetized with isoflurane (IsoFlo®, Esteve, Spain) due to sex differences in anaesthesia tolerance (Zambricki & Dalecy, 2004; Westenberg & Bolam, 1982). The previous decision was made after trying the anaesthesia in a preliminary pilot experiment, where nine female rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.) in phosphate-buffered saline (PBS). Seven of the rats were lost in this attempt (i.e., they never woke up from anesthesia).

The lesioning method was identical in Experiment 1 and in Experiment 2. An incision was made in the scalp, and the skin was retracted to expose the skull. A craniotomy was made directly above the target region with a Navfram N-120 micromotor (0.8 mm diameter drill bit, 20.000 rpm) on each side of the skull of every rat, according to the coordinates. Then, rats were injected with one injection in each cerebral hemisphere (Izaki et al., 2008). The injections were of ibotenic acid (Lesion group) or PBS (Sham group) at 0.1 µL⁄min using a 10 µL Hamilton syringe coupled to a 33G needle, at the following coordinates of the hippocampus according to Paxinos & Watson atlas (Paxinos & Watson, 1998): -5.3 mm anterior from bregma, ± 5 mm lateral from the midline and -4.2 mm below the skull. The injection needle was always held in place for 5 min following any injection to avoid fluid reflux and to allow the proper penetration of the injected liquid. Rats were monitored and weighed daily to ensure a proper recovery from the surgery. They were administered a post-surgery analgesic for three consecutive days (i.e., Meloxicam, a dose of 2 mg / kg).

Behavioural protocol. *Apparatus.* Following Rodríguez et al. (2010), the apparatus was a circular swimming pool made of plastic and fiberglass. It measured 1.58 m in diameter, 0.4 m deep and it was filled to a depth of 0.22 m with water rendered opaque by the addition of 1 cl/L of latex. The water temperature was maintained at 22 ± 1°C. The pool was situated in the middle of a large room and mounted on a wooden platform 0.43 m above the floor. On the landmark learning task, a single object (landmark) could be hung from a black false ceiling. When that was the case, it was suspended 35 cm above the surface of the water. The landmark was an empty plastic bottle, covered cylindrically with white paper, which measured 8.4 cm in diameter at the base and 29 cm in height. On the landmark learning task, the single landmark defined the location of the platform. To create the triangular geometry, two acrylic boards forming an angle of 90° were inserted in the pool resting on platforms at the base, which supported them vertically. The boards were 39.5 cm high, 0.5 cm thick and 112 cm long. The boards rose 17.5 cm above the water surface, so that their top coincided with the pool border. On the geometry learning task, the point formed by the corner of the pool with a straight wall to the left and the circular base of the triangle to the right defined the location of the platform. The pool was surrounded by black curtains reaching from the ceiling to the base of the pool and forming a circular enclosure of 2.4 m in diameter. In order to ensure that the rats used these two sources of information (the landmark or the geometry of the pool) to locate the platform, rather than any inadvertently remaining static room cues (like noises from pipes and air conditioning), the landmark (on the landmark learning task), the two boards (on the geometry learning task) and the platform were semi-randomly rotated with respect to the room (90°, 180°, 270°, or 360°) with the restriction that all four positions of the room were used each day. A closed-circuit video camera with a wide-angle lens was mounted 1.75 m above the centre of the pool inside the false ceiling, and its picture was relayed to recording equipment in an adjacent room. A circular platform 0.11 m in diameter and made of transparent Perspex was mounted on a rod and base and could be placed 0.38 m from either the landmark position or the point formed by the corner of the pool with a straight wall to the left, and the circular base of the triangle to the right, on a line that bisected the centre of the pool with its top 1 cm below the surface of the water. The hidden platform (*P*), the landmark (X), and the geometry of the pool were situated as shown in Figure 1, Top panels (left: landmark learning task; right: geometry learning task).

