

# Navigation With Two Landmarks in Rats (*Rattus norvegicus*): The Role of Landmark Salience

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In two experiments, male and female rats were trained in a Morris pool in the presence of 1 (Experiment 1) or 2 (Experiment 2) landmarks, which were placed relatively close in relation to a hidden platform. Experiment 1 established the relative salience of 3 landmarks. Two of them revealed a similar salience, and smaller than a third one, 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 2 landmarks and the effects of varying the salience of one of the landmarks were studied. Subsequent test trials without the platform revealed that finding the platform was controlled by different strategies and that the rats were taking advantage of this redundancy depending on the nature of the test trials. Surprisingly, in Experiment 2 a clear sex difference was found on escape trials only, with males reaching the platform faster than females.

*Keywords:* landmark learning, landmark salience, sex differences, Morris pool, rats

Animals can use different mechanisms and strategies while navigating (for reviews see Rodrigo, 2002; Tommasi, Chiandetti, Pecchia, Sovrano, & Vallortigara, 2012). But many questions remain to be answered yet. For example, in spite of a great amount of research in the last century, it is still unclear what rats learn in the presence of two landmarks. Do they learn like their close relatives, the gerbils? Collett, Cartwright, and Smith (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 the identity of the landmarks. In another test the distance between landmarks was doubled and the gerbils searched in two places defined by the direction and distance of the goal from each of the two training landmarks. These results show that the gerbils, at least the females, calculated the distance and direction of the food from each cue independently, something different to what pigeons seem to do Cheng, 1988; 1989).

Cheng (1988, 1989) trained pigeons to find food in a place that maintained a constant relationship with two landmarks (one bigger than the other one). After training, the birds were tested with the landmarks moved further away. Unlike gerbils, the pigeons did not search in two places. They searched in the middle of the line defined by the two positions of the goal as indicated by the landmarks. Such strategy, often called “middle rule,” 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 distribution of two or more landmarks in space also has geometrical properties. When landmarks were separated so that each one pointed out a different food position (Cheng, 1989), the pigeons searched in the middle of the line defined by these two positions. A possible explanation for these results is that they searched for food in terms of the geometrical relationship between the two landmarks, a line.

There is evidence that rats can learn both the geometrical properties defined by a configuration of landmarks as well as the landmarks’ identity (Greene & Cook, 1997). Greene and Cook (1997) trained male rats in a modified open field in which a fixed configuration formed by six different objects was presented to the animals. The rats searched for food, which was hidden in the upper part of six among 24 poles. Following acquisition, several test trials were conducted: with the same objects but in different positions, with different objects although occupying the same positions of the training objects, and with the training objects in their normal position. The results revealed that the number as well as the identity and the geometrical configuration formed by the objects were important factors when searching for food. Greene and Cook (1997) claimed that the animals learned the geometry formed by the objects and such learning included the objects identity as well as their specific position within the configuration they formed. Would these authors have found the same results

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while varying the salience of the different objects? Would they have found the same results if the animals had been females, instead of male rats, and the task aversive instead of appetitive? The aim of the present study is to answer these questions while using a configuration formed by two different objects or landmarks only: either of the same or of different salience.

The term *salience* refers to significance or noticeability. In the spatial domain Chamizo, Rodrigo, Peris, and Grau (2006) addressed this issue training rats in a Morris pool to find a hidden platform in the presence of a single landmark. Two aspects of the salience of the landmark were studied: relative size and relative distance from the platform. The results revealed that the effects of the two magnitudes studied were additive: It was found a better landmark control of the subjects' performance as the sum of the salience components of a landmark increased. Would the same happen if configurations of landmarks, instead of individual landmarks, are used (i.e., a better landmark control as the sum of the salience components of two landmarks—the total salience of a configuration—increases)? Would the total salience of a configuration affect how the animals learn or what they prefer?

It is worth mentioning that in a previous study (Rodríguez, Aguilar, & Chamizo, 2011) where the procedure and the landmarks were similar as those used in the present experiments, we examined the possibility that the estrus cycle of females could influence their performance and no significant effect was found. Given these null results, we did not measure the rats' estrus cycle in the present experiments in order to avoid unnecessarily stressing them. A significance level of .05 was adopted for all the statistical analysis. Only when the variable sex (or an interaction between sex and another variable) is significant does it appear reflected in the graphs.

