



Navigation with two landmarks relatively far from a goal in rats (*Rattus norvegicus*): The role of landmark salience

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ABSTRACT

Male and female rats were extensively trained in a Morris pool to find a hidden platform in the presence of a configuration formed by 2 landmarks and the effects of varying the salience of one of the landmarks were studied. The two landmarks could be either of different salience (i.e., B and c, Group Bc) or of the same salience (i.e., b and c, Group bc) and they were relatively far (110 cm) and equidistant from the hidden platform. Rats in Group Bc ended up being faster to reach the platform than rats in Group bc, and males were always faster to find the platform than females. No sex differences were found on subsequent test trials without the platform (i.e., when a different measure was used). The results showed a clear salience effect: The best rats' performance was when the more salient landmark, B, was present, either by itself or in compound with landmark c. A final test trial, a conflict test, was also conducted with the two landmarks in compound although reversing their relative positions, in order to test the preference for the geometry they formed, a straight line (independently of the landmarks' identities), vs. the landmarks identities, B or b. All rats showed a preference for the geometry formed by the two landmarks, independently of their identities.

1. Introduction

There are many ways by which animals can navigate (for reviews see Knierim & Hamilton, 2011; Rodrigo, 2002; Tommasi, Chiandetti, Pecchia, Sovrano, & Vallortigara, 2012). Two crucial ones by means of visual cues are landmarks and environmental geometry. Here we address, in a single experiment, the role of landmark salience (i.e., the significance or noticeability of landmarks) while navigating, although some geometry learning will also be discussed. The present experiment is a continuation of a previous study by Rodrigo, Gimeno, Ayguasanosa, and Chamizo (2014). Rodrigo et al. (2014) studied sex differences and landmark salience while training rats in a Morris pool in the presence of one (Experiment 1) or two (Experiment 2) landmarks. These landmarks were always placed relatively close in relation to a hidden platform (approximately 50 cm away). Experiment 1 addressed single landmark learning and established the relative salience of three different landmarks (i.e., b, c, and B). Two of them (b and c) revealed a similar salience, and smaller than the third one (B), the most salient landmark, both in training and on a test trial without the platform. Then in Experiment 2, rats were extensively trained to find a hidden platform in the presence of a configuration formed by landmarks B and c or by landmarks b and c (i.e., different salience, Group Bc, and same salience, Group bc, respectively). A strong sex difference emerged

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during acquisition, with males reaching the platform faster than females. This sex difference, however, was not found in any of the subsequent test trials, without the platform, where performance was always above chance (i.e., when the training configuration of landmarks was tested and when any single landmark was tested). On Group Bc, Test Bc > Test B > Test c; and on Group bc, Test bc > (Test b = Test c). These results suggested a clear additive effect between the two landmarks of each configuration. On Test B/b a clear salience effect was found: the best rats' performance was with Landmark B, Group Bc, which differed from Landmark b, Group bc. The reverse was true on Test c: Group bc performed better than Group Bc. These last results clearly showed that the high salience of Landmark B affected not only how well it could be used to locate the platform, but also its ability to reduce learning about Landmark c (i.e., interference by relative salience, a result well predicted by associative learning—see Mackintosh, 1976; Miles & Jenkins, 1973). Extended training (i.e., phase 2 of acquisition) did not affect what was initially learned. The previous results revealed that the rats knew the identity of the landmarks and that they were able to locate the hidden platform based on information about the relative position of one landmark to another, something that their close relatives, the gerbils, cannot do (Collett, Cartwright, & Smith, 1986).

Collett et al. (1986) trained female gerbils in a sort of open field to find hidden food in a place that maintained a fixed relationship with the position of two distinct and equidistant from the food landmarks. Once they had learned to locate the food, a series of test trials were given. When one of the landmarks was removed, the gerbils searched in two locations whose directions and distances corresponded to those between each of the landmarks and the goal during training. This suggests that the animals knew the direction and distance of the food from the landmarks, but that they did not know their identity. The tested landmark was treated as if it could have been either of the two training landmarks. In another test the distance between landmarks was doubled and again it was found that the gerbils searched in two places defined by the direction and distance of the goal from each of the two training landmarks. These results suggest that the gerbils, at least the females, calculated the distance and direction of the food from each cue independently; they were not able to locate the food based on information about the relative position of one landmark to another.

