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What makes a landmark effective in adolescent and adult rats?: Sex and age differences in a navigation task

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ABSTRACT

In three experiments rats of different age were trained in a circular pool to find a hidden platform whose location was defined in terms of a single a landmark, a cylinder outside the pool. Following training two main components of the landmark, its shape and pattern, were tested individually. Experiment 1 was with adolescent and adult rats (1a, males; 1b, females). Adult rats always learned faster than adolescent animals. On test trials interesting tendencies were found, mainly one favouring males on the shape test trial and another favouring females on the pattern test trial. Then Experiment 2 was conducted only with adolescent rats and males and females did not differ when learning the task. However on test trials, males learned more about the landmark shape component than about the landmark pattern component, while females learned equally about the two components of the landmark. Finally, Experiment 3 was conducted only with adult rats and again males and females did not differ when learning the task. However on test trials, males learned equally about the two components of the landmark (shape and pattern), although females learned more about the landmark pattern component than about the landmark shape component. This set of experiments supports the claim that male and female rats can learn rather different things about a landmark that signals the location of the platform, age being a critical variable.

Keywords: landmark learning, shape and pattern components, sex differences, adolescence and adulthood, rats.

There is evidence that males and females often show a predisposition to pay attention to different aspects of the environment. An early demonstration in chicks is the study by Vallortigara (1996). Vallortigara trained young male and female chicks to discriminate between two boxes. The correct box was indicated by a colour cue and a position cue. Following training the chicks were retrained to discriminate either on the basis of colour (irrespective of position) or on the basis of position (irrespective of colour). During re-training females performed better on the colour learning task and males performed better on the position learning task. Working with rodents, there are also many demonstrations showing that male and female rats often rely on different cues to solve spatial problems (Hawley, Grissom, Barratt, Conrad, & Dohanich, 2012; Keeley, Tyndall, Scott, & Saucier, 2013; Rodríguez, Torres, Mackintosh, & Chamizo, 2010; Roof & Stein, 1999; Williams, Barnett, & Meck, 1990). Males are more likely to rely on the geometry of the environment (or apparatus), while females more on local cues. The same claim has also been made while working with humans, using a variety of tasks (Dabbs, Chang, Strong, & Milun, 1998; Galea & Kimura, 1993; Jones & Healy, 2006; Sandstrom, Kaufman, & Huettel, 1998; Saucier, Green, Leason, MacFadden, Bell, & Elias, 2002).

Equally interesting is the finding that age may have different effects in males and females when solving a spatial task (in rats see Kanit et al., 2000; Krasnoff & Weston, 1976; Rodríguez, Chamizo, & Mackintosh, 2013. In humans and virtual navigation, Bohbot et al., 2012). The work by Krasnoff and Weston (1976) addressed sex differences in spontaneous wheel running and maze performance both pre- and post-puberty (adolescent and adults, respectively). No sex differences were found when adolescent in either task although in adulthood, females performed better than males in wheel running and males performed better than females in maze performance. In the Kanit et al. study (2000 –see also Kanit et al., 1998), adolescent and adult rats were trained in a Morris pool. After acquisition, a test trial was conducted in which the platform was moved to another quadrant and the time the animals spent in the quadrant where the platform was during training as well as the time to reach the visible platform in the new place, were both measured. The results showed that adult female rats went directly to the newly positioned visible platform, while adult males searched for the platform in its old location, as did both male and female adolescent rats. In the Rodríguez et al. study (2013), 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. After acquisition, the rats were given test trials without the platform. On one test trial the two sources of information were put into opposition. Two other trials were single-cue test trials. In the test trials males and females differed. The differences observed were mainly in their preferred mode of solution, with adult, but not adolescent females using the landmark in preference to geometry, and males using geometry regardless of age. Also important and relevant for the present study, was that males (both adolescent and adults) performed more accurately than females on the single-cue geometry test trial –for the same result see also Rodríguez et al. (2010). In both Kanit's et al. (2000) and Rodríguez's et al (2013) work, adult female rats solved the task differently than both adult male rats and adolescent rats whether male or female. Therefore, a clear "age effect" in the strategies used by female rats to solve a navigation task was found in the two studies.

If the results reported by Kanit et al. (2000) and by Rodríguez et al. (2013) represent a more general manifestation of the response patterns from male and female rats of different age to geometrical and non-geometrical visual cues, similar results should also be found with respect to other tasks or more restrictive tasks, like for example to the geometrical and non-geometrical visual features or components of a single landmark.

Experiment 1 (1a, males; 1b, females) reports such a test of the possibility that age may have different effects on landmark learning, specifically of what is learned about the two main components of a landmark, its shape and pattern (i.e., geometrical and non-geometrical visual information, respectively). In two subsequent experiments, Experiment 2 and Experiment 3, a direct comparison between male and female rats (Experiment 2, adolescents; Experiment 3, adults) is provided. No differences between the groups were expected in Experiment 1a and in Experiment 2, however they were expected in Experiment 1b as well as in Experiment 3. Would that be the case?

Importantly, because it has been recently found that the specific characteristics of a landmark cue can play a crucial role in females' preference when solving a spatial task (Torres, Rodríguez, Chamizo, & Mackintosh, 2014 ─see also Chamizo, Rodríguez, Torres, Torres, & Mackintosh, 2014), in the present set of experiments the landmark used was carefully chosen (i.e., it was always a white cylinder entirely covered with black dots). The main requirement to choose the landmark was that it had the same appearance when approached from different directions. This is important when rats are trained to find a hidden platform in a pool, since they are put into the pool at different points on its circumference on different trials and, once in the pool, are free to swim anywhere. It seems very probable that they will approach the platform from different directions on different trials.

Method

We describe first the apparatus and general procedures common to all experiments.

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 0.4 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. 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 all subjects landmark X was always a cylinder, 8.5 cm in diameter and 30 cm in height, entirely patterned with thirty two black dots mixed up on the white surface (2 cm diameter each dot). This single landmark was the only cue defining the location of the platform. In order to ensure that the rats used this landmark, X, as the source of information to locate the platform, rather than any inadvertently remaining static room cues (like noises from pipes and air conditioning), the landmark 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. Two additional objects were also used: the same cylinder but plain light brown, and a white drum entirely patterned with black dots mixed up on a white surface as in the training landmark (2 cm diameter each dot; and 21 cm diameter and 10.5 cm in height the drum). 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, 11 cm in diameter and made of white Perspex, was mounted on a base and could be placed in front of the landmark, X, 38 cm from the wall, with its top 1 cm below the surface of the water. The hidden platform, P, and landmark, X, were situated as shown in Figure 1A.

