

## **Landmark vs. geometry learning: Explaining female rats' selective preference for a landmark**

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Rats were trained in a triangular-shaped pool to find a hidden platform, whose location was defined in terms of two sources of information, a landmark outside the pool and a particular corner of the pool. Subsequent test trials without the platform pitted these two sources of information against one another. In Experiment 1 this test revealed a clear, although selective, sex difference. As in previous experiments, females spent more time in an area of the pool that corresponded to the landmark, but here only when it was a cone but not when it was a pyramid. Males, on the other hand, always spent more time in the distinctive corner of the pool. Experiments 2 and 3 were only with female rats. In Experiment 2 two identical shaped cylinders were used as landmark cues (one plain white and the other vertically patterned with four different patterns). The results of the preference test revealed that only the females trained and tested with the plain cylinder spent more time in the area of the pool that corresponded to the landmark than in the distinctive corner of the pool. Finally, Experiment 3 replicated the results of Experiment 2 while eliminating an alternative explanation in terms of differential contrast between the two cylinders and the black curtain.

Spatial tasks, such as mazes and the Morris pool, frequently employ different cues, for example, three-dimensional objects or landmarks and the shape of the room or apparatus. These sometimes produce unexpected results, for example qualitative sex differences (Rodríguez, Torres, Mackintosh, & Chamizo, 2010; Rodríguez, Chamizo, & Mackintosh, 2011;

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Williams, Barnett, & Meck, 1990). In the Rodríguez et al. study (2010, Experiment 2), two groups of male and female rats were trained in a triangular-shaped pool to find a hidden platform, whose location was defined in terms of two sources of information: a landmark outside the pool, a ninepin, and a particular corner of the pool with an unusual triangular shape (this particular corner had a straight wall to the left, and a circular wall to the right, and we refer to this throughout this paper as a geometrical cue). After acquisition, three test trials were conducted, without the platform: a preference test (where the two sources of information were pitted against one another) and two single-cue tests (where the two cues were presented individually). On any test trial there were two recording areas and the time the subjects spent in these two areas was measured. On the preference test, a clear sex difference was found: males spent more time in the distinctive corner of the pool, while females spent more time in the area of the pool next to the landmark, even although the single-cue tests revealed that both males and females had learned about the two cues. Moreover, a clear male advantage on geometrical learning was also found. These results imply that geometry is more salient for males, and landmarks for females. Subsequent related work by Rodríguez et al. (2011), where cue competition designs were used, confirmed this claim by showing that overshadowing was asymmetrical, both in males and in females. In males, geometry learning overshadowed landmark learning, but not vice-versa; while in females, landmark learning overshadowed geometry learning, but not vice-versa.

The studies by Rodríguez et al. (2010, 2011) imply that the geometry of the pool is more salient for males while the landmark cue is more salient for females, and subsequent work in our laboratory has confirmed this finding in adult male and female rats –but not in juvenile rats or ovariectomized females (Rodríguez, Chamizo, & Mackintosh, 2013). These results are in agreement with previous findings showing that male and female rats do not always use the same cues when solving spatial tasks (Williams, Barnett, & Meck, 1990; Roof & Stein, 1999; Hawley, Grissom, Barratt, Conrad, & Dohanich, 2012), and could have important implications concerning the way they perceive and represent the world. Interestingly, there are analogous data from human participants (Dabbs, Chang, Strong, & Milun, 1998; Chai & Jacobs, 2009; Lövdén, Herlitz, Schellenbach, Grossman-Hutter, Krüger, & Lindenberger, 2007). We were therefore surprised by the results of an unpublished study in our laboratory which found that females showed no preference for the landmark over the geometry provided by the pool when the landmark was a cube, rather than the ninepin or beach ball used in our other experiments. Since the cube was

approximately the same size as the beach ball, it did not seem likely that it was markedly less salient than the ninepin, so what was the critical difference?

Importantly, we should mention that in previous studies (Rodríguez et al., 2010, Experiment 1; Rodríguez et al., 2011a, 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 the present experiments in order to avoid unnecessarily stressing them.

## **EXPERIMENT 1**

In order to see whether females' preference for the landmark over geometry depends on the nature of the landmark, the first requirement is to provide a direct comparison of different landmarks. In Experiment 1 we used two different landmarks, a cone and a pyramid. The choice of these two landmarks was based on the tentative assumption that the critical feature of the cube that distinguished it from the ninepin and beach ball (or equally from a cone) is that it contains edges and corners. If this assumption is correct, then we should expect that rats trained and tested with the cone would replicate the results obtained by Rodríguez et al. (2010, 2011), while female rats trained and tested with the pyramid might show no preference for the landmark when landmark and geometry were pitted against each other.