Experiment 2 of the study by Rodrigo et al. (2014) ended with a final test, without the platform, in which the relative positions of the two training landmarks were reversed, thus pitting elemental learning and geometry (i.e., the configuration formed by a straight line between the two landmarks—learning in the absence of the landmarks' identities) against one another, and no sex differences were found. This final test is a measure of salience, already employed by Rodríguez, Torres, Mackintosh, and Chamizo (2010)—namely, opposing two cues or sources of information and seeing which one wins. Only in Group bc geometry learning was preferred. Bc rats did not differ between elemental and geometry preferences. A geometry strategy, following acquisition with two landmarks, one larger than the other, has been found in pigeons (1989, Cheng, 1988). When the landmarks were moved further away on test, the pigeons did not search in two places, like the gerbils. They searched in the middle of the line defined by the two positions of the goal as indicated by the landmarks. Such strategy is used by birds of a corvid species, Clark's nutcrackers (*Nucifraga Columbiana*; Kamil & Jones, 1997, 2000), as well as by adult humans but it is less frequent in children and in nonhuman primates (MacDonald, Spetch, Kelly, & Cheng, 2004).

The present study was designed to complement that of Rodrigo et al. (2014). It consists of a replication of Experiment 2, although with one main change. In the study by Rodrigo et al. (2014, Experiment 2) the distance between the two landmarks and the hidden platform was approximately 50 cm, while in the present experiment that distance was increased to approximately 110 cm. A greater distance between the landmarks and the hidden platform will reduce the salience of the landmarks (for a demonstration with single landmark learning see Chamizo, Rodrigo, Peris, & Grau, 2006) and might end up eliciting a different way of processing them, like pure configuration (i.e., a shape abstracted from the elements). Although we expected a worse performance overall due to the increased task difficulty, we predicted similar results in the two groups, Group Bc and Group bc, when the training configuration of landmarks was tested and when any single landmark was tested. However, that was not the case in the final conflict test, when the relative positions of the two training landmarks were reversed. We predicted that geometry learning (i.e., learning a straight line between the two landmarks, independently of their identities) will be preferred to elemental learning in all rats, even in Group Bc. Would that be the case? No prediction was made regarding the sex variable. It should be mentioned that the present study was inspired by work carried out with humans. Specifically, in the long debate related to Global-to-Local perceptual precedence (Navon, 1977). According to Navon (1977), global structuring of a visual scene precedes analysis of local features, although subsequent research has shown that many variables can affect this result (Grice, Canham, & Boroughs, 1983). For example, it has been claimed that greater distance from things can elicit a higher-level of thinking—a global processing instead of elemental processing (Henderson, Fujita, Trope, & Liberman, 2006; Trope & Liberman, 2010).

Importantly, the present experiment incorporates two procedural changes from the previous study (Rodrigo et al., 2014, Experiment 2). The first training phase lasted sixteen days and the second training phase six days (instead of twelve and four, as in the previous study). These changes were introduced to compensate for the fact that the task is now more difficult.

2. Method

2.1. Subjects

The subjects were naive Long Evans rats (*Rattus norvegicus*), 16 males and 16 females approximately three months old at the beginning of the experiment. They were housed in standard cages, 25 × 15 × 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 h of the light cycle.