General Procedure

There were four types of trial: pretraining, preexposure, training, and test trials. Pretraining consisted of placing a rat into the circular pool without the landmark 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 squads of eight and spent the intertrial interval (ITI) in small individual compartments. After pretraining, preexposure trials took place during one day in an open compartment (specifically, a round soup dish 21 cm in diameter and 3 cm in depth) which was always situated in the center of the pool. This compartment was placed on the platform with its base approximately at the water level. The animals were preexposed to each of the three objects used in the present experiments (cylinder with dots –the training landmark, X–, brown cylinder, and drum with dots) twice, individually (i.e., 6 trials in total –order of presentation of the objects: A, B, C, C, B, A). A preexposure trial consisted of placing a rat in this open compartment and leaving it there for 1 min facing the single object, which was always placed above the edge of the pool. The average ITI of preexposure trials was 8-10 min.

The procedure for training was similar to that of pretraining with the exception that the landmark, X, was always present. As in pretraining, the rat was placed in the pool in a different location on each trial, equally often with the platform to the right, to the left or in front (near or far) of where the rat was placed (at I, II, III, and IV of Figure 1A). Rats were given eight trials per day over three days (a total of 24 trials). These trials had an ITI of 8-10 minutes, and the platform and the landmark, were rotated between trials. Finally, there were two test days, with eight training trials (identical to those in the training phase), followed by one test trial without the platform, 60 s long. Rats received a different test trial each day, counterbalanced, one with the brown cylinder (i.e., the shape component of the training landmark), and the other one with the drum with dots (i.e., the pattern component of the training landmark). For purposes of recording the rat's behavior, on these trials the amount of time that the rats spent in two different but identically sized areas (target and control) was recorded. These areas were 22 cm in diameter—twice the hidden platform diameter. Each rat was placed in the pool from one specific position (at I and II of Figure 1B), and rats were, as much as possible, placed equally in the two starting positions. An alpha level of .05 was adopted for all statistical analyses. Only significant results are presented.

Experiment 1

The aim of Experiment 1 (1a, males; 1b, females) was to provide a direct comparison between two groups of same sex rats: adolescent and adults. The experiment was conducted to answer the following questions. Would the rats learn the spatial task equally fast despite having such a different age? Would the two age groups learn equally about the two components of the landmark, shape and pattern?

Subjects

The subjects were naive Long Evans rats (*Rattus norvegicus*), 10 adolescent and 10 adult males in Experiment 1a; and 10 adolescent and 10 adult females in Experiment 1b. Adolescent rats were approximately one month old at the beginning of the experiments, and adult rats were approximately three months old at the beginning of the experiments. The animals were housed in standard cages, 25 x 15 x 50 cm, in groups of two (same sex, same age) 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.

Results and Discussion

All of Experiment 1 (1a and 1b) was analysed together. Latencies (*sem*) to find the platform decreased over the course of the 5 initial pretraining trials. Adolescent males decreased from a mean of 113.0 (6.97) s on Trial 1 to a mean of 80.6 (11.88) s on Trial 5, adult males decreased from a mean of 106.2 (9.94) s on Trial 1 to a mean of 54.7 (11.74) s on Trial 5, adolescent females decreased from a mean of 120.0 (0.00) s on Trial 1 to a mean of 84.9 (11.00) s on Trial 5, and adult females decreased from a mean of 103.7 (6.78) s on Trial 1 to a mean of 67.7 (13.09) s on Trial 5. An ANOVA conducted on these data, taking into account the variables trials (1-5), sex (males, females), and age (adolescent, adult), showed that the variables trials, $F(4,144) = 13.24$ ($p < 0.001$, $\eta^2_p = 0.27$, 95% CIs = 0.14, 0.36; power > 0.99), and age, $F(1,36) = 22.63$ ($p < 0.001$, $\eta^2_p = 0.39$, 95% CIs = 0.14, 0.56; power > 0.99), were significant. No other main effect or interaction was significant, minimum $F(4,144) = 0.36$ ($p = 0.835$). All rats improved their performance as pretraining trials progressed, and adult rats reached the platform significantly faster than adolescent rats.

Latencies (*sem*) to find the platform also decreased over the course of the training days (see Figure 2A for Experiment 1a; and Figure 2B for Experiment 1b). An ANOVA conducted on these data, taking into account the variables days (1-3), sex (males, females), and age (adolescent, adult), showed that the variables days, $F(2,72) = 135.88$ ($p < 0.001$, $\eta^2_p = 0.79$, 95% CIs = 0.70, 0.84; power > 0.99), and age, $F(1,36) = 50.07$ ($p < 0.001$, $\eta^2_p = 0.58$, 95% CIs = 0.35, 0.71; power > 0.99), were significant. However, the interactions days x age, $F(2,72) = 3.11$ ($p = 0.051$), and age x sex, $F(1,36) = 3.15$ ($p = 0.084$), were nearly significant. No other main effect or interaction was significant, minimum $F(2,72) = 0.46$ ($p = 0.633$). An ANOVA conducted on the escape trials of the two test days (with variables days, sex, and age), showed that the three variables were significant: days, $F(1,36) = 9.37$ ($p = 0.004$, $\eta^2_p = 0.21$, 95% CIs = 0.02, 0.41; power =

0.85), sex, $F(1,36) = 6.99$ ($p = 0.012$, $\eta^2_p = 0.16$, 95% CIs = 0.01, 0.37; power = 0.73), and age, $F(1,36) = 80.04$ ($p < 0.001$, $\eta^2_p = 0.69$, 95% CIs = 0.49, 0.78; power > 0.99). However, no interaction was significant, minimum $F(1,36) = 0.19$ ($p = 0.664$). Although all rats improved their performance as the experiment progressed, adult animals reached the platform faster than adolescents, and males faster than females.