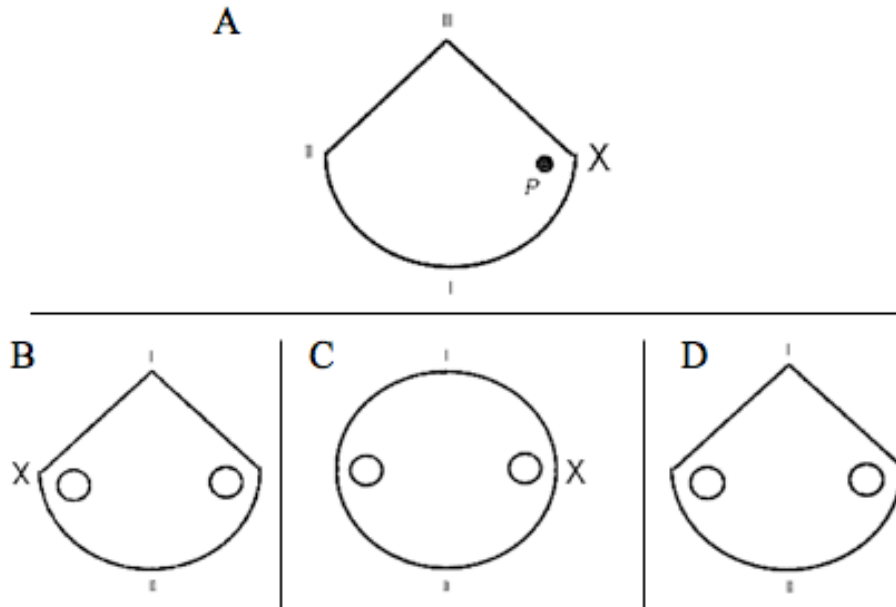
## **METHOD**

**Subjects.** The subjects were naive Long Evans rats: 10 males and 10 females, approximately three months old at the beginning of the experiment. The animals were housed in standard cages, 25 x 15 x 50 cm, in

groups of two and were maintained on *ad lib* food and water, in a colony room with a 12:12-hr light-dark cycle. They were tested within the first 8 hrs of the light cycle.

**Apparatus.** The apparatus was a circular swimming pool made of plastic and fibreglass and modelled after that used by Morris (1981). It measured 1.58-m in diameter and 0.65-m deep, and it was filled to a depth of 0.49-m with water rendered opaque by the addition of 1 cl/l of latex. The water temperature was maintained at  $22 \pm 1^\circ\text{C}$ . The pool was situated in the middle of a large room and mounted on a wooden platform 0.43-m above the floor. To create the triangular geometry, two acrylic boards forming an angle of  $90^\circ$  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 top of the boards was 9.5 cm above the water surface, i.e., at the same height as the outer wall of the pool. The pool was surrounded by black curtains reaching from the ceiling to the base of the pool and forming a circular enclosure 2.4-m in diameter. A single object, landmark X, was suspended from a black false ceiling inside this enclosure, 35-cm above the surface of the water and with its mid-line directly above the wall of the pool. For half of the subjects (i.e., five males and five females) landmark X was a white pyramid of 25 cm each side. For the other half, landmark X was a cone with black and white stripes, 58 cm tall and whose base was 16.5cm in circumference. The stripes were 1 cm wide at the base and narrowed to a point at the top of the cone. The single landmark X, as well as 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. In order to ensure that the rats used these two sources of information (the landmark and 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, the two boards and the platform were semi-randomly rotated with respect to the room ( $90^\circ$ ,  $180^\circ$ ,  $270^\circ$ , or  $360^\circ$ ) 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 which was placed 0.38-m from 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 center of the pool, with its top 1-cm below the surface of the water. The hidden platform, P,

landmark X, and the geometry of the pool were situated as shown in Figure 1A.



**Figure 1.** A schematic representation of the pool and the position of the landmark, X, as well as the hidden platform (P). A) for acquisition; B) for Preference test; C) for Learning test with the landmark and the circular pool; D) for Learning test with the geometry of the pool and without the landmark.

**Procedure.** There were three types of trials: pretraining, training, and test trials. Pretraining consisted of placing a rat into the circular pool 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, 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. Rats were given five such pretraining trials over two days, with two trials on Day 1, and three on Day 2. Rats were run in groups of ten and spent the intertrial interval (ITI) in small individual compartments.

The procedure for training was similar to that of pretraining with two exceptions. The landmark, X (either the pyramid or the cone), was always present, as well as the two boards forming the triangular pool, as shown in Figure 1A. As in pretraining, the rat was placed in the pool in a different location on each trial, as far as possible equally often with the platform to the right, to the left or in front of where the rat was placed (at I, II, and III of the previous figure). Rats were given eight trials per day over five days (a total of 40 trials). These trials had an ITI of 8-10 minutes, and the platform, landmark, and triangular geometry were rotated between trials.

There were three consecutive test days, each starting with eight training trials (identical to the training phase), followed by one test trial without the platform. Test trials were always 60 s long. On one test trial the two sources of information, the landmark and the triangular geometry, were presented 180° apart, as shown in Figure 1B. The amount of time the rat spent in two different areas (each of them 0.22-m in diameter – twice the hidden platform diameter), one in front of the landmark and one in front of the correct corner, was recorded. Each rat was placed in the pool from one specific position (at I and II only, as shown in Figure 1B). In the other two test trials the rats were tested in the circular pool with the landmark or in the triangular-shaped pool with no landmark (Figures 1C and 1D, respectively). These three different test trials were counterbalanced over the three days. The amount of time that the rats spent in the two different but identically sized areas (i.e., the target area close to either the landmark or the previously correct corner and a control area 180° apart, see Figures 1C and 1D) was recorded in each test. The reason for measuring the time spent in the control area as well as the target area was to check whether the geometry test rats could discriminate between these two corners of the triangle, and whether on the landmark test they were simply swimming in a circle at a certain distance from the wall of the pool.

An alpha level of .05 was adopted for all statistical analyses.

## RESULTS AND DISCUSSION

Latencies (*sem*) to find the platform decreased over the course of the 5 initial pretraining trials (see Table 1). An ANOVA conducted on these data taking into account the variables trials (1-5), group (Pyramid, Cone), and sex showed that the only variable significant was trials,  $F(4,144) = 9.30$ . No other main effect or interaction was significant ( $F_s < 1.5$ ).