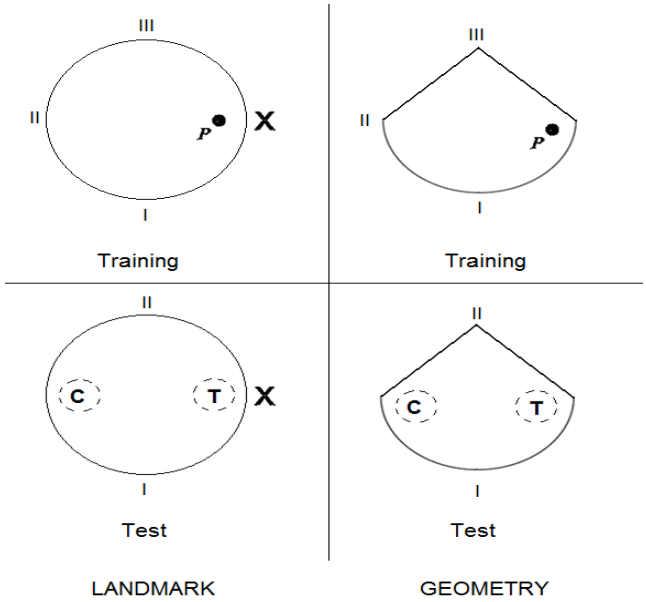

Figure 1. Left-hand panels: Schematic representations of the pool for the landmark task, showing the position of the landmark, X. Top left: for the training trials (showing the hidden platform, *P*, and the starting positions: I, II, and III). Bottom left: for test (where the "T" and "C" circles reflect the target and the control areas, respectively; and I and II the starting positions). Right-hand panels: Schematic representations of the pool for the geometry task. Top right: for the training trials (showing the hidden platform, *P*, and the starting positions: I, II, and III). Bottom right: for test (where the "T" and "C" circles reflect the target and the control areas, respectively; and I and II the starting positions).

Procedure. There were three types of trials: pretraining, training (i.e., landmark learning or geometry learning), and test trials. Pretraining started after the end of the third week of recovery from the surgery. It consisted of placing a rat into the circular pool, facing the wall, without the landmark or boards, but with the hidden platform present. The rat was given 120 s to find the platform, and once the rat had found it, it was allowed to stay on it for 30 s. If it had not found the platform within the 120 s, it was picked up, placed on it, and left there for 30 s. The platform was moved from one trial to the next, and the rat was placed in the pool in a different location on each trial (at I, II, III, and X in Figure 1, Top left), as far as possible equally often on the same or opposite side of the pool from the platform, and with the platform to the right or to the left of where the rat was placed. There were five such pretraining trials spread over 2 days, at a rate of two trials on Day 1, and three trials on Day 2. Rats were run in squads of ten (Experiment 1) or eleven (Experiment 2), and spent the time between trials in small opaque individual compartments. Latencies (time in seconds) to reach the platform were recorded.

The procedure for training was exactly the same as for pretraining, with two exceptions. The landmark, X, was always present for the landmark learning task (half of the rats for the Lesion group, the other half for the Sham group), and the two boards forming the triangular pool were always present for the geometry learning task (half of the rats for the Lesion group, the other half for the Sham group). As in pre-training, the rat was placed in the pool in a different location on each trial (at I, II, and III of Figure 1, top left for the landmark learning task and top right for the geometry learning task). All rats were given eight trials per day over four days (a total 32 trials). These trials had an ITI of 10-12 minutes, and the platform and the cue (either the landmark in the circular pool or the boards that form the triangular geometry) were rotated between trials.

Following training, rats received a test day. This day started with eight training trials (which were identical to the training phase), followed by one test trial without the platform, which was 60 s long (see Figure 1, Bottom panels). The amount of time that the rats spent searching for the platform in two different but identically sized areas (0.22 m in diameter, twice the platform diameter) was recorded in each test. The recording areas, target and control (i.e., T and C in Figure 1, Bottom panels), were 180° apart. In the landmark test the target area was in front of the landmark and in the geometry test, in front of the previously correct corner of the pool. The reason for measuring the time spent in the control area as well as in the target area was to check that on the geometry test rats could discriminate between the two corners of the triangle, and in the landmark test to check whether they were simply swimming in circles at a certain distance from the wall of the pool. On test trials, each rat was placed in the pool from one specific position (at I and II of Figure 1, Bottom panels −left: landmark test trial; right: geometry test trial).

A significance level of .05 was adopted for the statistical tests reported in both experiments. Analysis of variance (ANOVA) were performed as well as standard and Bayesian *t*-tests for pairwise comparisons. When Bayesian *t*-tests were performed we used JASP's default Cauchy prior width, $r = 0.707$ (Jasp Team, 2017).

Histology. In Experiment 1, after the behavioral protocol, rats were deeply anesthetized with an i.p. injection of pentobarbital 60 mg/kg and perfused transcardially with 0.1 M PBS followed by 4% paraformaldehyde (PFA) in PBS at 4ºC. Brains were carefully removed from the skulls and they were post-fixed in 4% paraformaldehyde overnight at 4 ºC, following incubation with 30% sucrose solution for three days. Finally, the brains were frozen and kept at -20 ºC. Exactly the same procedure was repeated in

Experiment 2, although with one exception. After removing the brains, they were stored in dry ice before performing their histology.

Eight coronal, 30 μm thick, hippocampus sections were cut using a cryostat. Sections were mounted on Superfrost slides (Menzel, Germany) and they were stained using a solution of 2.5 g/mL cresyl violet (Nissl staining) in distilled water for 15 min. Then, the slides were subsequently washed in distilled water and dehydrated with 70% ethanol, 95% ethanol and 100% ethanol, respectively (1 min for each reagent). Finally, the slides were dried and cleaned twice with xylene before mounting with DPX. Precision of the lesion was investigated in all rats and only rats with bilateral lesion were included in the analysis.