Figure 3 (A, for Experiment 1a; and B, for Experiment 1b) shows the time spent in the target and control areas by the groups during the two test trials (i.e., one with the brown cylinder –the shape component of the training landmark in the absence of the pattern component, and the other one with the drum with dots –the pattern component of the training landmark with a different shape). Student's *t* tests were used to compare rats' performance in each target area with the corresponding control area. On both test trials, all rats spent a significantly longer time in the target than in the control area [minimum $t(9) = 3.18$, and all *p*s < 0.02, $d = 1.01$, 95% CIs = 0.22, 1.76]. The above results imply that all rats (both males and females, adolescents and adults) had learned about the two components of the landmark (shape and pattern). An ANOVA was conducted on the time spent in the target area on the two test trials, taking into account the variables type of component tested (shape, pattern), sex (males, females), and age (adolescent, adult). The results showed that the variable age was significant, $F(1,36) = 8.94$ ($p = 0.005$, $\eta^2_p = 0.20$, 95% CIs = 0.02, 0.40; power = 0.83), as well as the interactions type of component tested x sex, $F(1,36) = 4.26$ ($p = 0.046$, $\eta^2_p = 0.11$, 95% CIs = 0.00, 0.30; power = 0.52), and type of component tested x age, $F(1,36) = 5.90$ ($p = 0.020$, $\eta^2_p = 0.14$, 95% CIs = 0.00, 0.34; power = 0.66). The analysis of the interaction type of component tested x sex showed only two tendencies: one favouring males on the shape test trial, $F(1,38) = 3.42$ ($p = 0.072$), and the second one showing that females had a better performance on the pattern test trial than on the shape test trial, $F(1.19) = 3.96$

(p = 0.061). The analysis of the interaction type of component tested x age revealed that adult rats performed better on the pattern test trial than adolescent rats, $F(1,38) = 14.07$ ($p = 0.001$, $\eta^2_p = 0.27$, 95% CIs = 0.06, 0.46; power = 0.96). In addition, adult rats showed a tendency favouring a superior performance on the pattern test trial than on the shape test trial, $F(1,19) = 3.32$ ($p = 0.084$).

In conclusion, Experiment 1 tentatively supports the claim that, depending on age, male and female rats could learn rather different things about the two main components (shape and pattern) of a landmark that signals the location of a hidden platform.

Following this experiment, Experiments 2 and 3 address the variable sex in two different age groups (adolescents and adults, respectively), in an attempt to optimize the number of subjects per experiment (Russell & Burch, 1959).

Experiment 2

The aim of Experiment 2 was to provide a direct comparison between two groups of adolescent rats: males and females. The experiment was conducted to answer the following questions. Would the two sexes learn the spatial task equally fast? Would females learn equally about the two components of the training landmark, as Experiment 1b suggests? Would males learn more about the shape component than about the pattern component of the training landmark, as Experiment 1a suggests?

Method

Subjects and apparatus

The subjects were 24 naive Long Evans rats, 12 males and 12 females, approximately one month old at the beginning of the experiment. The animals were kept and maintained as in Experiment 1. The apparatus (a Morris pool) and the objects used in this experiment [the cylinder with black dots (landmark, X), the brown cylinder, and the drum with dots] were also exactly the same as in Experiment 1.

Procedure

The general procedure was exactly the same as that used in Experiment 1, with one important exception. In this experiment only adolescent rats (males and females) were used.

Results and Discussion

Latencies (*sem*) to find the platform decreased over the course of the 5 initial pretraining trials. Males decreased from a mean of 120.0 (0.0) s on Trial 1 to a mean of 69.1 (11.50) s on Trial 5, while females decreased from a mean of 107.2 (8.79) s on Trial 1 to a mean of 72.7 (10.48) s on Trial 5. An ANOVA conducted on these data, taking into account the variables trials (1-5), and sex (male, female), showed that the only significant variable was trials, $F(4,88) = 5.82$ ($p < 0.001$, $\eta^2_p = 0.21$, 95% CIs = 0.05, 0.32; power = 0.98). No other main effect or interaction was significant, minimum $F(1,22) = 0.03$ ($p = 0.857$). Thus, both males and females improved their performance as pretraining trials progressed, reaching the platform equally fast.

Latencies (*sem*) to find the platform also decreased over the course of the training days (see Figure 4A). An ANOVA conducted on these data, taking into account the variables days (1-3), and sex (male, female), showed that the only significant variable was days, $F(2,44) = 132.44$ ($p < 0.001$, $\eta^2_p = 0.86$, 95% CIs = 0.76, 0.90; power > 0.99). No other main effect or interaction was significant, minimum $F(2,44) = 0.04$ ($p = 0.963$). An ANOVA conducted on the escape trials of the two test days (with variables days and sex), showed that no main effect or interaction was significant, minimum $F(1,22) = 0.02$ ($p = 0.893$). Both males and females improved their performance as the experiment progressed, reaching the platform equally fast.

Figure 4B shows the time spent in the target and control areas by the groups during the two test trials (i.e., one with the brown cylinder and the other one with the drum with dots –shape and pattern components of the training landmark, respectively). Student's *t* tests were used to compare rats' performance in each target area with the corresponding control area. On both test trials, male and female adolescent rats spent a significantly longer time in the target than in the control area [minimum $t(11) = 4.34$, and all *p*s < 0.005, $d = 1.25$, 95% CIs = 0.47, 2.00]. This implies that all rats had learned about both components of the landmark (shape and pattern). An ANOVA conducted on the time spent in the target area on the two test trials, taking into account the variables type of component tested (shape, pattern), and sex (male, female), showed that the variables type of component tested, $F(1,22) = 8.65$ ($p = 0.008$, $\eta^2_p = 0.28$, 95% CIs = 0.24, 0.52; power = 0.80), and sex, $F(1,22) = 12.26$ ($p = 0.002$, $\eta^2_p = 0.36$, 95% CIs = 0.06, 0.57; power = 0.92) were significant, as well as the interaction type of component tested x sex, $F(1,22) = 21.67$ ($p < 0.001$, $\eta^2_p = 0.50$, 95% CIs = 0.17, 0.67; power = 0.99). The analysis of the interaction type of component tested x sex revealed that males performed better than females in the shape test trial, $F(1,22) = 22.38$ ($p < 0.001$, $\eta^2_p = 0.50$, 95% CIs = 0.18, 0.68; power > 0.99). Moreover, male rats performed better in the shape test trial than in the pattern test trial, $F(1,11) = 21.84$ ($p < 0.001$, $\eta^2_p = 0.67$, 95% CIs = 0.21, 0.81; power = 0.99). These results show that male rats had performed more accurately in the shape test trial than in the pattern test trial, while female rats performed equally well in both test trials.