**Table 1. Latencies (sem) to find the platform (sec.) during pretraining (trials 1 and 5) of Experiments 1-3.**

<b>Experiment 1</b>	<b>Trial 1</b>	<b>Trial 5</b>
<b>Pyramid</b> ♂	97.4 (10.46)	53.4 (10.17)
<b>Pyramid</b> ♀	83.1 (12.65)	61.6 (12.69)
<b>Cone</b> ♂	109.8 (4.98)	60.5 (13.43)
<b>Cone</b> ♀	114.4 (3.73)	48.5 (11.70)

<b>Experiment 2</b>	<b>Trial 1</b>	<b>Trial 5</b>
<b>Plain</b> ♀	97.4 (13.22)	42.3 (9.84)
<b>4-Patterns</b> ♀	79.1 (15.03)	58.5 (16.44)

<b>Experiment 3</b>	<b>Trial 1</b>	<b>Trial 5</b>
<b>1-Pattern</b> ♀	76.5 (16.59)	49.5 (12.45)
<b>4-Patterns</b> ♀	86.6 (15.70)	33.6 (8.17)

Latencies (*sem*) to find the platform also decreased over the course of the training days (see Table 2). An ANOVA conducted on these data taking into account the variables days (1-5), group (Pyramid, Cone), and sex showed that the variables days,  $F(4,144) = 173.90$ , group,  $F(1,36) = 11.51$ , and sex,  $F(1,36) = 6.92$ , were all significant, as well as the interactions days x group,  $F(4,144) = 4.31$ , and days x sex,  $F(4,144) = 3.70$ . No other main effect or interaction was significant ( $F_s < 0.5$ ). The analysis of the interaction days x group showed that the groups differed on days 1 and 2 only,  $F_s(1,38) = 5.64$  and  $8.39$ , respectively; reflecting that animals in the Pyramid group reached the platform faster than animals in the Cone group. The analysis of the interaction days x sex showed that males and females differed on day 1 only,  $F(1,38) = 4.69$ , reflecting that male rats reached the platform faster than female rats. An ANOVA conducted on the escape trials of the three test days, taking into account the variables days (6-8), group (Pyramid, Cone), and sex, showed that the only variable significant was days,  $F(2,72) = 24.29$ . No other main effect or interaction was significant ( $F_s < 2.0$ ).

**Table 2. Latencies (sem) to find the platform (sec.) during training (days 1 and 5) of Experiments 1-3.**

<b>Experiment 1</b>	<b>Day 1</b>	<b>Day 5</b>
<b>Pyramid</b> ♂	34.8 (3.87)	7.8 (0.38)
<b>Pyramid</b> ♀	26.7 (2.05)	7.7 (0.50)
<b>Cone</b> ♂	42.7 (3.17)	7.9 (0.34)
<b>Cone</b> ♀	35.5 (3.97)	7.6 (0.45)

<b>Experiment 2</b>	<b>Day 1</b>	<b>Day 5</b>
<b>Plain</b> ♀	18.3 (2.24)	7.3 (0.41)
<b>4-Patterns</b> ♀	30.4 (4.50)	7.9 (0.68)

<b>Experiment 3</b>	<b>Day 1</b>	<b>Day 5</b>
<b>1-Pattern</b> ♀	27.9 (3.51)	9.8 (2.00)
<b>4-Patterns</b> ♀	28.5 (2.80)	9.6 (1.09)

Figure 2A shows the time spent in the two recording areas (i.e., landmark area and geometry area) by the groups on the preference test trial. It is apparent that animals trained and tested with the cone replicated our previous results: males preferred the geometrical cue, while females preferred the landmark. But when animals were trained and tested with the pyramid, both males and females preferred the geometrical cue (although this preference was less marked in females). An ANOVA conducted on these data taking into account the variables type of cue tested (landmark or geometry), group (Pyramid, Cone), and sex showed that the variable cue was significant,  $F(1,36) = 11.57$ ; the cue x group and cue x sex interactions were also significant,  $F_s(1,36) = 5.93$ , and  $45.59$ , respectively, as well as the triple interaction cue x group x sex,  $F(1,36) = 10.99$ . No other main effect or interaction was significant ( $F_s < 4.0$ ). The analysis of the triple interaction cue x group x sex showed that the interaction cue x sex was significant in the Cone group only,  $F(1,18) = 80.62$ . Simple main effects of this interaction showed that males and females differed in the amount of time spent both in the landmark and in the geometry areas,



$F_s(1,18) = 63.76$ , and  $37.30$ , respectively. In addition, while male rats spent more time in the geometry area than in the landmark area,  $F(1,9) = 35.89$ , the reverse was true for females,  $F(1,9) = 50.61$ .

Figure 2B shows the time spent in the two target and control areas by the groups during the two single cue test trials. Student  $t$  tests were used to compare rats' performance in each target area with its control area. On both kinds of test trial (geometry or landmark), with both landmarks (cone or pyramid), both males and females spent significantly more time in the target than in the control area [minimum  $t(9) = 5.35$ ]. The implication is that males and females of both groups, Pyramid and Cone, had learned about both the landmark and the correct corner. An ANOVA conducted on the time spent in the target area on landmark and geometry tests, taking into account the variables type of cue tested (geometry or landmark), group (Pyramid, Cone), and sex showed that only the interaction cue  $\times$  sex,  $F(1,36) = 5.30$  was significant. No other main effect or interaction was significant ( $F_s < 2.0$ ). The analysis of the interaction showed that males performed better on the geometry test than females,  $F(1,36) = 6.47$ , while sexes did not differ in the landmark test ( $F < 1.0$ ). In addition, while females spent more time in the landmark area than in the geometry area,  $F(1,19) = 6.70$ , males did not differ on the two tests ( $F < 1.0$ ).