### Experiment 1

Experiment 1 tries to establish the relative salience of three single three-dimensional (3D) objects, the landmarks of this study. The aim of the experiment was to check if two of them had a similar salience, and smaller than that of a third one, the supposedly most salient landmark. Because salient landmarks control navigation to a higher degree than less salient landmarks (Chamizo et al., 2006), it was expected that the supposedly less salient landmarks would acquire the same control of the rats' performance and less than the third landmark, which would acquire a higher control than the previous ones. Would that be the case?

### Method

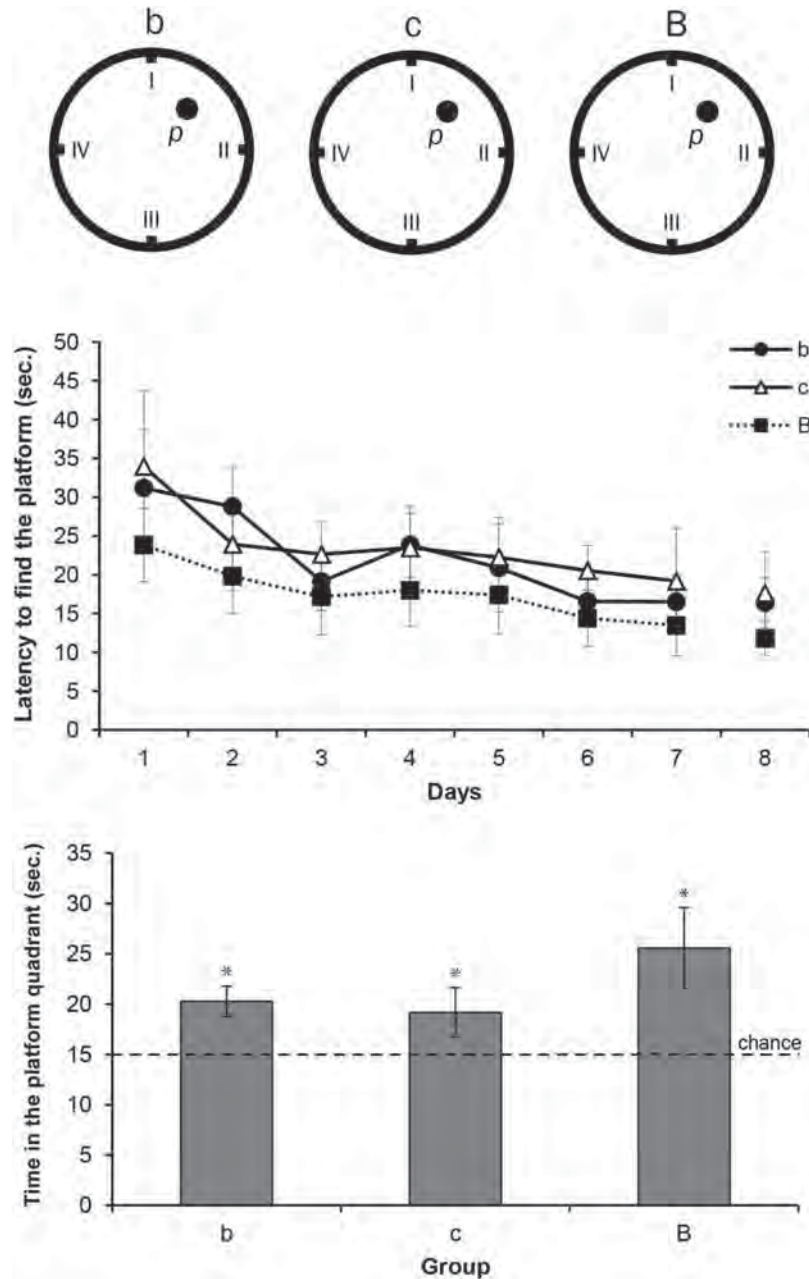
**Subjects.** The subjects were 42 Long Evans rats, 21 males and 21 females, between 3- and 6-months-old at the beginning of the experiment; 12 rats had previously participated in a taste aversion study. They were maintained on ad lib food and water, in a colony room which had a 12:12-hr light–dark cycle, and were tested within the first 8 hr of the light cycle. The animals were divided into three groups of seven males and seven females each, matched for age, previous experience, and latency to find the platform on pretraining trials. One group was trained and tested with Landmark b (Group b), a second group with Landmark c (Group c), and the third group with Landmark B (Group B). All rats learned in the presence of a single landmark (i.e., b, c, or B) which was placed

approximately 40–50 cm from the hidden platform (as shown in Figure 1, top panel).