In conclusion, when the two landmark components (shape and pattern) were tested one by one, Experiment 2 showed that males learned more about the shape component than about the pattern component (as Experiment 1a suggested), while females learned equally about the two landmark components (as Experiment 1b suggested). In addition, a clear male advantage was found when learning the shape component, while the two sexes did not differ when learning the pattern component.

Experiment 3

The aim of Experiment 3 was to provide a direct comparison between two groups of adult rats: males and females. The experiment was conducted to answer the following questions. Would the two sexes learn the spatial task equally fast? Would males learn equally about the two components of the training landmark, shape and pattern, as Experiment 1a suggest? Would females learn more about the pattern component than about the shape component of the training landmark as Experiment 1b suggests?

Method

Subjects and apparatus

The subjects were 36 naive Long Evans rats, 18 males and 18 females, approximately three months old at the beginning of the experiment. The animals were kept and maintained as in the previous experiments. The apparatus (a Morris pool) and the objects used in this experiment [the cylinder with black dots (landmark, X), the brown cylinder, and the drum with dots] were exactly the same as in Experiments 1 and 2.

[Initially, this experiment was carried out with groups of 12 rats (replication 1). However, on the test trials without the platform, although the two main variables sex and component were significant, the interaction did not reach significance ($p = 0.11$). Then, 6 more rats per group were added (replication 2) to see whether the interaction could be significant, as was the case.]

Procedure

The general procedure was the same as that in Experiments 1 and 2, although with two important exceptions. In this experiment only adult rats (males and females) were used. Secondly, this experiment was conducted in two replications.

Results and Discussion

All the statistical analysis were carried out with the variable replication. However, because neither in the acquisition nor in the test trials this variable was significant or interacted with another variable, the results and statistics for this experiment are reported without this variable in order to make them less cumbersome.

Latencies (*sem*) to find the platform decreased over the course of the 5 initial pretraining trials. Males decreased from a mean of 79.9 (10.13) s on Trial 1 to a mean of 46.5 (10.17) s on Trial 5, while females decreased from a mean of 99.7 (8.44) s on Trial 1 to a mean of 57.9 (10.32) s on Trial 5. An ANOVA conducted on these data, taking into account the variables trials (1-5), and sex (male, female), showed that the only significant variable was trials, $F(4,136) = 4.60$ ($p = 0.002$, $\eta^2_p = 0.12$, 95% CIs = 0.02, 0.20; power = 0.94). No other main effect or interaction was significant, minimum $F(4,136) = 0.13$ ($p = 0.973$). Both males and females improved their performance as pretraining trials progressed, reaching the platform equally fast.

Latencies (*sem*) to find the platform also decreased over the course of the training days (see Figure 5A). An ANOVA conducted on these data, taking into account the variables days (1-3), and sex (male, female), showed that the only significant variable was days, $F(2,68) = 27.52$ ($p < 0.001$, $\eta^2_p = 0.45$, 95% CIs = 0.26, 0.57; power > 0.99). No other main effect or interaction was significant, minimum $F(2,68) = 0.29$ ($p = 0.746$). An ANOVA conducted on the escape trials of the two test days (with variables days and sex), showed that the only significant variable was days, $F(1,34) = 8.02$ ($p = 0.008$, $\eta^2_p = 0.19$, 95% CIs = 0.01, 0.40; power = 0.79). No other main effect or interaction was significant, minimum $F(1,34) = 1.19$ ($p = 0.283$). Both males and females improved their performance as the experiment progressed, reaching the platform equally fast.

Figure 5B shows the time spent in the target and control areas by the groups during the two test trials (i.e., one with the brown cylinder and the other one with the drum with dots –shape and pattern components of the training landmark, respectively). Student's *t* tests were used to compare rats' performance in each target area with the corresponding control area. The time in the target areas differed significantly from that in the control areas on all test trials [minimum $t(17) = 6.46$, and all *p*s < 0.001, $d = 1.52$, 95% CIs = 0.83, 2.20], with the exception of females on the shape test trial, $t(17) = 1.41$ ($p = 0.178$). This implies that all rats had learned about both components of the landmark (shape and pattern), except for the females, who only learned about the pattern component. An ANOVA conducted on the time spent in the target area on the two test trials, taking into account the variables type of component tested (shape, pattern), and sex (male, female), showed that the variables type of component tested, $F(1,34) = 18.87$ ($p < 0.001$, $\eta^2_p = 0.36$, 95% CIs = 0.11, 0.54; power = 0.99), and sex, $F(1,34) = 8.23$ ($p = 0.007$, $\eta^2_p = 0.20$, 95% CIs = 0.02, 0.40; power = 0.80), were significant, as well as the interaction type of component tested x sex, $F(1,34) = 7.75$ ($p = 0.009$, $\eta^2_p = 0.19$, 95% CIs = 0.01, 0.39; power = 0.77). The analysis of the interaction type of component tested x sex revealed that males performed better than females in the shape test trial, $F(1,34) = 15.40$ ($p < 0.001$, $\eta^2_p = 0.31$, 95% CIs = 0.08, 0.51; power = 0.97). Moreover, females performed better in the pattern test trial than in the shape test trial, $F(1,17) = 19.23$ ($p < 0.001$, $\eta^2_p = 0.53$, 95% CIs = 0.16, 0.71; power = 0.99).

In conclusion, when the two landmark components (shape and pattern) were tested one by one, Experiment 3 showed that males learned equally about the two landmark components, shape and pattern (as suggested in Experiment 1a), while females learned more about the pattern component than about the shape component (as suggested in Experiment 1b). In addition, as in Experiment 2, a clear male advantage was found when learning the shape component, while the two sexes did not differ when learning the pattern component.