In conclusion, the results of the preference test clearly showed that only the females trained and tested with the cone spent more time in the area of the pool that corresponded to the landmark than in the distinctive corner of the pool.

## EXPERIMENT 2

The results of Experiment 1 showed that female rats spent more time in the landmark area than in the geometry area when the landmark used was the cone, but this preference disappeared when the landmark used was the pyramid. It does not seem likely that this was because the pyramid was a very unsalient cue. The only difference between the pyramid and cone groups in initial training was that the pyramid group found the platform rather faster than the cone group on the first two days of training. Nor was there any suggestion that either males or females performed less accurately on the landmark alone test trials when the landmark was a pyramid rather than a cone.

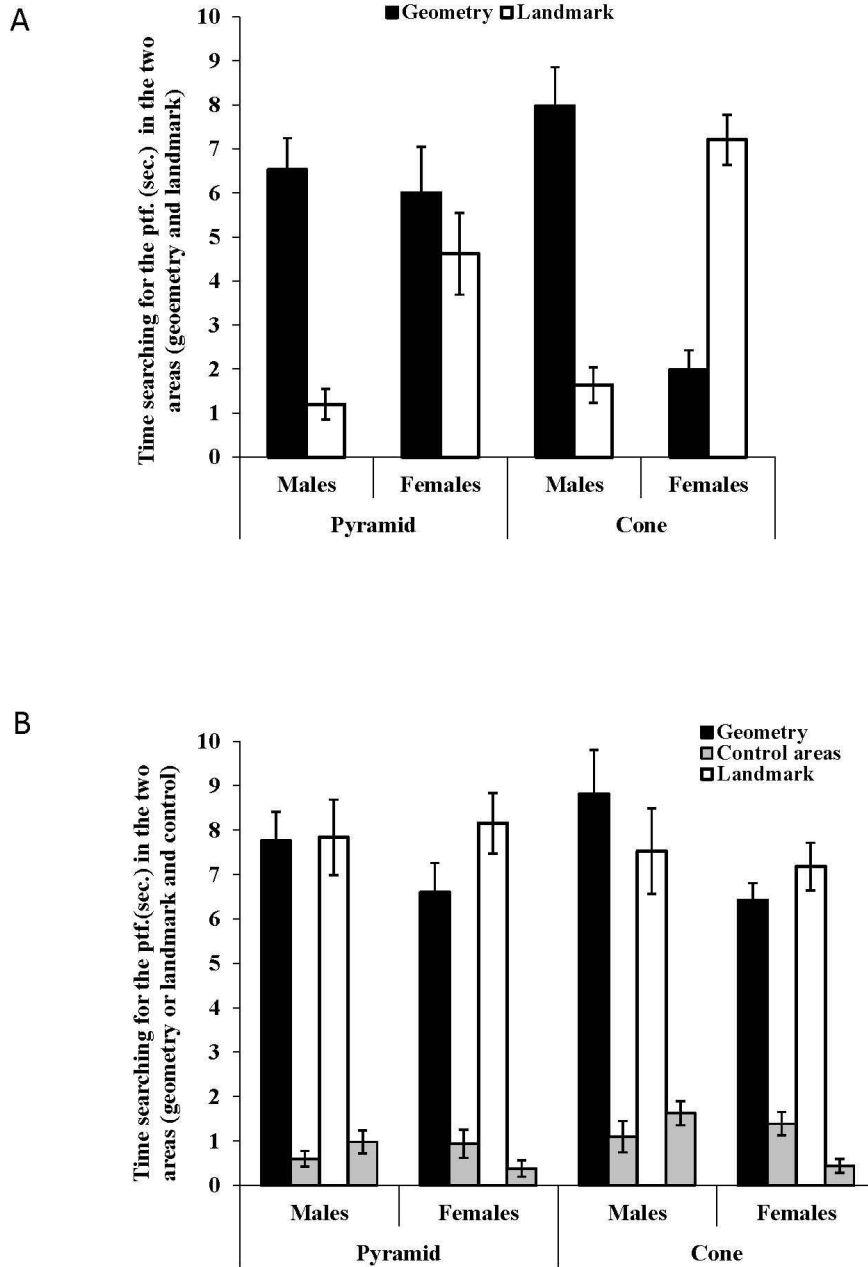


Figure 2. A) Mean time spent in the two recording areas (geometry and landmark) by the subjects during the Preference test trial of Experiment 1. Error bars denote standard error of the means. B) Mean time spent in the two recording areas (geometry or landmark and control) by the subjects during the two Learning test trials (geometry and landmark) of Experiment 1. Error bars denote standard error of means.

So our assumption that females would be less likely to show a preference for a landmark with edges and corners seems to be borne out. But why should such features be so important? Is it because they make the object somehow more complex? But what does that mean, and why should it make the pyramid a somehow less effective cue? Perhaps a simpler possibility is that the pyramid (or a cube) looks different from different perspectives, so that it does not seem to be the same object when approached from different directions? To test this possibility, in Experiment 2, with female rats only, all animals were trained and tested with a cylinder as the landmark cue (i.e., an object without corners and edges), but for one group the cylinder was completely white (plain), while for a second its surface was divided into four equally wide vertical segments, each of them "patterned" differently –with vertical lines, dots, horizontal lines and plain white. This ensured that it looked different from different positions in the pool. If this is the critical variable that determines females' behaviour, the prediction is that when the landmark looks the same from different perspectives (i.e., the plain cylinder), females should show a preference for it in the preference test; on the contrary, if the landmark looks different from different perspectives (i.e., the patterned cylinder), females' preference for the landmark cue should disappear.