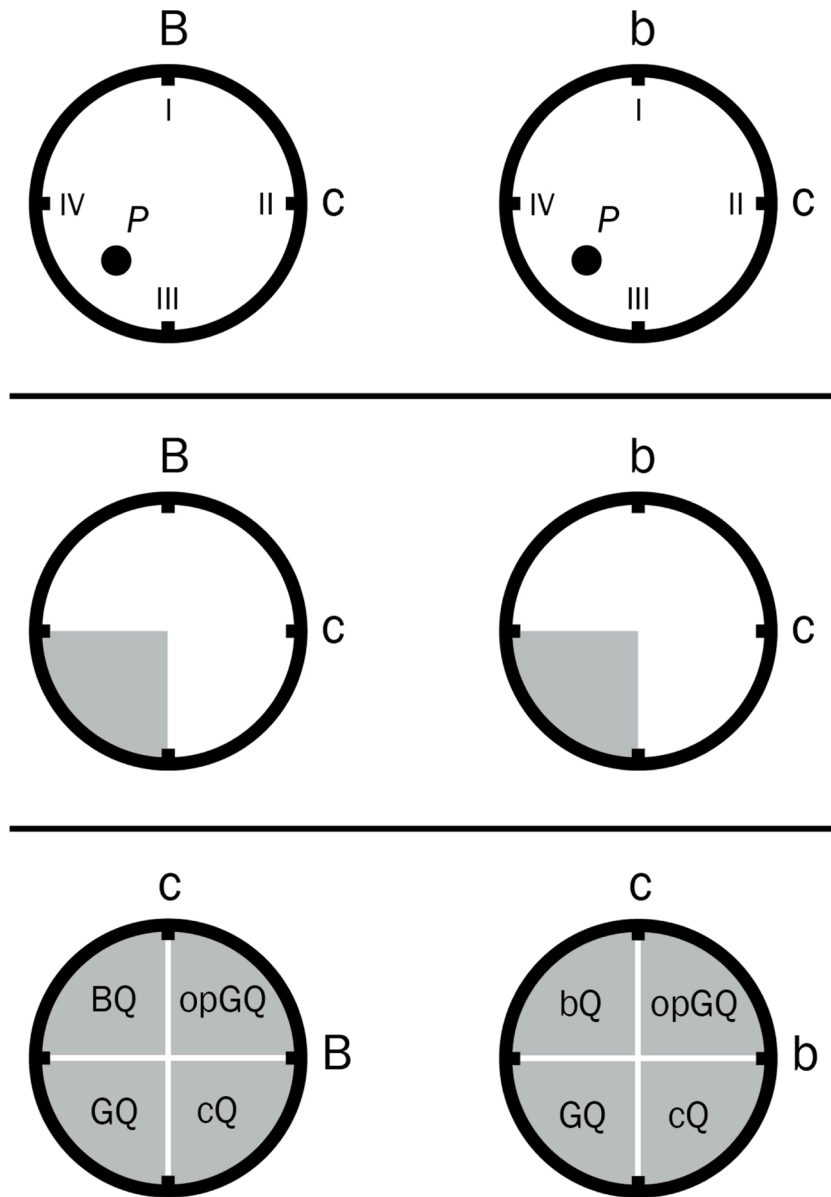


Fig. 1. Left, Group Bc; right, Group bc. Top panel: A schematic representation of the pool and the position of the two landmarks (left: B and c; right: b and c), as well as the hidden platform, *p*, and the starting positions (I, II, III, IV). Middle panel: Platform quadrant registered on test trials for Bc and bc rats. Bottom panel: Preference test. GQ, quadrant controlled by the geometry, a straight line, formed by the tested landmarks; bQ and BQ, quadrants controlled by landmarks b and B; opGQ, shows the rats' performance on the quadrant opposite to GQ.

2.2. Apparatus

The apparatus was a circular swimming pool, made of plastic and fiber glass, modeled after that used by [Morris \(1981\)](#). It measured 1.58-m in diameter and 0.65-m deep, and 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 ± 1 °C. The pool was situated in the middle of a large room, mounted on a wooden platform 0.43-m above the floor, and it was surrounded by black curtains reaching from ceiling to the base of the pool and forming a circular enclosure 2.4-m in diameter. Inside the black enclosure, around the curtains, and hanging from a black false ceiling, three objects or landmarks could be placed, suspended from the false ceiling, 23-cm above the surface of the water and had the midline directly above the wall of the pool. In order to ensure that the animals used these landmarks, rather than any inadvertently remaining static room cues, to locate the platform, between each trial the landmarks and platform were semirandomly rotated with respect to the room (90°, 180°, 270°, or 360°), with the restriction that all parts of the room were equated each day. A closed-circuit video camera with a wide-angle lens was mounted 1.75-m above the center of the pool inside the false ceiling, and its picture was relayed to