**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  $22 \pm 1$  °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 recording equipment in an adjacent room. All measures were automatically registered by a computer. 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 16.5-cm diameter plastic ball with mixed colors; (Landmark c) a green plastic plant approximately 35-cm in diameter and 30-cm in high; and (Landmark B) a 32-cm diameter plastic beach ball with alternate blue, white, yellow, white, red, and white vertical segments.

**Procedure.** There were three types of trial: pretraining, landmark learning, and a test trial. 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 Figure 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 the single landmark, either Landmark b, c, or B, was always present. Animals were given eight trials per day over 7 days (a total of 56 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 the landmark were rotated between trials.

After the landmark learning phase, all rats received a test day. This test day consisted of eight training trials (which were identical to the landmark learning phase), followed by one test trial, without the platform, 60 s long. On the test trial, the amount of time (in



*Figure 1.* Top panel: A schematic representation of the pool and the position of the single landmark in the three groups (left: b; center: c; right: B), as well as the hidden platform, *p*, and the starting positions (I, II, III, IV) for Experiment 1. Middle panel: Mean escape latencies during all the escape trials for the rats of Experiment 1. Bottom panel: Rats' performance on the test trial in the three groups (left: b; center: c; right: B). A small asterisk above each test indicates whether each group differed significantly from chance. Error bars denote 95% CI.

seconds) the rat spent in the platform quadrant was recorded (i.e., the dependent measure). Each animal was placed in the pool from one specific position (I, II, III, and IV, as shown in Figure 1, top panel) and rats were, as much as possible, placed equally in the four starting positions. When present, the platform was always situated as shown in Figure 1 (top panel).

## Results and Discussion

Figure 1 (middle panel) shows the mean escape latencies of rats during all the experiment (Days 1–8). Two independent ANOVAs were conducted to analyze these latencies. The ANOVA of the landmark learning phase (Days 1–7), taking into account the variables Group, Sex, and Days, showed that the variable Group

was significant,  $F(2, 36) = 4.23$ ,  $p = .22$ ,  $\eta_p^2 = .19$ , as well as Days,  $F(6, 216) = 14.63$ ,  $p < .001$ ,  $\eta_p^2 = .29$ . Newman–Keuls post hoc comparisons revealed that Group b and Group c, which did not differ from one another, differed from Group B. No other main effect or interaction was significant ( $F_s < 1.5$ ). The ANOVA of the escape trials during the test day (Day 8), taking into account the variables Group and Sex, showed that the variable Group was close to differ,  $F(2, 36) = 2.82$ ,  $p = .073$ ,  $\eta_p^2 = .14$ . No other main effect or interaction was significant ( $F_s < .5$ ).

Figure 1 (bottom panel) shows the time spent in the platform quadrant on the test trial: b, c, B (Group b, Group c, and Group B, respectively). Student  $t$  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. Rats performed above chance on all tests trials; minimum  $t(13) = 3.66$ ,  $p = .003$ ,  $d = .98$ . An ANOVA was conducted to analyze these data taking into account the variables Group and Sex. The results revealed that the variable Group was significant,  $F(2, 36) = 6.29$ ,  $p = .005$ ,  $\eta_p^2 = .26$ . Newman–Keuls comparisons revealed that Group b and Group c, which did not differ from one another, differed from Group B,  $p = .013$  and  $p = .007$ , respectively. No other main effect or interaction was significant ( $F_s < .5$ ).

In conclusion, as expected, both escape trials and test trial revealed that the salience of the landmarks determined the rats' performance: The most salient landmark, B, acquired a better control than the two less salient ones, b and c, which did not differ from each other. Moreover, males and females did not differ.

## Experiment 2

Experiment 1 established the relative salience of three landmarks. As expected (Chamizo et al., 2006), the supposedly less salient landmarks acquired the same control of the rats' performance and less than the third landmark, which acquired a higher control than the previous ones, both in males and in females. In Experiment 2 training was in the presence of two landmarks, which could have either the same or different salience. The aim of the experiment was to investigate the effects of varying the salience of one of the two landmarks in each configuration. Would the animals learn elementally or in a configural way? (i.e., in the first case rats would use individual landmarks to locate the hidden platform, and in the second case they would use the configuration formed by the two landmarks). Would they learn, simultaneously, the geometry formed by the two landmarks? After acquisition, different test trials without the platform were planned to answer these questions.