**Subjects, apparatus and procedure.** The subjects were 16 naive female Long Evans rats, approximately three months old at the beginning of the experiment. The animals were kept and maintained as in Experiment 1. The general procedure was the same as that used in Experiment 1 with the exception of the landmarks used. In this experiment, for half of the subjects (Plain group) landmark X was a plain white cylinder, 8.5 cm in diameter and 30 cm in height, while for the other half (Patterned group) landmark X was an identical cylinder but with four vertical segments, each 7 cm wide, with different vertical patterns: one was plain white, one with four black horizontal lines (2-cm thick each line), one with eight black dots mixed up on the white surface (2-cm diameter each dot), and the fourth one with two vertical black lines (2-cm thick each line). The apparatus, the experimental room, the platform and the two walls forming the triangular pool were the same as those used in Experiment 1.

## RESULTS AND DISCUSSION

Latencies (*sem*) to find the platform decreased over the course of the 5 initial pretraining trials (see Table 1). An ANOVA conducted on these data taking into account the variables trials (1-5), and group (Plain,

Patterned) showed that no variable was significant ( $F_s < 3.0$ ), although the variable trials was close to significance ( $p = .09$ ). Latencies (*sem*) to find the platform also decreased over the course of the training days (see Table 2). An ANOVA conducted on these data taking into account the variables days (1-5) and group (Plain, Patterned) showed that the variables days,  $F(4,56) = 28.37$ , and group,  $F(1,14) = 9.21$ , were both significant, as well as the interaction days x group,  $F(4,56) = 3.70$ . The analysis of the interaction days x group showed that the groups differed on day 1 only,  $F(1,14) = 5.82$ , reflecting that females in the Plain group reached the platform faster than females in the Patterned group. An ANOVA conducted on the escape trials of the three test days, taking into account the variables days (6-8) and group (Plain, Patterned), showed that no variable was significant ( $F_s < 2.0$ ).

Figure 3A shows the time spent in the two recording areas (i.e., landmark area and geometry area) by the two groups on the preference test trial. It is apparent that when trained and tested with the plain cylinder, females preferred the landmark, but this preference was not evident in those trained and tested with the patterned cylinder. An ANOVA conducted on these data taking into account the variables type of cue tested (landmark or geometry), and group (Plain, Patterned), showed that the variable cue was significant,  $F(1,14) = 18.00$ , as well as the interaction cue x group,  $F(1,14) = 28.09$ . No other main effect or interaction was significant ( $F_s < 1.0$ ). The analysis of the interaction cue x group showed that the groups differed in the amount of time spent both in the landmark and in the geometry areas,  $F_s(1,14) = 10.82$ , and  $19.92$ , respectively. In addition, while rats in the Plain group spent more time in the landmark area than in the geometry area,  $F(1,7) = 40.59$ , those in the Patterned group did not differ on the two tests ( $F < 1.0$ ).

Figure 3B shows the time spent in the two target and control areas by the groups during the two single cue test trials. Student *t* tests were used to compare rats' performance in each target area with its control area. On both kinds of test trial (geometry or landmark), with both landmarks (plain or patterned), both groups spent significantly more time in the target than in the control area [minimum  $t(7) = 5.07$ ]. The implication is that both groups, Plain and Patterned, had learned about both the landmark and the correct corner. An ANOVA conducted on the time spent in the target area on landmark and geometry tests, taking into account the variables type of cue (geometry or landmark), and group (Plain, Patterned), showed that no variable was significant ( $F_s < 0.5$ ).