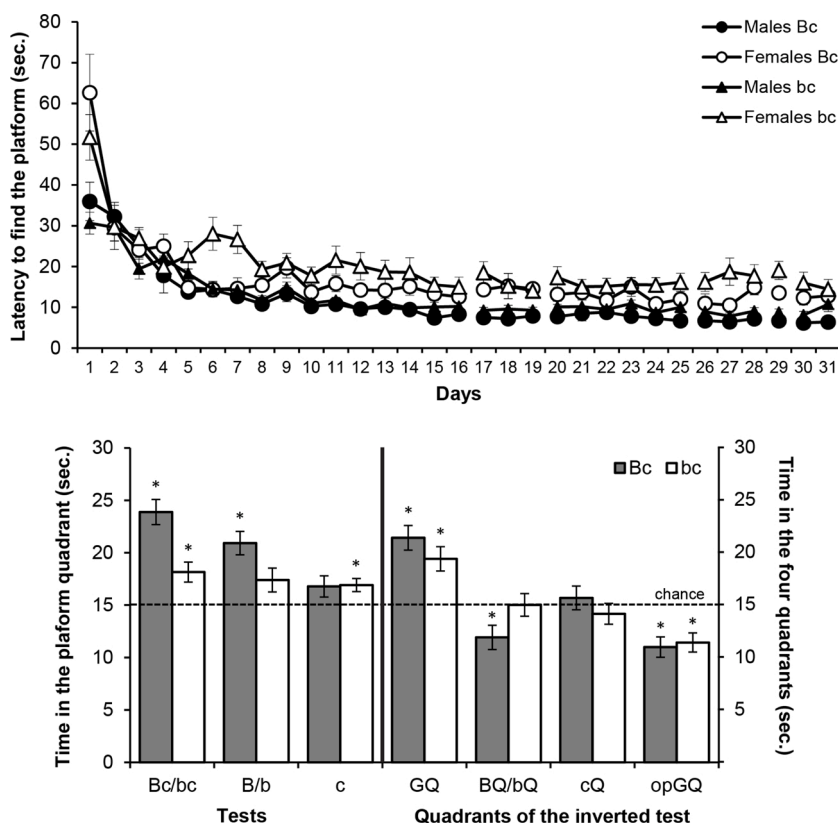


Fig. 2. Top: Mean escape latencies during all the escape trials. Bottom, left: Time searching in the platform quadrant during the averaged test trials, Bc/bc, B/b, and c. Bottom, right: Time searching in the four quadrants (GQ, middle quadrant formed by the two tested landmarks—either c and B or c and b; BQ/bQ, quadrant controlled by Landmarks B or b; cQ, quadrant controlled by Landmark c; and opGQ, which shows performance on the quadrant opposite to GQ, at a short distance from the tested landmarks) during the last preference test (Day 1 and Day 2 averaged). A small asterisk above each test indicates whether each group differed significantly from chance. Error bars denote SEM.

recording equipment in an adjacent room. All measures were automatically registered by a computer using the SMART video tracking software (2.5.19) (Panlab, 2007). A circular platform, 0.11-m in diameter and made of transparent Perspex, was mounted on a rod and base, and could be placed in one quadrant of the pool, 0.38-m from the side, with its top 1-cm below the surface of the water. The three landmarks used were as follows: (Landmark B) a 32-cm diameter plastic beach ball with alternate blue, white, yellow, white, red, and white vertical segments; (Landmark b) a 16.5-cm diameter plastic ball with mixed colors; (Landmark c) and a green plastic plant approximately 35-cm in diameter and 30-cm in high.

The landmarks, the hidden platform, *P*, and the pool were situated as shown in Fig. 1 (top).

2.3. Procedure

There were three types of trial: pretraining, landmark learning, and test trials. Pretraining consisted of placing a rat into the pool, facing the wall, without landmarks but with the platform present. The rat was given 120 s to find the platform, and once it had found 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 the platform, 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 IV in Fig. 1, top), 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 over 2 days, at a rate of two trials on Day 1, and three on Day 2. Thus, the dependent measure was time to reach the platform (in seconds). The procedure for landmark learning was exactly the same as for pretraining except that two landmarks were always present on each trial, either B and c (Group Bc) or b and c (Group bc). The platform was always situated relatively far (approximately 110 cm away) from B and c for Group Bc and relative far from b and c for Group bc, as shown in Fig. 1, top. Animals were given eight trials per day over 16 days—days 1–16 in Fig. 1 (a total of 128 trials), with the exception of the first 2 days, where rats received only four trials (i.e., the first experimental day was run in two working days). These trials had an inter-trial-interval (ITI) of 8–10 min, and the platform and landmarks rotated between trials with the platform always maintaining a fixed position in relation to the landmarks.

After the landmark learning phase, all rats received three test days—days 17–19 in Fig. 1 (Test Phase 1). Each test day started with eight landmark learning trials, followed by a single test trial, 60-s long, on which the rats were placed in the pool with one or two

landmarks present, but without the platform. The same four starting positions were used as in training. For purposes of recording the rat's behaviour, on test trials the pool was divided into four quadrants. On these test trials the amount of time the rat spent in the platform quadrant (Quadrant III–IV in Fig. 1, middle panel) was recorded automatically by the program. On the first test day, all rats were tested with the training configuration: with B and c simultaneously present (Group Bc), and with b and c simultaneously present (Group bc). On the following test days, all rats were tested with the same landmarks but individually presented, counterbalanced (i.e., on the second test day half in each group were tested with either B or b alone, and half with c alone, and vice versa on the third test day).

Following Test Phase 1, all rats received six further days of landmark learning -days 20–25 in Fig. 1 (a total of 48 trials), the procedure being exactly the same as before, followed by a further three test days -days 26–28 in Fig. 1 (Test Phase 2) which were exactly the same as Test Phase 1 (i.e., a repetition of the previous test trials).

Finally, all rats received one further day of landmark learning (day 29 in Fig. 1) followed by two further test days -days 30 and 31 in Fig. 1 (Inverted Position Test Phase). On both test days, the rats were tested with the two training landmarks simultaneously present: with B and c (Group Bc), and with b and c (Group bc), but reversing the relative positions of the two landmarks, Test cB/cb—in order to test the geometry that they formed (a straight line, G, independently of the landmarks identities). On these test trials the amount of time the rat spent in the four quadrants of the pool, GQ,¹ BQ/bQ, cQ, and opGQ (i.e., the quadrant controlled by the geometry of the two landmarks, the quadrant controlled by Landmarks B or b, the quadrant controlled by Landmark c, and the quadrant opposite to the geometry quadrant, between the two landmarks, respectively—Quadrants III–IV, IV–I, III–II, and I–II in Fig. 1, bottom panel) was recorded automatically by the program (i.e., a different measure compared to that of the escape trials). Only the two target quadrants were further analyzed (i.e., GQ and BQ/bQ; a preference test between the geometrical properties defined by the two testing landmarks independently of their identities vs. Landmarks B or b).