In Experiment 2 the rats were trained until they had reached asymptotic performance with the purpose of eliminating possible sex differences (see Crespo, Rodríguez, & Chamizo, 2012, Experiment 2; and Chamizo, Rodríguez, Espinet, & Mackintosh, 2012, Experiment 1). Surprisingly, on training the groups did not differ and a strong sex difference was observed. Following Schenk and Morris's (1985, p. 20) suggestion that "... escape latency is a poor measure of spatial localization capacity" (p. 20), the rats were tested, without the platform, with the training landmarks, in the hope to find a group effect. Although the rats trained with the configuration with more total salience (Group Bc) spent more time searching for the platform in the platform quadrant than those animals trained with

the configuration with less total salience (Group bc), the two groups did not statistically differ. Then the animals were also tested with the training landmarks, although individually presented (i.e., Test Phase 1). A clear interference effect of the smaller landmark (c) by the bigger one (B) was expected in Group Bc only. The rats received a second training phase followed by exactly the same test trials (i.e., Test Phase 2). Following Test Phase 2, all rats received one further day of escape training, and then two final test trials with the training landmarks simultaneously present, although with their positions reversed (i.e., Inverted Position Test Phase). In the Inverted Position test trials, the time spent in the four quadrants of the pool was registered (i.e., although only two were analyzed): Elemental and geometrical preferences were put into conflict.

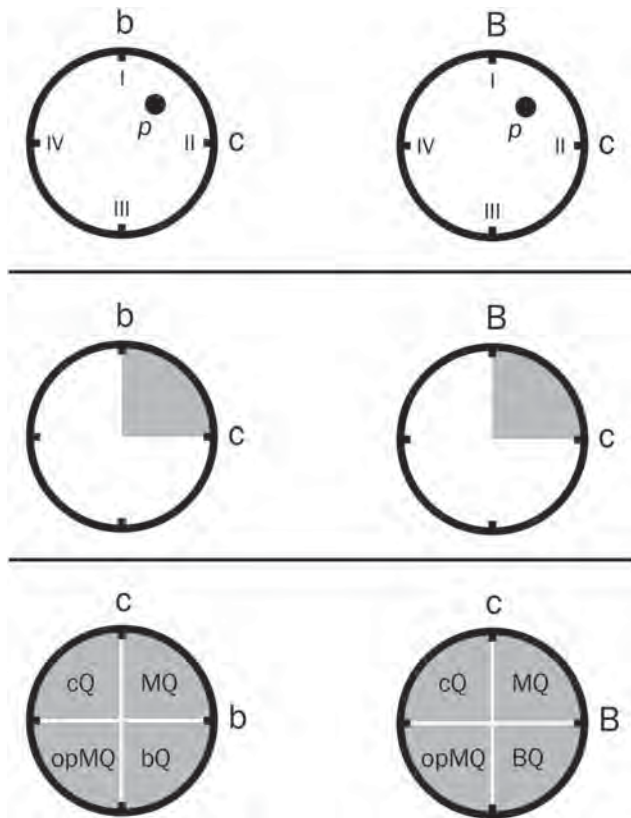
## Method

**Subjects and apparatus.** The subjects were 32 Long Evans rats, 16 males and 16 females, approximately 5 months old at the beginning of the experiment, that had previously participated in a taste aversion study. They were housed and maintained as in Experiment 1. The apparatus and the landmarks were also the same. The animals were divided into two groups, matched for sex and for latency to find the platform on pretraining trials, with one group being trained and tested with Landmarks b and c (Group bc) and the other with Landmarks B and c (Group Bc).

**Procedure.** There were three types of trial: pretraining, landmark learning, and test trials. Pretraining was exactly the same as in Experiment 1, with the hidden platform and no landmark present. 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 between B and c for Group Bc and between b and c for Group bc, as shown in Figure 2 (top panel). Animals were given eight trials per day over 12 days (a total of 96 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 (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 behavior, 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 I–II in Figure 2, 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).