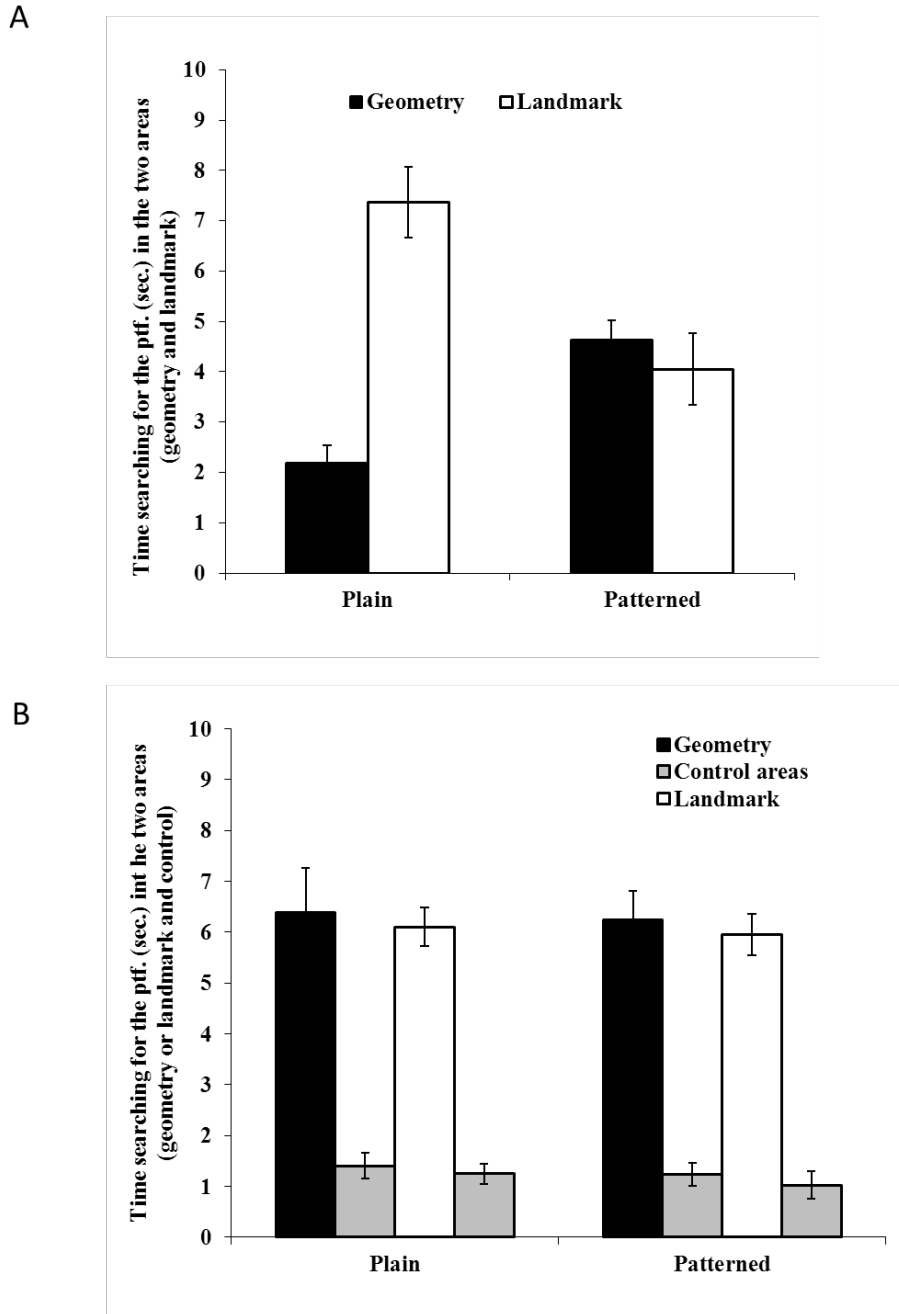


Figure 3. A) Mean time spent in the two recording areas (geometry and landmark) by the subjects during the Preference test trial of Experiment 2. Error bars denote standard error of the means. B) Mean time spent in the two recording areas (geometry or landmark and control) by the subjects during the two Learning test trials (geometry and landmark) of Experiment 2. Error bars denote standard error of means.

In conclusion, the results of the preference test clearly showed that only the females trained and tested with the plain cylinder spent more time in the area of the pool that corresponded to the landmark than in the distinctive corner of the pool.

### **EXPERIMENT 3**

The results of the preference test of Experiment 2 revealed that female rats showed a preference for the plain cylinder, which disappeared with the vertically patterned cylinder. This last result is consistent with the suggestion that the critical factor determining the females' behaviour toward the landmark was whether it looked the same or different when approached from different directions. There is, however, an alternative possible explanation. In all the experiments reported here, the pool was surrounded by black curtains. It is possible that there was a greater contrast between the all-white cylinder and the background than between the patterned cylinder and the background, making the former more salient. Experiment 3, with two groups of female rats (nine rats in each group), addressed this issue. One group was trained and tested with the same vertically patterned cylinder used in Experiment 2. The second group was divided into three subgroups: for three rats the cylinder was entirely patterned with vertical lines, for three with dots, and for the final three with horizontal lines. This should be sufficient to equate the contrast between the cylinder and the background curtains in the two groups.

### **METHOD**

**Subjects, apparatus and procedure.** The subjects were 18 naive female Long Evans rats, approximately three months old at the beginning of the experiment. The animals were kept and maintained as in Experiment 1. The general procedure was the same as that used in Experiment 1 with the exception of the landmarks used. In this experiment, as described above, one group of nine rats (4-Patterns group) landmark X was the same cylinder with the four different patterns used in Experiment 2. The 1-Pattern group was divided into three subgroups of three rats, one trained and tested with the landmark entirely covered with vertical lines, one with horizontal lines, and the third with dots. The apparatus, the experimental room, the platform and the two walls forming the triangular pool were the same as those used in Experiment 1.

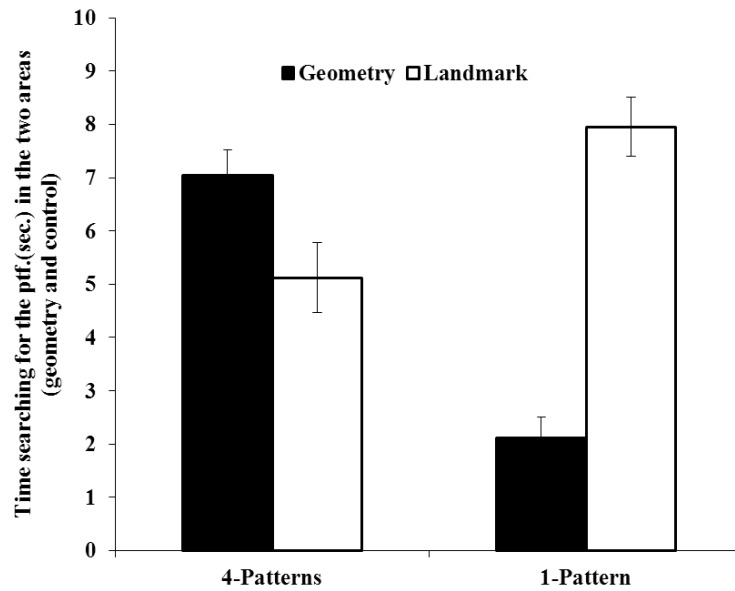
## RESULTS AND DISCUSSION

Latencies (*sem*) to find the platform decreased over the course of the 5 initial pretraining trials (see Table 1). An ANOVA conducted on these data taking into account the variables trials (1-5), and group (4-Patterns, 1-Pattern) showed that the only variable significant was trials,  $F(4,64) = 2.95$ . No other main effect or interaction was significant ( $F_s < 2.0$ ).