2.4. Data analysis

We carried out mixed analyses of variance (ANOVA) and one sample *t*-tests to assess our hypotheses. Significant interactions were analyzed through simple main effects and post-hoc pairwise comparisons. Statistical analysis was performed in SPSS (IBM Corp., 2017), except for post-hoc pairwise comparisons, which were performed in R (R Core Development Team, 2020) using the 'emmeans' (Lenth, 2020) and 'effectsize' (Ben-Shachar, Makowski, & Lüdtke, 2020) packages.

3. Results and discussion

Fig. 2 (top panel) shows the mean escape latencies of rats during all the experiment (days 1–31). Six independent mixed ANOVAs were conducted to analyse these latencies, taking into account the variables Group (Group Bc, with landmarks of different salience vs. Group bc, with landmarks of the same salience), Sex and Days (although with the exception of Day 29, where the variables were Group and Sex, only). The ANOVA of the first landmark learning phase (Days 1–16) showed that the assumption of sphericity had been violated, Mauchly's test $\chi^2(119) = 340, p < .001$, therefore degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\epsilon = .30$). The ANOVA showed that the variable Sex was significant, $F(1, 28) = 20.25, p < .001, \eta^2_p = .42$ (males reached the platform faster than females), as well as were Days, $F(4.53, 126.89) = 50.78, p < .001, \eta^2_p = .65$, and the interaction Sex $\times$ Days, $F(4.53, 126.89) = 4.63, p = .001, \eta^2_p = .14$. The interaction Group $\times$ Days was close to the level of significance, $F(4.53, 126.89) = 2.24, p = .060, \eta^2_p = .07$. Additional simple effects analysis of the interaction Sex $\times$ Days revealed that males outperformed females on Days 1 and 6–15, minimum $F(1, 30) = 5.15, p = .031, \eta^2_p = .15$. No other main effect or interaction was significant ($F_s \leq 1.82$). The analysis of the escape latencies during Test Phase 1 (Days 17–19) revealed that the only significant variable was Sex, $F(1, 28) = 27.07, p < .001, \eta^2_p = .49$ (males reached the platform faster than females). No other main effect or interaction was significant ($F_s \leq 1.32$). The ANOVA of the second landmark learning phase (Days 20–25) showed that the variable Sex was significant, $F(1, 28) = 30.60, p < .001, \eta^2_p = .52$ (males reached the platform faster than females), as well as Group, $F(1, 28) = 6.91, p = .014, \eta^2_p = .20$ (rats in Group Bc, with landmarks of different salience, were faster to reach the platform than rats in Group bc, with landmarks of the same salience). No other main effect or interaction was significant ($F_s \leq 1.1$). The analysis of the escape latencies during Test Phase 2 (Days 26–28) revealed that the only significant variables were Sex, $F(1, 28) = 20.40, p < .001, \eta^2_p = .42$ (males reached the platform faster than females), and Group, $F(1, 28) = 5.24, p = .030, \eta^2_p = .16$ (rats in Group Bc, with landmarks of different salience, were faster to reach the platform than rats in Group bc, with landmarks of the same salience). The variable Days was close to the level of significance, $F(2, 56) = 2.91, p = .063, \eta^2_p = .094$. No other main effect or interaction was significant ($F_s \leq 2.34$). The ANOVA of the escape latencies during Day 29 included only factors Sex and Group, and showed that the variable Sex was significant, $F(1, 28) = 38.21, p < .001, \eta^2_p = .58$ (males outperformed females), as well as Group, $F(1, 28) = 7.50, p = .011, \eta^2_p = .21$ (Bc rats were faster than bc rats). No other main effect or interaction was significant ($F_s \leq 1.53$). Finally, the ANOVA of the escape trials during the Inverted Position Tests (Days 30–31) showed that the variable Sex was significant, $F(1, 28) = 13.51, p = .001, \eta^2_p = .33$ (males reached the platform faster than females). The interaction Sex $\times$ Days was close to the level of significance, $F(1, 28) = 3.13, p = .088, \eta^2_p = .10$, and the triple interaction Sex $\times$ Group $\times$ Days was nearly significant, $F(1, 28) = 4.09, p = .053, \eta^2_p = .13$. No other main effect or interaction was significant ($F_s \leq 0.88$). In conclusion, on escape trials, the performance of the rats improved as days went on with male rats finding the platform faster

¹ GQ was termed MQ, middle quadrant, in the previous study (Rodrigo et al., 2014).

than females. Moreover, with extended training Group Bc showed a better performance than Group bc.