RESULTS

Validation of ibotenic acid lesions. The method of the validation of the lesion was identical in experiments 1 and 2. Hippocampal stained sections were examined to verify the extent of the lesions. Histology analysis revealed that in Experiment 1 one rat of the Lesion group had no signs of tissue injury in the hippocampus and therefore it was removed from the experiment. Thus, the final sample analysed in Experiment 1 (males) was n=10 sham rats and n=9 hippocampal lesioned animals (specifically, in the geometry learning task, 4 Lesion rats and 5 Sham animals; and in the landmark learning task, 5 Lesion rats and 5 Sham animals). In Experiment 2 (females), the histology analysis revealed that seven rats of the Lesion group had no signs of tissue injury in the hippocampus and therefore they were removed from the experiment. Consequently, the final sample analysed in Experiment 2 was n=10 sham rats and n=5 hippocampal lesioned animals (specifically, in the geometry learning task, 4 Lesion rats and 5 Sham animals; and in the landmark learning task, 1 Lesion rat and 5 Sham animals). Figure 2 shows the final sample of lesioned rats in each experiment (A, 9 males in Experiment 1; B, 5 females in Experiment 2). Notice that the images of each individual rat's lesions are overlayed in each of the eight coronal sections of the hippocampus.

Results and Discussion of the Behavioural protocol: Experiment 1 (male rats). During the course of the five initial pre-training trials, latencies (*SEMs*) to find the platform decreased. Lesion group decreased from means of 108.5 (8.74) s on Trial 1 to means of 49.5 (12.73) s on Trial 5, and Sham group decreased from means of 89.9 (12.50) s on Trial 1 to means of 33.5 (12.15) s on Trial 5. An ANOVA conducted on these data taking into account the variables trials (1-5) and group (Lesion, Sham) showed that the only significant variable was trials, $F(4,68) = 7.08$ ($p < 0.001$, $\eta^2_p = 0.29$). No other