Latencies (*sem*) to find the platform also decreased over the course of the training days (see Table 2). An ANOVA conducted on these data taking into account the variables days (1-5) and group (4-Patterns, 1-Pattern) showed that the only variable significant was days,  $F(4,64) = 26.33$ . No other main effect or interaction was significant ( $F_s < 0.5$ ). An ANOVA conducted on the escape trials of the three test days, taking into account the variables days (6-8) and group (4-Patterns, 1-Pattern), showed that no variable was significant ( $F_s < 1.0$ ). Additional ANOVAs were performed to compare the performance of the three subgroups in the 1-Pattern group; they revealed that the three subgroups did not differ either in the course of the training days ( $F < 0.5$ ) or in the escape trials of the three test days, ( $F < 1.0$ ). Figure 4A shows the time spent in the two recording areas (i.e., landmark area and geometry area) by the two groups on the preference test trial. When the cylinder had a single pattern, rats preferred the landmark to the geometrical cue, but this preference disappeared when the cylinder had four distinct patterns. An ANOVA conducted on these data taking into account the variables type of cue tested (landmark or geometry), and group (4-Patterns, 1-Pattern), showed that the variable cue was significant,  $F(1,16) = 18.83$ , as well as the interaction cue x group,  $F(1,16) = 74.06$ . No other main effect or interaction was significant ( $F_s < 3.0$ ). The analysis of the interaction cue x group showed that the groups differed in the amount of time spent both in the landmark and in the geometry areas,  $F_s(1,16) = 10.72$ , and 65.63, respectively. In addition, while rats in the 1-Pattern group spent more time in the landmark area than in the geometry area,  $F(1,8) = 125.51$ , the reverse was true for rats in the 4-Patterns group,  $F(1,8) = 6.83$ . An additional ANOVA taking into account the data of the three subgroups of females in the 1-Pattern group revealed that the three subgroups did not differ ( $F < 1.0$ ).

Figure 4B shows the time spent in the two target and control areas by the groups during the two single cue test trials. Student *t* tests were used to compare rats' performance in each target area with its control area. On both kinds of test trial (geometry or landmark), with both landmarks (4-patterns or 1-pattern), both groups spent significantly more time in the target than in the control area [minimum  $t(7) = 5.25$ ]. The implication is that

A



B

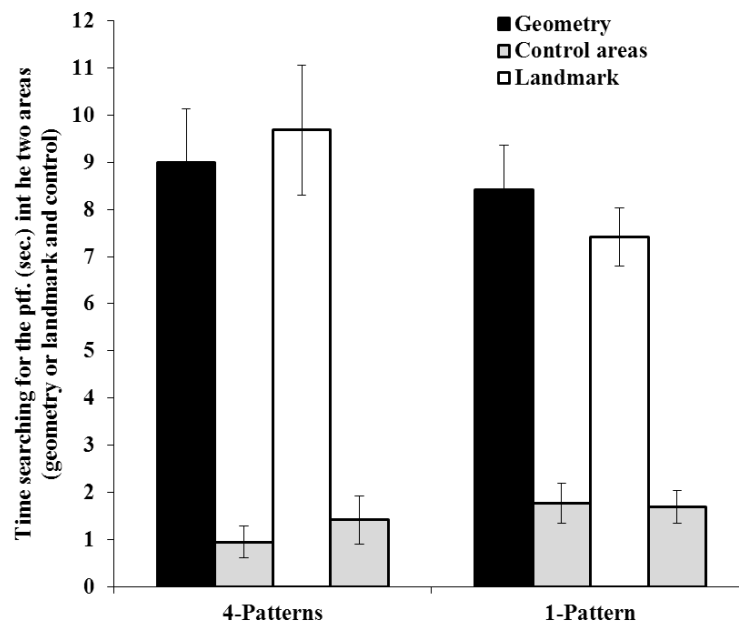


Figure 4. A) Mean time spent in the two recording areas (geometry and landmark) by the subjects during the Preference test trial of Experiment 3. Error bars denote standard error of the means. B) Mean time spent in the two recording areas (geometry or landmark and control) by the subjects during the two Learning test trials (geometry and landmark) of Experiment 3. Error bars denote standard error of means.



both groups, 4-Patterns and 1-Pattern, had learned about both the landmark and the correct corner. An ANOVA conducted on the time spent in the target area on landmark and geometry tests, taking into account the variables type of cue (geometry or landmark), and group (4-Patterns, 1-Pattern), showed that no variable was significant ( $F$ 's  $< 1.5$ ), revealing that there was no difference in learning between the two groups. An additional ANOVA taking into account the data of the three subgroups of females in the 1-Pattern group revealed that the three subgroups did not differ ( $F < 1.5$ ).

In conclusion, the present experiment replicated the results of Experiment 2 while using an optimal procedure. The results of the preference test clearly revealed that only the females trained and tested with the 1-pattern cylinder spent more time in the area of the pool that corresponded to the landmark than in the distinctive corner of the pool.