**Figure 2.** 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) for Experiment 2. Middle panel: Platform quadrant registered on test trials for bc and Bc rats. Bottom panel: Preference test. MQ, quadrant controlled by the geometry, a straight line, formed by the tested landmarks; bQ and BQ, quadrants controlled by landmarks b and B; opMQ, shows the rats' performance on the quadrant opposite to MQ.

Following Test Phase 1, all rats received four further days of landmark learning (a total of 32 trials), the procedure being exactly the same as before, followed by a further three test days (Test Phase 2) which were exactly the same as Test Phase 1.

Finally, all rats received one further day of landmark learning followed by two further test days (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. On these test trials the amount of time the rat spent in the four quadrants of the pool, MQ, BQ/bQ, cQ, and opMQ (i.e., the quadrant in the middle of the two landmarks, the quadrant controlled by Landmarks B or b, the quadrant controlled by Landmark c, and the quadrant opposite to the middle quadrant, respectively—Quadrants I–II, II–III, and IV–I in Figure 2, bottom panel) was recorded automatically by the program. Only the two target quadrants were further analyzed (i.e., MQ and BQ/bQ; a preference test between Landmarks B or b vs. the geometrical properties defined by the two testing landmarks, independently of their identities).

## Results and Discussion

Figure 3 (top panel) shows the mean escape latencies of rats during all the experiment (Days 1–25). Six independent ANOVAs were conducted to analyze these latencies, taking into account the variables Group, Sex, and Days (although with the exception of Day 23, where the variables were Group and Sex, only). The ANOVA of the first landmark learning phase (Days 1–12) showed that the variable Sex was significant,  $F(1, 30) = 18.24, p < .001, \eta_p^2 = .38$ , as well as Days,  $F(11, 330) = 25.22, p < .001, \eta_p^2 = .46$ , and the interaction Sex  $\times$  Days was also significant,  $F(11, 330) = 1.87, p = .043, \eta_p^2 = .06$ . No other main effect or interaction was significant ( $F_s < 1.5$ ). Additional analysis of this interaction, Sex  $\times$  Days, revealed that males outperformed females on Days 2, 3, 5, 7, 8, 10, and 12; minimum  $F(1, 32) = 6.67, p = .015, \eta_p^2 = .17$ . The analysis of the escape latencies during Test Phase 1 (Days 13–15) revealed that the only significant variable was Sex,  $F(1, 30) = 16.01, p < .001, \eta_p^2 = .35$ . No other main effect or interaction was significant ( $F_s < 3.0$ ). The ANOVA of the second landmark learning phase (Days 16–19) showed that the variable Sex was significant,  $F(1, 30) = 16.36, p < .001, \eta_p^2 = .35$ , as well as Days,  $F(3, 90) = 6.52, p < .001, \eta_p^2 = .18$ , and the interaction Sex  $\times$  Days was also significant,  $F(3, 90) = 4.91, p = .003, \eta_p^2 = .14$ . Simple effects analysis of this interaction, Sex  $\times$  Days, revealed that males outperformed females on all 4 days; minimum  $F(1, 32) = 10.64, p = .003, \eta_p^2 = .25$ . No other main effect or interaction was significant ( $F_s < 1.0$ ). The analysis of the escape latencies during Test Phase 2 (Days 20–22) revealed that the only significant variable was Sex,  $F(1, 30) = 9.48, p = .004, \eta_p^2 = .24$ . The triple interaction Group  $\times$  Sex  $\times$  Days was also significant,  $F(2, 60) = 3.23, p = .047, \eta_p^2 = .10$ . No other main effect or interaction was significant ( $F_s < 2.0$ ). Additional analyses of the triple interaction revealed that on Group Bc males outperformed females on Days 1 and 3; minimum  $F(1, 31) = 5.55, p = .025, \eta_p^2 = .15$ ; although on Group bc males outperformed females on Days 1 and 2; minimum  $F(1, 31) = 5.39, p = .027, \eta_p^2 = .15$ . Males outperformed females on Day 23 also,  $F(1, 30) = 5.49, p = .026, \eta_p^2 = .16$ . No other main effect or interaction was significant ( $F_s < 1.5$ ). Finally, the ANOVA of the escape trials during the Inverted Position Tests (Days 24–25) showed that the only significant variable was Sex,  $F(1, 30) = 12.93, p = .001, \eta_p^2 = .30$ . No other main effect or interaction was significant ( $F_s < 3.5$ ). In conclusion, on escape trials, the performance of the rats improved as days went on with male rats finding the platform faster than females. Extended training did not affect this result.