General Discussion

In all the present experiments a submerged platform was located near the wall of a circular pool, in front of a landmark. Circular black curtains surrounded the pool, which was rotated from trial to trial, along with the landmark and the platform, so that the only indicator of the location of the platform was the landmark. Once the rats had learned to find the platform, they were given two test trials, without the platform, on which the two main components of the landmark (its shape and pattern) were individually presented: on one the landmark had a different pattern (thus testing shape, the geometric component of the training landmark), and on the other the pattern was presented on a different shape (thus testing pattern, the non-geometric component of the training landmark). The present study found that adult males performed equally well when tested on both components, while adult females performed much less accurately when tested with the geometric component of the training landmark. However adolescent males performed much less accurately when tested with the pattern component of the training landmark, while adolescent females behaved like adult males (i.e., they learned equally about the two components of the landmark), but unlike adult females, when searching for the platform in the Morris pool.

It is worth noting that the present experiments with adolescent rats (Experiments 1a and 1b, and Experiment 2) took place when the rats were between 31 and 39 days old, before the period of vaginal opening (around the 42nd day). Moreover, in relation to hormonal changes that appear at the onset of puberty, there is reason to believe (see Pleil & Williams, 2010; Williams & Meck, 1991) that the critical factor is the rats' hormonal state during acquisition rather than at the time of test. The literature on sex differences in spatial navigation (and in humans in mental rotation) has largely focused on differences in speed of learning or accuracy of performance. There was no suggestion of any such differences in the present experiments when males and females were directly compared (Experiment 2 and Experiment 3). Male and female rats did not differ during the course of initial training (for the same result, see also Rodríguez et al., 2013). However they differed on test trials. In both adolescence (Experiment 2) and adulthood (Experiment 3), a sex difference was found when landmark learning. In males, selective learning by the shape component was evident only in adolescence, while in females a selective learning for the pattern component was observed only in adulthood. Therefore, age seems to determine selective learning by one or the other component of the training landmark both in males and in females. We are forced to conclude that the two sexes, both when adolescent and when adults, differed in the amount learned about the two main components (shape and pattern) of a landmark that signalled the location of a platform (for related results see Kanit et al., 2000; Rodríguez et al. 2013). Most importantly in the present set of experiments is the demonstration for the first time that the differential response patterns from males and females of different age can apply to the geometrical and non-geometrical components of a single landmark (its shape and pattern).

When rats are trained in a circular pool to find a hidden platform whose location is defined in terms of a single landmark outside the pool, Chamizo et al. (2014) have shown that adult male and female rats do not seem to learn the same about the landmark (for a similar result see also Torres et al., 2014). Chamizo et al. (2014) found that the nature of the landmark affected only the behavior of females, and not that of males. In this study, two identical cylinders were used as landmarks, one plain white and the other divided into four vertical segments, each patterned differently. Adult male rats learned about the 4-patterns cylinder as rapidly as about the cylinder with a single pattern. However, that was not the case with the females. Adult female rats learned to swim to the platform more rapidly with the plain white cylinder than with the 4-patterns cylinder. It was concluded that male rats, being less likely than females to rely on landmarks to solve a spatial problem, do not pay very close attention to the specific features of any landmark; that they simply learned that the platform was to be found next to the only large object suspended above the pool. The test results of the present study with adult male rats contrast sharply with the previous conclusion. They show that adult male rats pay attention to the main components (shape, pattern) of a single landmark. Other studies have indeed shown that, depending on the salience of the cues, adult male rats may in some circumstances prefer to use the visual features of a landmark rather than its spatial location (e.g., Arain & Cohen, 2013) or rather than local geometry provided by the apparatus (Mesa, Osorio, Ballesta, Marimon, & Chamizo, 2017).

What is the explanation of the change in the behavior of rats, both males and females, as they grow older? It could be argued that the two sexes differ in cognitive style when adolescent and when adults, thus learning differently about the two main components (shape and pattern) of a landmark that signals the location of a platform. Selective landmark learning was found in the two sexes, but at a different age –in males, in adolescence; in females, in adulthood. In males (but not in females), a natural predisposition to geometry could explain the results of Experiment 2 (Izard, Pica, Dehaene, Hinchey & Spelke, 2011). If this is correct, then Experiment 3 reveals that such a predisposition seem to disappear with age. In any case it is clear that the present results offer little support to the claim that animals recognize objects primarily on the basis of their shapes, regardless of task demands (Izard et al., 2011). In females, the obvious answer, suggested by the observation that ovariectomized females behave like younger rather than older rats (Rodríguez et al., 2013), is that the hormonal changes associated with the onset of puberty changed the females' learning and attention. Both in males and females, age could capture different aspects of the landmark, although we do not know exactly why. Sex differences in cognitive patterns probably arose because they proved to be evolutionarily advantageous (for reviews in spatial cognition see Halpern, 2012; Jones, Braithwaite, & Healy, 2003; Mackintosh, 2011).

In the literature of spatial abilities it has been repeatedly said that sex differences, both in rodents and in humans, appear after puberty (for reviews see Kimura, 1999; Voyer, Voyer, & Briden, 1995). However, recent research, carried out with babies using mental rotation tasks with 3D objects (Lauer, Udelson, Jeon, & Lourenco, 2015; Moore & Johnson, 2008; Quinn & Liben, 2008, 2014), has shown that the previous statement is inaccurate. Undoubtedly, more research is needed to understand how spatial tasks (or other tasks with spatial components) interact with sex differences throughout the life cycle. For example, the different natural selection pressures often exerted during centuries in males and females of different species is a subject that would have to be addressed more in the future, given its many implications (Lauer & Lourenco, 2016).

According to Mackintosh (1975), the attention paid to a cue increases if it is a better predictor of the outcome than all other cues present on a trial and decreases if it is no better a predictor of the outcome than all the other cues present on a trial. Thus, cues which are good predictors of subsequent events will enjoy an increase in their salience (i.e., an increase in their attention). Irrelevant cues that are poor predictors of subsequent events, however, suffer a reduction in their attention (see also Esber & Haselgrove, 2011; Le Pelley, 2004). We believe that the previous analysis can also be applied to the two main components (shape and pattern) of a landmark. Most importantly, both sex and age seem to be able to determine the gains or losses in the attention given to these components.