## GENERAL DISCUSSION

In Experiment 1, male and female rats were trained to find a hidden platform located in one corner of a triangular pool next to a white cone serving as a landmark. A test trial without the platform pitted these two sources of information (landmark and geometrical cue) against one another. Consistent with several previous findings (Rodríguez et al., 2010, 2011, 2013), adult female rats, unlike males, searched for the platform next to the landmark, while adult males searched in the previously correct corner of the pool. When the landmark was a white pyramid, approximately the same size as the cone, however, females' preference for the landmark disappeared, and, like males, they searched in the previously correct corner. What is it about the pyramid that changes the female rats' behavior? Is it because the pyramid, unlike the cone (or the beach ball or the ninepin used in earlier experiments) is full of angles and therefore somehow a more complex object? Or is it simply that, because the pyramid has angles, it looks different from different perspectives? In Experiment 2, two identical cylinders were used as landmarks, one plain white and the other divided into four vertical segments, each "patterned" differently. On the test trial, female rats preferred the plain white cylinder to the geometrical cue, but this preference was reversed when the cylinder was divided into four different patterns. The implication is that the landmark wins out over the geometrical cue only when it looks the same from all perspectives. Experiment 3 confirmed the results of Experiment 2: when the 4-patterns landmark of Experiment 2 was used, females preferred the geometrical cue

to the landmark; but when the cylinder was covered entirely with a single pattern, they preferred the landmark to the geometrical cue.

Over the course of five pretraining trials in the circular pool and without the landmark, there was no difference between males and females in latency to find the platform in Experiment 1. This suggests that females were not more stressed than males. They both spent the same time exploring the pool and swimming directly to the platform.

We conclude that the specific characteristics of the landmark cue play a crucial role in females' preference when solving the present spatial task. Specifically, when the landmark looks the same from different perspectives, females show a preference for it. But when the landmark looks different from different perspectives (because of the angles or because of the decoration), females' preference for the landmark cue disappears. With the advantage of hindsight, it may not be surprising that a landmark that is seen as the same object from whatever direction it is approached should be more effective than one that looks different when approached from different directions, but as far as we know this is the first demonstration of such an effect. Therefore, the appearance of the objects used as landmarks can be critical for exploring sex differences in spatial learning.

It is not only in navigation tasks with rats that the nature of the objects used can have a marked effect on the outcome of an experiment. A related finding has been found on mental rotation tasks with humans, where men tend to outperform women in the speed of mental rotation (for a review see Voyer, Voyer, & Bryden, 1995). Several experiments (Heil & Jansen-Osmann, 2008; Jansen-Osmann & Heil, 2007) have questioned this claim. In the study by Jansen-Osmann and Heil (2007), sex differences were investigated as a function of stimulus material. Five types of stimuli (i.e., alphanumeric characters, PMA symbols, animal drawings, polygons and 3D cube figures) were used. Polygons were the only material that produced substantial and reliable sex differences in mental rotation speed. Subsequent work by Heil and Jansen-Osmann (2008) replicated the previous results and, in addition, revealed that the sex effects reflected a difference in strategy, with women mentally rotating the polygons in an analytic, piecemeal fashion while men using a holistic mode of mental rotation. These results could have important implications concerning the way men and women perceive and represent the world.

Interestingly, in SOR (spontaneous object recognition memory tasks), it has been recently claimed (Gámiz & Gallo, 2012) that one factor that deserves further research is the type of stimuli used. These tasks have used a great variety of three-dimensional objects as stimuli (like in many spatial

tasks), often with contradictory results. For example, in the three experiments of the study by Gámiz and Gallo (2012), with adult and elderly male rats, the critical variable among the experiments was the stimuli they used. Two standard objects, a plastic apple and a porcelain jar were used in Experiment 1; two similar geometric figures, two different pyramids, were used in Experiment 2; and finally, two complex forms built of Lego bricks were employed in Experiment 3. Adult rats outperformed elderly rats when the more complex forms built of Lego bricks were used only (in Experiment 3).

A main implication of all the previous studies, as well as the present experiments, our second main conclusion, is that the type of stimulus material used in a variety of tasks (like mental rotation in humans, and SOR and the Morris pool in rats) can be a critical variable that certainly deserves further research.

## RESUMEN

**Aprendizaje basado en un punto de referencia vs. aprendizaje de la geometría: Explicando la preferencia selectiva de las ratas hembra por un punto de referencia.** Se entrenó a unas ratas en una piscina con forma triangular a que encontrasen una plataforma oculta, cuya ubicación estaba definida en base a dos fuentes de información, un punto de referencia y una parte de la piscina con una forma distintiva. Ensayos de prueba posteriores, sin la plataforma, enfrentaron la forma y el punto de referencia. En el Experimento 1 esta prueba reveló una diferencia de sexo clara, aunque selectiva. Como en experimentos anteriores, las hembras pasaron más tiempo en el área de la piscina que se correspondía con el punto de referencia, aunque sólo cuando este era un cono no cuando era una pirámide. Por otro lado, los machos siempre pasaron más tiempo en el área de la piscina que se correspondía con la forma distintiva. Los Experimentos 2 y 3 se llevaron a cabo sólo con ratas hembra. En el Experimento 2 se emplearon como puntos de referencia dos formas cilíndricas idénticas (una de color blanco y la otra verticalmente dividida en cuatro segmentos con trama diferente). Los resultados de las pruebas de preferencia revelaron que solamente las hembras entrenadas y puestas a prueba con el cilindro blanco pasaron más tiempo en el área de la piscina que se correspondía con el punto de referencia que en el área de la piscina que se correspondía con la forma distintiva. Por último, el Experimento 3 replicó los resultados de los Experimentos 1 y 2 eliminando una explicación alternativa basada en el contraste diferente de los dos cilindros respecto a las cortinas negras.

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