Figure 3 (bottom panel, left) shows the time spent in the platform quadrant on each type of test trial (i.e., 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). Student *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. Rats performed above chance on all tests trials; minimum  $t(15) = 3.41, p < .004, d = .85$ . The spatial task could be solved with all the

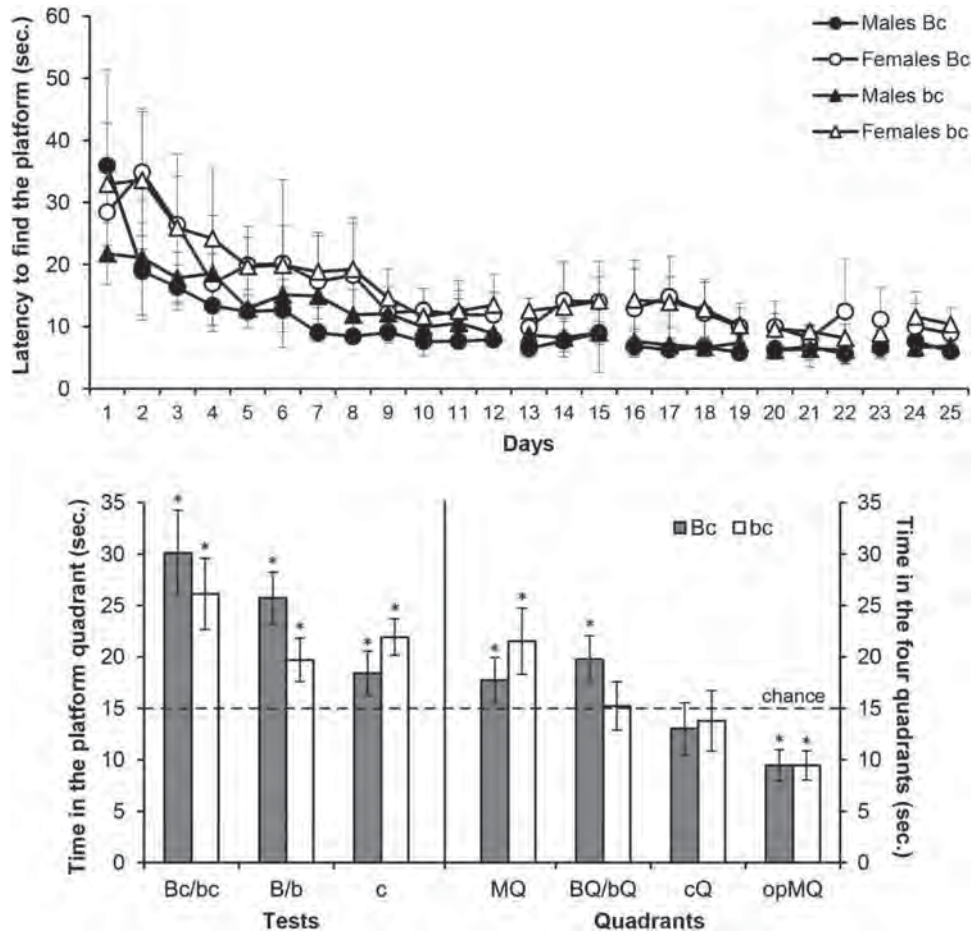


Figure 3. Top: Mean escape latencies during all the escape trials for the rats of Experiment 2. Bottom, left: Time searching in the platform quadrant by rats of Experiment 2 during the averaged test trials, Bc/bc, B/b, and c. Bottom, right: Time searching in the four quadrants (MQ, middle quadrant formed 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 opMQ, which shows performance on the quadrant opposite to MQ) by rats of Experiment 2 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 95% CI.