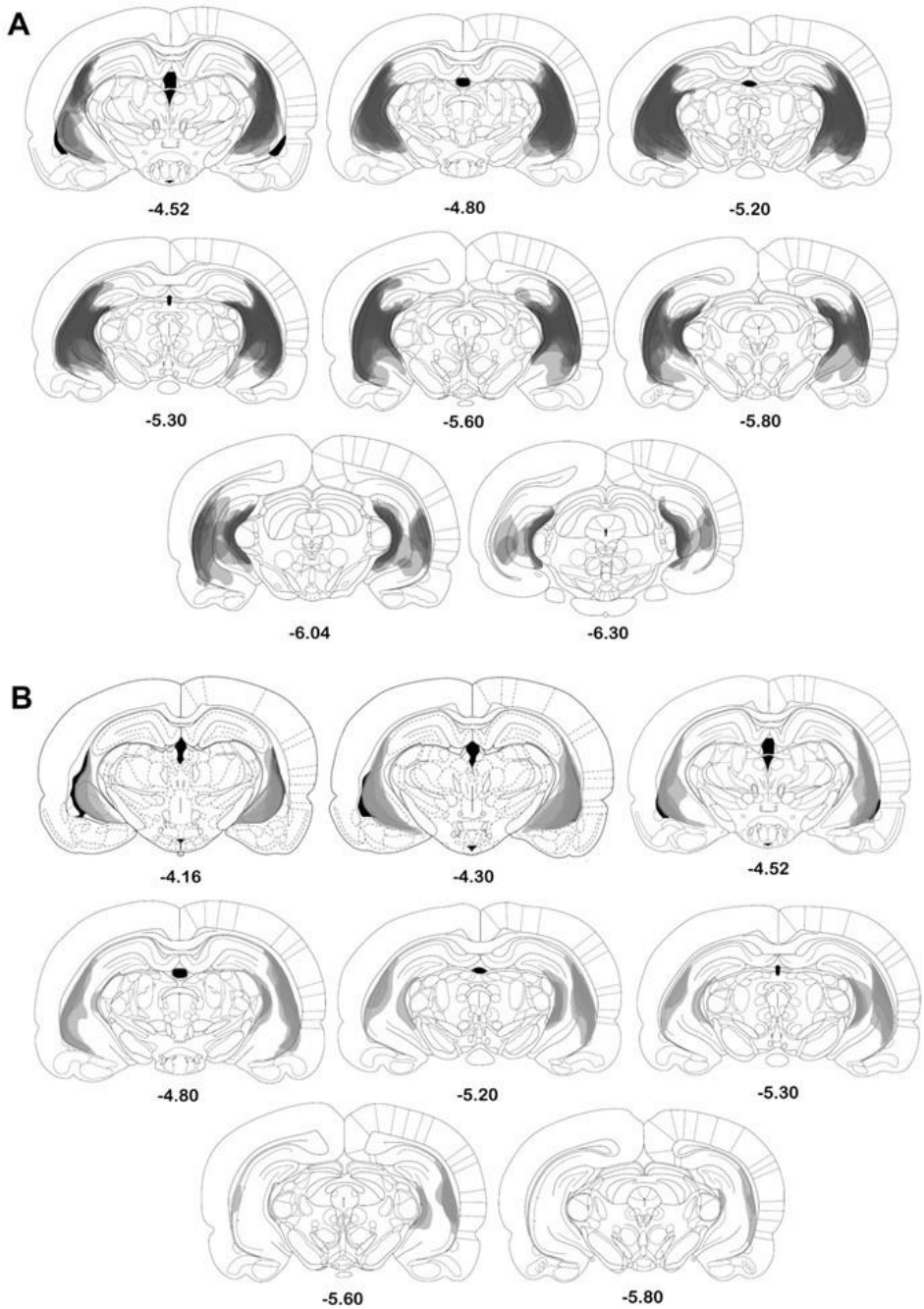

Figure 2. Overlay of the extent of the lesions in eight coronal sections of the hippocampus of the 9 lesioned male rats in Experiment 1 (A) and of the 5 lesioned female rats in Experiment 2 (B). Precision of the injection was evaluated by a cresyl violet staining. The numbers on each drawing are the anteroposterior coordinates (in mm) from bregma of each section.

main effect or interaction was significant ($F < 2.5$). These results reflect that all rats improved their performance as trials went by.

Latencies (*SEM*s) to find the platform also decreased over the course of the training days (Figure 3, Top). An ANOVA conducted on these data taking into account the variables days (1-4), group (Lesion, Sham) and type of cue (geometry, landmark) showed that the variables days, $F(3,45) = 5.72$ ($p = 0.002$, $\eta^2_p = 0.28$), and group, $F(1,15) = 9.93$ ($p = 0.007$, $\eta^2_p = 0.40$), were significant. No other main effect or interaction was significant ($F < 2.5$). These results reflect that all rats improved their performance as days progressed, with Sham rats reaching the platform faster than Lesion animals. An ANOVA conducted on the escape trials of the test day taking into account the variables group (Lesion, Sham) and type of cue (geometry, landmark) revealed that no main effect or interaction was significant ($F < 1.0$). All the rats had learned the task by the test day.

Figure 4 (Top) shows the time spent in the two recording areas (i.e., the target area and the control area) on each test (i.e., landmark and geometry) by the two groups of Experiment 1 during the 60 s of both test trials. Student's within-subjects *t*-tests were used to compare rats' performance in each target area with its control area in order to evaluate whether the test results reflected significant spatial learning in each condition. The time in the target area differed significantly from that in the control area only for group Sham on the geometry test trial, $t(4) = 2.87$ ($p = 0.046$). That was not the case for group Lesion on the geometry test trial, $t(3) = 0.75$ ($p = 0.509$), for group Lesion on the landmark test trial, $t(4) = 2.56$ ($p = 0.063$), and for the group Sham on the landmark test trial, $t(3) = 1.91$ ($p = 0.130$). However, bayesian one-sided *t*-test analysis (Wagenmakers et al., 2017) suggested moderate evidence favouring the alternative hypothesis ($BF_{10} = 3.60$) for group Lesion and anecdotal evidence favouring the alternative hypothesis ($BF_{10} = 2.08$) for group Sham, both in the landmark test trial. Finally, anecdotal evidence was suggested favouring the null hypothesis ($BF_{01} = 1.89$) for group Lesion in the geometry test trial. Thus, it seems sensible to accept that in the landmark test trial both the Lesion group and the Sham group spent more time in the target area than in the control area. The implication is that the two groups (Lesion, Sham) had learned about the landmark cue, but only group Sham had learned about the correct corner −the geometry cue. A final complementary ANOVA on the time spent in the target and control areas on landmark and geometry tests, taking into account the variables area (target, control), group (Lesion, Sham), and type of cue (geometry, landmark), showed that only the variable area was significant, $F(1,15) = 15.23$ ($p = 0.001$, $\eta^2_p = 0.50$). No other main effect or interaction was significant ($F < 2.0$).