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FOOTNOTE TO TITLE PAGE

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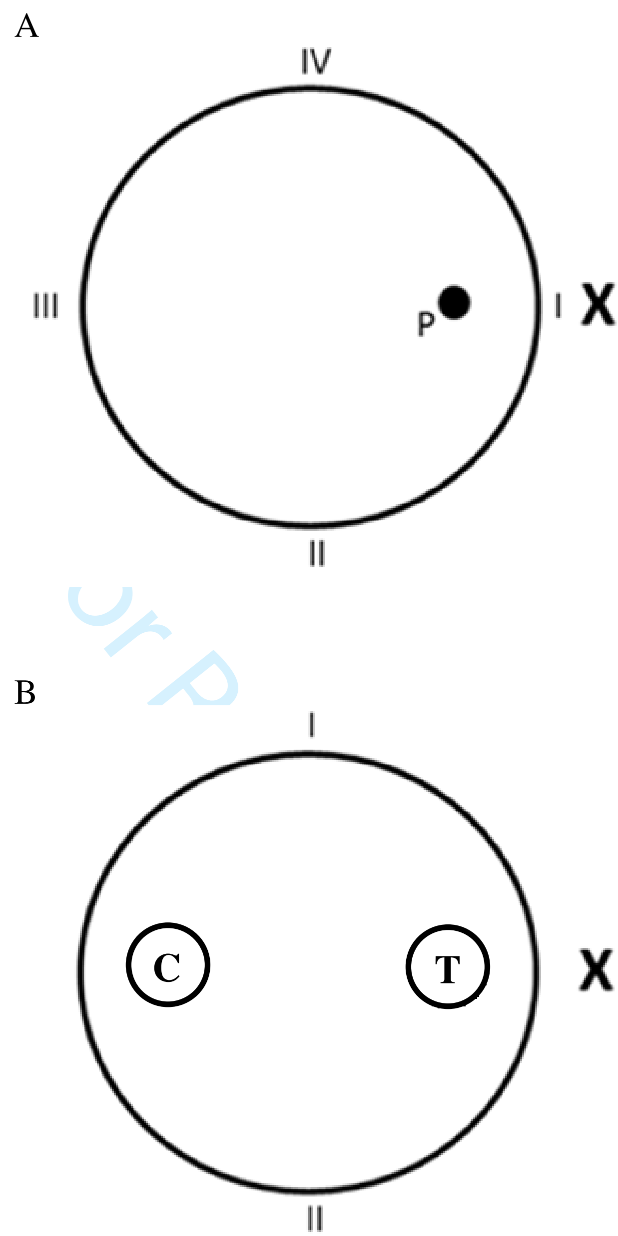

Figure 1. A: Schematic representation of the pool and the position of the landmark, X, and the hidden platform (P) during Experiments 1-3. I, II, II, and IV indicate the four starting positions. B: Schematic representation of the pool and the position of the component of the landmark, X, the target area (T), and the control area (C) during the test trials. I and II indicate the starting positions.

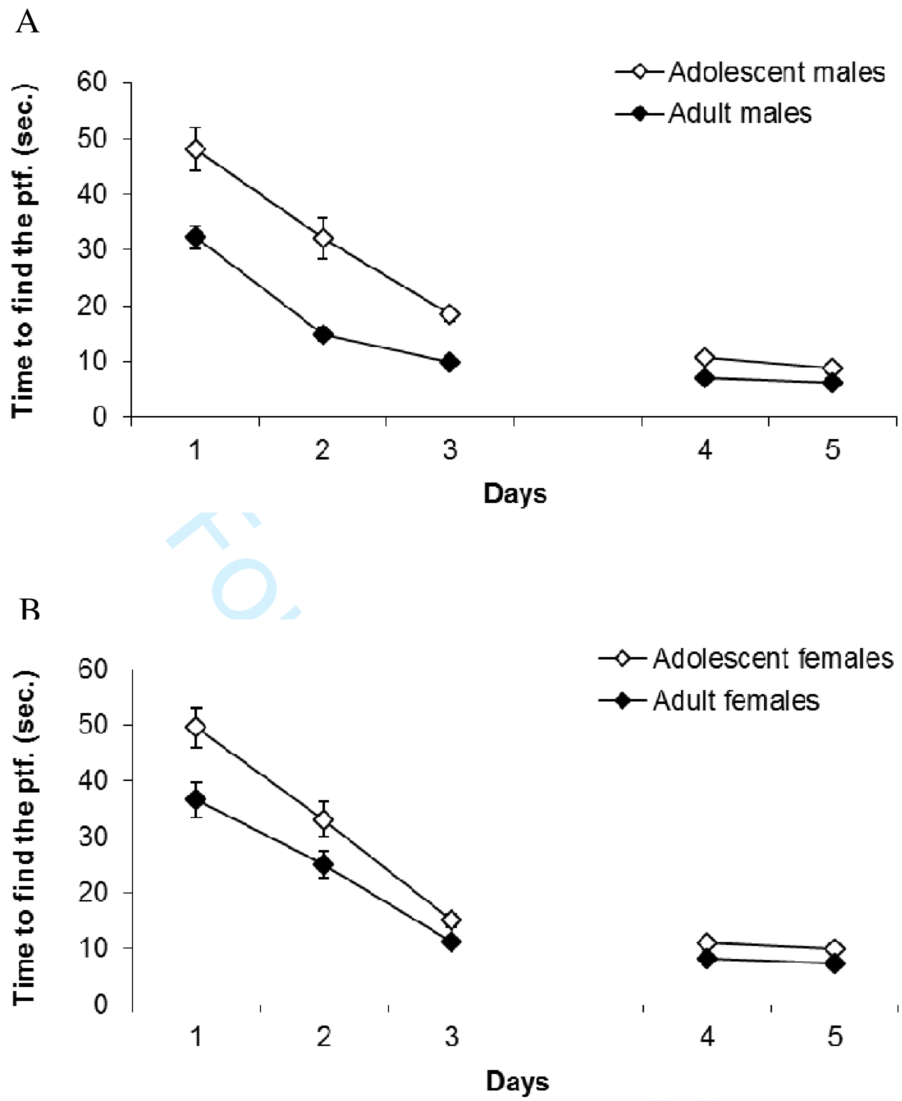

Figure 2. A: Mean escape latencies for the groups of Experiment 1 (males) during the training and the test days. Error bars denote standard error of the means. B: Mean escape latencies for the groups of Experiment 1 (females) during the training and the test days. Error bars denote standard error of the means.