Fig. 2 (bottom panel, left) shows the time spent in the platform quadrant on each type of test trial (i.e., three in total) during Test Phase 1 and Test Phase 2. For Group Bc: Test Bc (the training configuration), Test B (Landmark B alone—the most salient landmark), and Test c (Landmark c alone); for Group bc: Test bc (the training configuration), Test b (Landmark b alone), and Test c (Landmark c alone). These data are averaged over Test Phase 1 and Test Phase 2 with those landmarks (because the two phases did not differ) and over sex (because males and females did not differ). One sample *t* tests were used to compare rats' performance with chance (i.e., 15.0 s searching in the platform quadrant) in order to evaluate whether the test results reflected significant spatial learning. For Group Bc: rats performed above chance on all tests trials except for Test c, minimum $t(15) = 5.29, p < .001, d = 1.32$; for Group bc: rats performed above chance on all tests trials except for Test b, minimum $t(15) = 3.05, p = .008, d = 0.76$ (see Appendix Table A1), although performance on Test b was nearly significant, $t(15) = 2.09, p = .054, d = 0.52$. The spatial task could not be solved with all the individual landmarks, which shows that the rats did not know all the landmarks' identity (specifically, landmark c in Group Bc; and, to a lesser degree, landmark b in Group bc). An ANOVA was conducted to analyze these data taking into account the variables Group, Sex, Tests (Bc/bc, B/b, and c), and Test Phase (1 and 2). The results revealed that the variable Tests was significant, $F(2, 56) = 10.81, p < .001, \eta^2_p = .28$, as well as Group, $F(1, 28) = 8.98, p = .006, \eta^2_p = .24$, and the interaction Group $\times$ Tests was also significant, $F(2, 56) = 5.42, p = .007, \eta^2_p = .16$. No other main effect or interaction was significant ($F_s \leq 2.43$). Simple effects analysis of the interaction Group $\times$ Tests showed that Group Bc spent more time in the platform quadrant than Group bc both in Test Bc/bc and in Test B/b, $F(1, 30) = 13.97, p = .001, \eta^2_p = .32$ and $F(1, 30) = 4.87, p = .035, \eta^2_p = .14$, respectively. These results show a clear salience effect: The best rats' performance was with landmark B (the big ball), Group Bc, which differed from Landmark b (the small ball), Group bc. Moreover, the variable Tests differed only in Group Bc, $F(2, 60) = 15.25, p < .001, \eta^2_p = .34$. Subsequent pairwise comparisons using the Bonferroni correction revealed that the best rats' performance was with Test Bc and Test B, which were close to differ from one another [$t(56) = 2.33, p = .070, d = 0.62$] but both differed from Test c, $t(56) = 5.59, p < .001, d = 1.49$ and $t(56) = 3.26, p = .006, d = 0.87$, respectively. Because, at least visually, in group Bc the rats' best performance was with Test Bc, this result suggests an additive effect between the two landmarks of the configuration formed by B and c.

Fig. 2 (bottom panel, right) also shows the time spent in the four quadrants of the pool (quadrants GQ, BQ/bQ, cQ, and opGQ) by the animals during the Inverted Position Test Phase, Tests cB/cb, in order to evaluate whether the rats' preference in the four quadrants differed from chance (both above and below chance). For Group Bc, rats differed from chance on quadrants GQ, BQ, and opGQ, minimum $t(15) = -2.67, p < .017, d = -0.67$; while for Group bc, rats differed from chance on quadrants GQ and opGQ, minimum $t(15) = 3.80, p = .002, d = 0.95$. None of the groups differed from chance on Quadrant cQ, the quadrant controlled by Landmark c (see Appendix Table A1). These results suggest that the two groups, Bc and bc, had a clear preference for Quadrant GQ; that is, a geometry preference (i.e., a pure configuration in which the shape, a straight line, is abstracted from the landmarks). A subsequent ANOVA conducted on these data, taking into account the variables Group (Bc, bc), Sex, Quadrants (GQ, BQ/bQ) and Tests Repetition (1, 2) confirmed this preference. This analysis revealed that the variable Quadrant was significant, $F(1, 28) = 27.60, p < .001, \eta^2_p = .50$, and the interaction Quadrant $\times$ Group was close to the level of significance, $F(1, 28) = 3.72, p = .064, \eta^2_p = .12$. No other main effect or interaction was significant ($F_s \leq 2.60$). The rats' performance was controlled by the geometry formed by the two landmarks (GQ), independently of their identities.