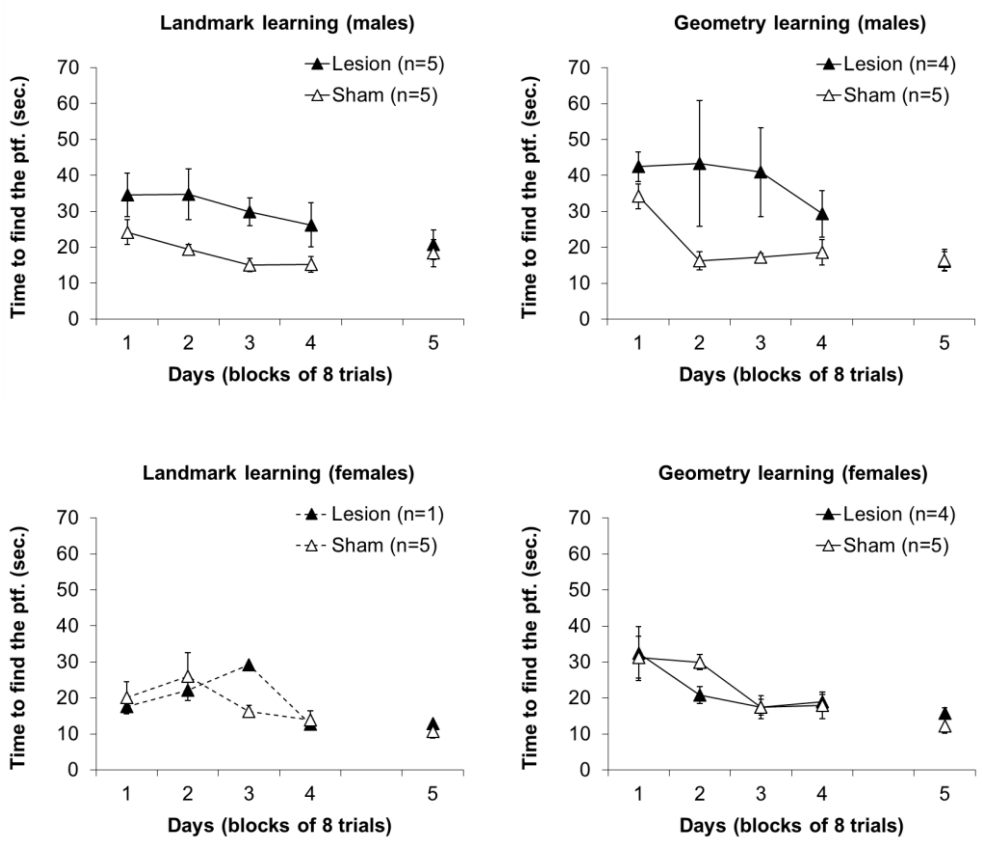

Figure 3. Top: Mean escape latencies for the groups of Experiment 1 (males). Bottom: Mean escape latencies for the groups of Experiment 2 (females). Left-hand panels: For landmark learning task. Right-hand panels: For geometry learning task. Error bars denote standard errors of the means.

In conclusion, the results of Experiment 1 show that Sham male rats learned significantly faster than Lesion male animals, both with the landmark cue and with the geometry cue. Of most importance, the final test trial revealed that Lesion rats had not learned about the geometry cue. The clear implication is that a complete dorsal hippocampus seems to be crucial in the acquisition of the geometry learning strategy in male rats.