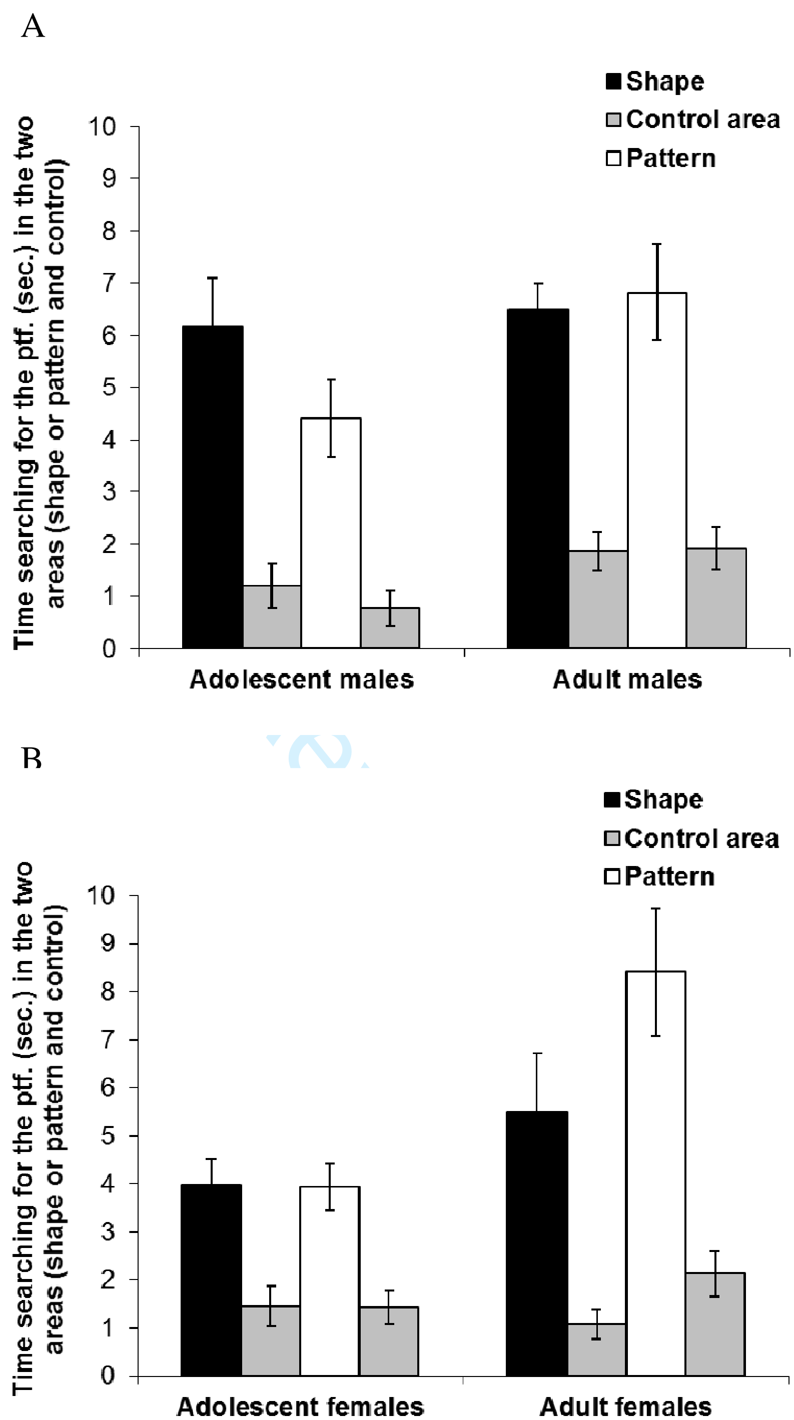

Figure 3. A: Mean time spent in the target and control areas by the subjects during the two test trials of Experiment 1 (males). Error bars denote standard error of the means. B: Mean time spent in the target and control areas by the subjects during the two test trials of Experiment 1 (females). Error bars denote standard error of the means.