3.1. General discussion

When rats are trained with two landmarks, both equidistant from a hidden platform, they do not need to know their identity. Often, if the landmarks are close to the platform they simply need to learn to swim toward them, and if they are far away they just need to learn to swim away from them. However, with prolonged training we know that the identities of landmarks can be learned. At least this is the case when the landmarks are relatively close to the platform (Rodrigo et al., 2014, Experiment 2). Conversely, the present experiment shows that this is not always the case when the landmarks are relatively far from the platform. In the present experiment (in which the experimental room, Morris pool, and landmarks were exactly the same as those used in the mentioned study), the distance between the two landmarks and the hidden platform was increased, therefore rendering the task more difficult (for single landmark learning, see Forcano, Santamaría, Mackintosh, & Chamizo, 2009). Our main objective was to check whether the tests results of the present experiment would differ from those by Rodrigo et al. (2014), Experiment 2) and if, eventually, the landmarks themselves could be interfered by their geometrical configuration, the shape of a straight line (i.e., learning in the absence of the landmarks' identities) in Group Bc.

A strong sex difference was found on escape trials (males always reached the platform faster than females), as was also the case in the previous study (Rodrigo et al., 2014), although in the present experiment the two groups ended up differing (Group Bc > Group bc), but not in the previous work (Rodrigo et al., 2014). However, sex differences were not found on any of the subsequent test trials without the platform of Phases 1 and 2 of testing (when a new measure was used), as in Rodrigo et al. (2014), although the results of these test trials varied considerably between the two studies. In the present experiment spatial learning was not found on all test trials, unlike what happened in the work by Rodrigo et al. (2014, Experiment 2), where all test trials differed from chance. Specifically, when the training configuration of landmarks was tested (Tests Bc/bc), performance was above chance in both groups, Group Bc and Group bc. That was not the case when any single landmark was tested (Tests B/b/c); in Group Bc, performance was above chance on Test B only, while in Group bc, on Test c only. In Group bc the variability on Tests b and c could explain these differences. A clear salience effect was found on both Test Bc/bc and Test B/b: the best rats' performance was when Landmark B (the big ball) was present, Group Bc, which differed from when Landmark b (the small ball) was present, Group bc. The two groups did not differ on Test c -which eliminates any explanation that has to do with interference or overshadowing by landmark B in the present experiment. The results on

this test, Test c, were markedly different from those obtained in Rodrigo et al. (2014, Experiment 2), where Group bc performed better than Group Bc—thus showing that the high salience of Landmark B in Group Bc affected not only how well it could be used to locate the platform, but also its ability to reduce learning about the other landmark, Landmark c (i.e., interference by relative salience, Mackintosh, 1976; Miles & Jenkins, 1973). In the present experiment, test trials differed in Group Bc only [(Test Bc = Test B) $\neq$ Test c—although a clear tendency was found between Test Bc and Test B, Test Bc > Test B)]. Therefore, in Group Bc the contribution of Landmark c was less clear than expected. Admittedly, there is the possibility that any difference between the two tests, Bc and B, could be due to generalization decrement—the result of a change between training and testing (Pearce, 1987, 1994). In Group bc the test trials did not differ (Test bc = Test b = Test c). We must conclude that when the distance between the two landmarks and the hidden platform is increased, the previous test trials suggest elemental learning based on the most salient landmark, Landmark B (the big ball), in Group Bc and, surprisingly, Landmark c in Group bc. It is possible to explain this hypothetical “elemental learning” by appealing to within compound associations (Durlach & Rescorla, 1980) between these landmarks (Landmark B in Group Bc and Landmark c in Group bc) and the pool geometry, in addition to knowing the landmarks identity. Specifically, it could be the case that these landmarks had formed a within-compound association with the wall of the pool (see Austen, Kosaki, & McGregor, 2013), with the consequence that the rats could learn the distance of the platform not only from each individual landmark but also from the wall of the pool and then, knowing the landmarks identity (Landmark B in Group Bc and Landmark c in Group bc), right or left of one specific landmark, could be a relatively easy task. The main idea is that by viewing the landmarks while swimming, rats could learn a specific vector between them, and then a further vector between the landmarks and the position of the hidden platform, the goal. Thus, when a rat enters the pool, it could perceive the vector between its current position and that of the landmarks, then recover the stored vector which refer to the landmarks and the goal, and finally calculate a navigational vector that specify the distance and direction of the goal from its current position.