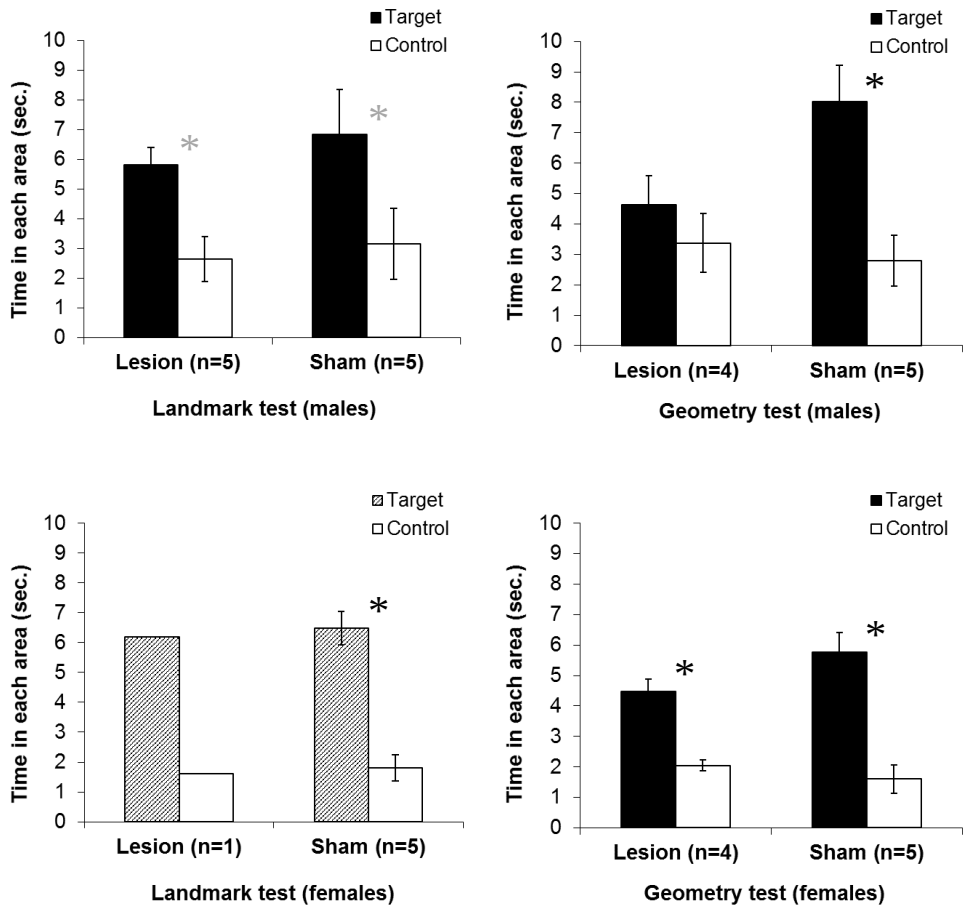

Figure 4. Top: Mean time spent in the two recording areas (target and control) by the subjects in Experiment 1 (males) during the test trials (landmark and geometry). Error bars denote standard errors of the means. Bottom: Mean time spent in the two recording areas (target and control) by the subjects in Experiment 2 (females) during the test trials (landmark and geometry). Error bars denote standard errors of the means.

Results and Discussion of the Behavioural protocol: Experiment 2 (female rats). During the course of the five initial pre-training trials, latencies (*SEMs*) to find the platform decreased. Lesion group decreased from means of 66.2 (22.63) s on Trial 1 to means of 37.6 (16.76) s on Trial 5, and Sham group decreased from means of 109.4 (8.88) s on Trial 1 to means of 36.7 (9.78) s on Trial 5. An ANOVA conducted on these data taking into account the variables trials (1-5) and group (Lesion, Sham) showed that the only significant variable was trials, $F(4,52) = 6.10$ ($p < 0.001$, $\eta^2_p = 0.32$). No other

main effect or interaction was significant ($F < 2.0$). These results reflect that all rats improved their performance as trials went by.

Because unfortunately the groups (Lesion, Sham) that remained in the landmark learning task were totally unbalanced (with a single lesioned rat and five sham animals), the results of the females in the landmark task will be presented but not analysed. Therefore, all the next results will address the geometry learning task. Latencies (*SEM*s) to find the platform also decreased over the course of the training days on the geometry learning task (Figure 3, Bottom). An ANOVA conducted on these data taking into account the variables days (1-4), and group (Lesion, Sham) revealed that the only significant variable was days, $F(3,21) = 4.69$ ($p = 0.012$, $\eta^2_p = 0.40$). No other main effect or interaction was significant ($F < 1.0$). These results reflect that all rats improved their performance as days progressed. An ANOVA conducted on the escape trials of the test day revealed that the variable group (Lesion, Sham) was not significant ($F < 0.5$).

Figure 4 (Bottom) shows the time spent in the two recording areas (i.e., the target area and the control area) on each test (i.e., landmark and geometry) by the two groups of Experiment 2 during the 60 s of both test trials. Student's within-subjects *t*-tests were used to compare rats' performance in each target area with its control area in the geometry test trial. In both groups it was found that the time in the target area differed significantly from that in the control area [Lesion group, $t(3) = 4.35$ ($p = 0.022$); and Sham group, $t(4) = 3.55$ ($p = 0.024$)]. The implication is that both groups had learned about the correct corner. Moreover, an additional Student's *t*-test were used to compare rats' performance in the target and control areas in the landmark test trial, only for Sham Group, $t(4) = 12.40$ ($p < 0.001$). Sham group spent more time in the target than in the control area on the landmark test trial. A final complementary ANOVA on the time spent in the target and control areas on the geometry test, taking into account the variables area (target, control) and group (Lesion, Sham), showed that only the variable area was significant, $F(1,7) = 21.62$ ($p = 0.002$, $\eta^2_p = 0.76$). No other main effect or interaction was significant ($F < 2.0$). All rats spent more time in the target than in the control area on the geometry test trial.

In conclusion, the results of Experiment 2 show that both Sham and Lesion female rats learned equally fast the geometry learning task. Of most importance, the final test trial revealed that both groups (Sham, Lesion) had learned about the geometry cue. The clear implication is that a complete dorsal hippocampus does not seem to be crucial in the acquisition of the geometry learning strategy in female rats.