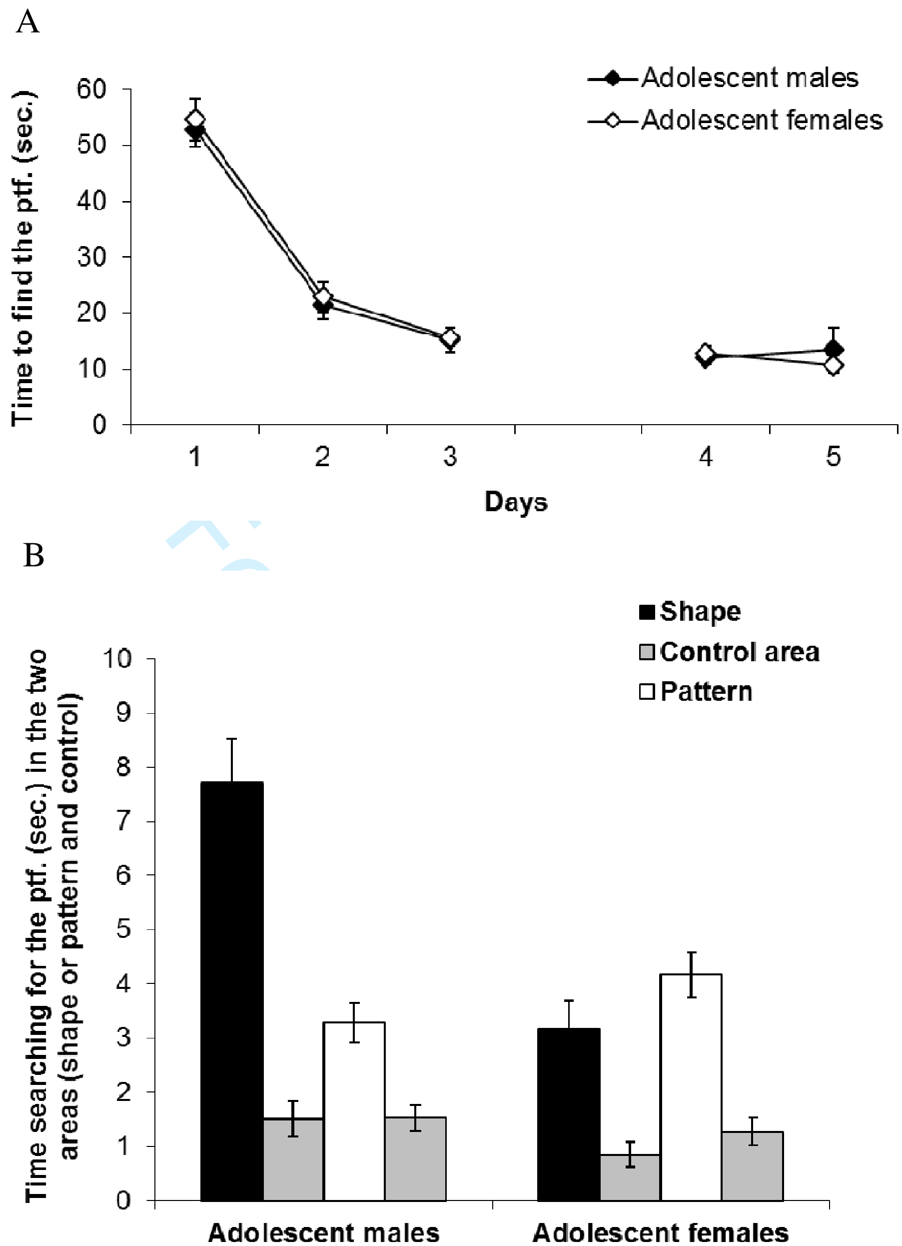

Figure 4. Experiment 2. A: Mean escape latencies for the groups during the training and the test days. Error bars denote standard error of the means. B: Mean time spent in the target and control areas by the subjects during the two test trials. Error bars denote standard error of the means.

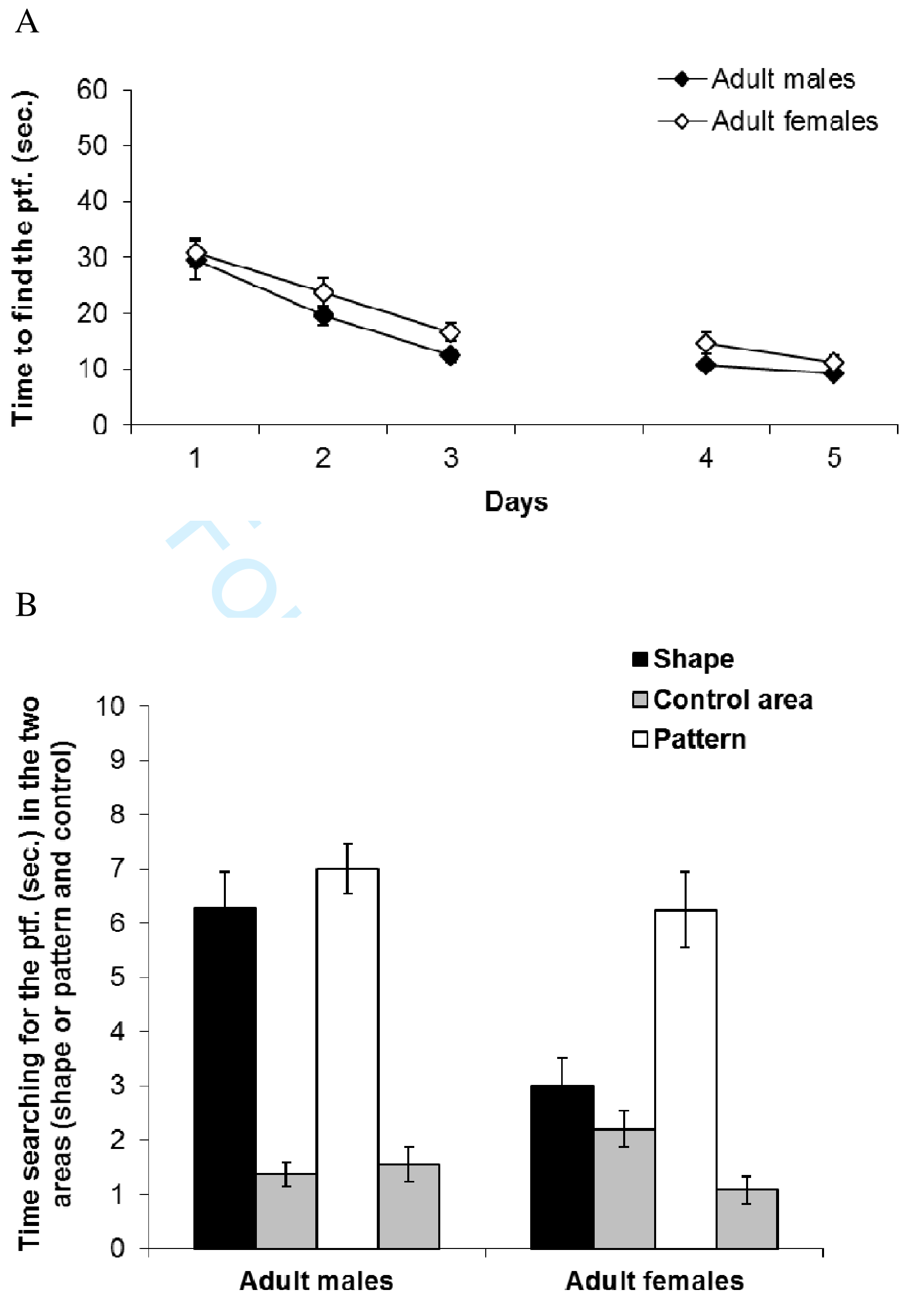

Figure 5. Experiment 3. A: Mean escape latencies for the groups during the training and the test days. Error bars denote standard error of the means. B: Mean time spent in the target and control areas by the subjects during the two test trials. Error bars denote standard error of the means.