How can the sex differences found on escape trials be explained? As Rodrigo et al. (2014) already claimed, several factors might have affected such a result. For example, there is evidence (Torres, Rodríguez, Chamizo, & Mackintosh, 2014) that the specific nature of a landmark cue plays a crucial role in females' preference—but not in males—when solving a spatial task in the presence of two sources of information (i.e., a specific landmark and the geometrical information provided by the shape of the pool). Only when the landmark looks the same from different perspectives (like a plain cone, but not a pyramid), females show a preference for it. In addition, male and female rats can learn rather different things about a single landmark that signals the location of a hidden platform (Chamizo, Rodríguez, Torres, Torres, & Mackintosh, 2014; Chamizo, Rodríguez, Torres, Torres, & Mackintosh, 2019). In addition, female rats are often more exploratory than males (for classical studies in the open field see Archer, 1975; Broadhurst, 1975; Gray, 1971), with the consequence that any increased tendency to explore might interfere with swimming directly to the platform. Therefore, the fact that females are slower to find the platform than males on landmark learning trials may reflect a difference in exploratory tendencies rather than any difference in spatial ability.

A final test trial measuring salience was also conducted at the end of the experiment by reversing the position of the two training landmarks (i.e., Inverted Position Test). In Group Bc, this test pitted two sources of information against one another, elemental preference based on landmark B vs. geometry preference (i.e., the geometry, a straight line, formed by the two tested landmarks, independently of their identities); in Group bc, this test addressed geometry preference only. A good and the same performance (i.e., well above the level of chance) was found in both groups, Group Bc and Group bc, in the geometry quadrant, GQ (and their time swimming in quadrants BQ/bQ either fell to chance, in Group bc, or was below the level of chance, in Group Bc). In Group Bc, this result reveals a clear geometry preference (i.e., a preference for the geometry formed by the two tested landmarks, a straight line, independently of their identities). In Group bc, the result on the Inverted Position Test reveals good geometry preference. Therefore, the present results do not replicate those obtained by Rodrigo et al. (2014) in Group Bc. They found a good performance (i.e., above the level of chance) and no significant preference in Group Bc in the two target quadrants, GQ and BQ (i.e., the geometry quadrant and the B quadrant); however, they found a clear preference for the geometry formed by the two tested landmarks in Group bc (rats spent significantly more time in the quadrant of the pool that corresponded to the geometry formed by the two landmarks, GQ, and their time swimming in quadrant bQ fell to chance). Therefore, when the two landmarks have the same or a similar salience (i.e., in Group bc), a geometry preference seems to prevail, both when the landmarks are placed relatively close in relation to the hidden platform (Rodrigo et al., 2014) and when they are placed relatively far from it, in the present experiment. However, when the two landmarks have a different salience (i.e., in Group Bc), a geometry preference seems to prevail only when the landmarks are placed relatively far from the hidden platform (i.e., in the present experiment). In other words, in Group Bc the individual landmarks seemed interfered by their geometrical configuration, the shape of a straight line. Thus, greater distance of the landmarks from the hidden platform ended up eliciting a higher-level of processing in rats (i.e., a global processing instead of elemental processing), a result that seems consistent with human participants (Henderson et al., 2006; Trope & Liberman, 2010). Moreover, the present results could shed some additional light on the role that relatively distal from the platform landmarks can play in the Morris pool because they show that many factors can contribute to control an animal's behaviour, a crucial one being the salience of the components of the tested cues or sources of information.

Admittedly, it could be argued that in the present experiment both, extended training and distance of the landmarks from the hidden platform, could be responsible for the final greater control by geometry on the inverted landmark test (i.e., a preference test). Future research should address this issue.

In conclusion, the results of the present experiment agree with the claim that after extended training with a configuration of two landmarks (both relatively far and equidistant from a hidden platform), the searching behaviour of rats can come under the control of different strategies, as claimed by Rodrigo et al. (2014). However, looking at the final preferences of the two studies (Rodrigo et al., 2014, Experiment 2, and the present experiment), it is clear that moving landmarks away from the hidden platform clearly benefits a

Table A1One sample *t* tests to compare rats' performance with chance (15 s) for each group on all quadrants.

Group	Quadrant	<i>t</i>	<i>P</i>	<i>d</i>	Mean Difference
Bc	Bc	7.39	<.001	1.85	8.88
	B	5.29	<.001	1.32	5.92
	C	1.76	.099	0.44	1.78
	GQ	5.44	<.001	1.36	6.42
	BQ	-2.67	.017	-0.67	-3.08
	cQ	0.60	.558	0.15	0.68
	opGQ	-4.12	.001	-1.03	-4.01
	bc	3.30	.005	0.82	3.15
	b	2.09	.054	0.52	2.39
	c	3.05	.008	0.76	1.91
Bc	GQ	3.81	.002	0.95	4.41
	bQ	0.01	.991	0.00	0.01
	cQ	-0.84	.412	-0.21	-0.84
	opGQ	-3.93	.001	-0.98	-3.57

geometry preference, a global processing (i.e., a straight line, independently of the landmarks' identities) at the cost of elemental processing (i.e., individual landmarks).

Author statement

Neither the manuscript nor the data have been published previously. All listed authors have significantly contributed to the manuscript and consent to their names on it.

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Appendix A

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