GENERAL DISCUSSION

In the present study male (Experiment 1) and female (Experiment 2) rats were employed in order to tentatively elucidate what is the role of the hippocampus in the two learning strategies, pool-geometry and landmark, of the Rodríguez et al. (2010, Experiment 2) protocol (see also Chamizo et al., 2016; Keeley et al., 2013; Rodríguez et al., 2011, 2013). As expected, a single injection of ibotenic acid per hemisphere was sufficient to selectively damage the dorsal hippocampus (Izaki et al., 2008). In the two experiments, there was no difference between Lesion and Sham animals in their initial speed of learning to find the hidden platform in the Morris pool during the pre-training, in the circular pool with no landmark present (i.e., in the absence of the two cues subsequently used during the training phases). This could suggest both in males (Experiment 1) and in females (Experiment 2), that Lesion rats are no more likely than Sham animals to spend time exploring the pool rather than swimming directly to the platform.

In Experiment 1, however, this was not the case in the presence of the target cues (geometry, landmark), in the two learning tasks. When male rats had to find the platform whose location in the pool was indicated only by a landmark next to it (i.e., in the landmark learning task), Sham and Lesion animals differed in their latencies to reach the platform, as was also the case when the animals were trained in a triangular-shaped pool to find the platform, whose location was defined now by a particular corner of the pool (i.e., in the geometry landmark task). Therefore, both when landmark learning and when geometry learning, Sham male rats learned significantly faster than Lesion male animals. These results show that the hippocampal lesion altered the rate of conditioning (i.e., the speed at which males' localized the hidden platform by means of the two cues, the landmark cue and the geometry cue).

In Experiment 2 Sham and Lesion female animals did not differ in their latencies to reach the platform in the geometry learning task. This result suggests that, unlike what happened in males, the hippocampal lesion did not seem to affect females' ability to localize the hidden platform by means of the geometry cue. Following training, all the rats were tested, without the platform, to the target cue (landmark or geometry). In Experiment 1, when tested with the landmark cue both groups (Lesion, Sham) spent more time in the target area than in the control area, thus reflecting landmark learning. That was not the case when tested with the geometry cue. Only group Sham spent more time in the target area than in the control area, thus reflecting geometry learning based on the correct corner of the pool. Therefore, the control exerted by the different cues (geometry and landmark) was different in the

lesioned male rats. On the contrary, in Experiment 2 the control exerted by the geometry cue was the same in the two groups (Lesion, Sham). All female rats spent more time in the target area than in the control area, thus reflecting both groups the same geometry learning based on the correct corner of the pool. What could the Lesion males from Experiment 1 have learned? Because the platform was always located at a fixed distance from the walls of the pool, it could be argued that the rats could have extracted very precise information about the distance of the hidden platform from the walls or corners of the pool. Therefore they could have learned to swim at a certain distance from the walls. This strategy always results in the platform being found. The main implication of the present experiments is that the use of the dorsal hippocampus, mainly while learning the geometry task, could be influenced by the rats' sex, being more important in males than in females.

Several features of our results make us think about previous work by Roof, Zhang, Glasier, & Stein (1993) on a working memory task in a Morris water maze. In this study male and female rats were used. Roof et al. (1993) found that unilateral lesions of the entorhinal cortex (EC), the main input region to the hippocampus, resulted in a differential performance in males and females. Specifically, males' performance was drastically impaired whereas females displayed only a slight deficit, independently of the size of the lesion. These results already suggested that the role of both the hippocampus and EC in spatial learning seems to be different in males and females. Roof et al. (1993) ended their work by claiming that "Whatever the mechanism underlying the recovery or lack of impairment in females, it is clear from these results that more attention must be paid to the study of gender differences in determining or specifying structure-function relationships in the central nervous system. It is also clear that the issue of gender must be given more weight in planning and developing treatment and rehabilitation strategies for brain-damaged patients" (p. 50). Unfortunately, at present, most basic research is still conducted with males only.

Other experiments in the literature have shown that male rats with lesions in the hippocampus can learn about different landmarks (McGregor et al., 2004; Morris et al., 1982; Rice, Wallace, & Hamilton, 2015), usually a visible platform or an object, suspended from a false ceiling, indicating the location of the goal ─in the second case, a hidden platform. With regard to geometry learning, the existing literature is more controversial (Cheng, 1986, 2008). The neural domains that the geometry processing seems to require are not well understood yet. The difficulty of hippocampus-lesioned male rats to learn about the geometry of the environment found in the present Experiment 1 is also reported in other studies. For example, some authors have found that hippocampal lesioned rats were impaired when discriminating long versus

short walls of a rectangular pool (McGregor et al., 2004; Pearce et al., 2004) and that they could not discriminate a right-angled corner from its mirror image (Jones et al., 2007). In humans, activation of the hippocampal formation appears in boundary-based learning, rather than in landmark-based learning (Bird, Capponi, King, Doeller, & Burgess, 2010; Doeller & Burgess, 2008). Both the rat and the human data seem consistent (mainly with male participants) with the claim that the hippocampus may play a role in geometry learning but it is less clear in landmark learning. The results of Experiment 1 agree with these statements. Research with hippocampal lesioned female rodents is scarce (although see Roof et al., 1993).

There is one difference worth noting between the present protocols (based in Rodríguez el al., 2010, Experiment 2) and other related studies (like Williams et al., 1990). In the present experiments, the landmark and the correct corner of the pool could be regarded simply as beacons which the rats learned to approach (i.e., beacons are "objects" situated so near the goal that the animal simply has to perceive them in order to locate the goal), whereas in the Williams et al. (1990) experiment the landmarks and the geometry of the room could hardly have acted as beacons. The implication is that as an animal makes its way to the hidden platform in the present experiments, the swimming response, an instrumental response, could be elicited by a specific stimulus, the beacon (either the landmark or the correct corner of the pool). Therefore, the rats could learn an instrumental stimulus-response (S-R) association, or response learning. There is evidence suggesting that hippocampal lesions have a minor impact on the formation of these S-R associations (Jones et al., 2007; McGregor et al., 2004; Packard & McGaugh, 1996). Packard and McGaugh have shown that the hippocampus and caudate nucleus selectively mediate expression of place and response learning, respectively. The results of Experiment 2 suggest that this possibility could be more important for females (who are more caudate-dependent animals) than for males (who are more hippocampal-dependent animals), thus explaining, at least partly, the different results in the two experiments of the present study when geometry learning. Further research should address if the caudate nucleus is compensating a decreased hippocampal function in the females. Research in rodents has suggested that the caudate nucleus and the hippocampus can function independently (Packard & McGaugh, 1996; White & McDonald, 2002 −in human navigation see Bohbot, Del Balso, Conrad, Konishi, & Leyton, 2013; Iaria, Petrides, Dagher, Pike, & Bohbot, 2003).

Admittedly, Experiments 1 and 2 have many things which are not common. Firstly, the male rats were anesthetized with sodium pentobarbital, while female rats were anesthetized by means of spontaneous inhalation of isoflurane. Secondly, a close look at Figure 2 clearly shows that the lesions

in males and females do not overlap (in other words, that a slightly different area of the posterior hippocampus has been lesioned in both sexes). Finally, unfortunately the number of animals in each lesioned group is different: 9 males (Experiment 1) versus 5 females (Experiment 2), with the result that we have to ignore the landmark learning task in the females. All these differences could be affecting the present results. Of most importance, a direct comparison between the two sexes, necessary for strong conclusions, was precluded here by their data coming from separate experiments. A sex difference in spatial learning has often uncertain results. However, males seem more likely to rely on geometrical information to reach a goal, while females are more likely to use landmarks. The exact reasons implicated are still a matter of debate. But the present research goes beyond the work in this literature by showing that the use of the hippocampus by males and females could be different. If that is the case, the previous statement has a myriad of implications (Konishi & Bohbot, 2013; Li & Singh, 2014; Persson et al., 2017; Will et al., 2017) that can by no means be ignored any longer.

RESUMEN

Dos estrategias que se utilizan para resolver una tarea de navegación: ¿un uso diferente del hipocampo en machos y en hembras?. Un estudio preliminar con ratas. Hay mucha investigación (tanto en roedores como en humanos) que muestra que los machos y las hembras a menudo usan diferentes tipos de información en la navegación espacial. Los machos prefieren la geometría como fuente de información, mientras que las hembras utilizan más los puntos de referencia (que a menudo son objetos que se encuentran cerca de una meta). Sin embargo, al considerar el papel del hipocampo, la investigación se centra casi exclusivamente en los machos. En el presente estudio, basado en el protocolo de navegación de Rodríguez, Torres, Mackintosh y Chamizo (2010, Experimento 2), llevamos a cabo dos experimentos, uno con machos y otro con hembras, para tener una primera aproximación del papel del hipocampo dorsal en la adquisición de dos tareas: una basada en el aprendizaje en base a un punto de referencia y la otra basada en el aprendizaje de un vértice de la piscina (aprendizaje de la geometría). Tanto en el aprendizaje basado en el punto de referencia como en el aprendizaje de la geometría, las ratas de control macho aprendieron significativamente más rápido que los machos lesionados. Este no fue el caso con las ratas hembra. Estos resultados sugieren que el hipocampo dorsal podría jugar un papel importante solo en los machos.

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