individual landmarks, which shows that the rats knew the landmarks' identity. 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 only significant variable was Tests,  $F(2, 56) = 30.82, p < .001, \eta_p^2 = .52$ , and the interaction Group  $\times$  Tests was also significant,  $F(2, 56) = 11.77, p < .001, \eta_p^2 = .30$ . No other main effect or interaction was significant ( $F_s < 1.0$ ). Simple effects analysis of the interaction Group  $\times$  Tests revealed that the variable Tests differed in the two groups, Bc and bc,  $F(2, 60) = 32.56, p < .001, \eta_p^2 = .52$ , and  $F(2, 60) = 9.93, p < .001, \eta_p^2 = .25$ , respectively. Subsequent pairwise comparisons on Group Bc revealed that the best rats' performance was with Test Bc, which differed from Test B and from Test c, which differed from one another ( $ps \leq .014$ ). On Group bc, the best rats' performance was with Test bc, which differed from Test b and from Test c ( $ps \leq .006$ ), which did not differ from one another. Moreover, on Test Bc/bc, with the training configuration of landmarks, although Bc subjects spent more time

in the platform quadrant than bc rats, this difference was not significant; on Test B/b a clear salience effect was found: The best rats' performance was with Landmark B (the big ball), Group Bc, which differed from Landmark b (the small ball), Group bc,  $F(1, 30) = 15.21, p = .001, \eta_p^2 = .34$ . Most importantly, the groups also differed on Test c: Group bc performed better than Group Bc on test trials to c alone,  $F(1, 30) = 7.26, p = .011, \eta_p^2 = .19$ . This result shows 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 the other landmark, Landmark c. No differences were found between males and females and extended training did not affect these results. In these phases—Test Phase 1 and Test Phase 2—we also performed two independent ANOVAs (one with only Test Bc/bc, and the second one with Tests B/b and c), and the same results were found.

It could be argued that, for example when testing the training landmarks individually presented (i.e., Test B/b, or Test c), the rats approached the landmark without differentiating between the

quadrants on either side of the landmark. To control for this possibility, two independent ANOVAs were conducted (one for Test B/b, and the second one for Test c), taking into account the variables Group, Sex, Phase, and Quadrant (i.e., the two quadrants on either side of each tested landmark—target vs. adjacent). The interactions Group  $\times$  Quadrant were significant,  $F(1, 28) = 8.12$ ,  $p = .008$ ,  $\eta_p^2 = .23$ , and  $F(1, 28) = 16.33$ ,  $p < .001$ ,  $\eta_p^2 = .37$  (Test B/b and Test c, respectively). Simple effects analysis of this interaction for Test B/b, revealed that Bc rats outperformed bc animals in the target quadrant,  $F(1, 30) = 15.21$ ,  $p = .001$ ,  $\eta_p^2 = .34$ . Moreover, the two groups, Bc and bc, showed a better performance in the target quadrant than in the adjacent one,  $F(1, 30) = 49.64$ ,  $p < .001$ ,  $\eta_p^2 = .62$ , and  $F(1, 30) = 9.33$ ,  $p = .005$ ,  $\eta_p^2 = .24$ , respectively. Simple effects analysis of the interaction Group  $\times$  Quadrant for Test c, revealed that bc rats outperformed Bc animals in the target quadrant,  $F(1, 30) = 7.26$ ,  $p = .011$ ,  $\eta_p^2 = .19$ , although the reverse was true in the adjacent quadrant—where Bc rats had a better performance than bc animals,  $F(1, 30) = 10.74$ ,  $p = .003$ ,  $\eta_p^2 = .26$ . Moreover, only Group bc showed a better performance in the target quadrant than in the adjacent one,  $F(1, 30) = 40.27$ ,  $p < .001$ ,  $\eta_p^2 = .57$ . After training with multiple landmarks, finding the platform when only a single landmark is available can be seen as a little surprising result (Prados & Trobalon, 1998; Rodrigo, Chamizo, McLaren, & Mackintosh, 1997), because a single, symmetrical, distal landmark cannot unambiguously define the location of the platform (although it can define the distance to the platform). Nevertheless, it is possible to explain this hypothetical “elemental learning” by appealing to within-compound associations (Durlach & Rescorla, 1980) between the landmarks and the pool geometry, in addition to knowing the landmarks identity. Specifically, it could be the case that each landmark 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, right or left of one specific landmark, is an easy task. Moreover, when testing the training landmarks individually presented a configural explanation is difficult to rule out because once a configural representation has been constructed, it may be activated by one of the elemental components (Sutherland & Rudy, 1989).

Figure 3 (bottom panel, right) also shows the time spent in the four quadrants of the pool (quadrants MQ, BQ/bQ, cQ, and OpMQ) 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. All rats differed from chance on quadrants MQ and OpMQ; that is, they performed above and below chance, respectively—minimum  $t(15) = 2.63$ ,  $p = .019$ ,  $d = .66$ ; on quadrant BQ/bQ, only Bc rats differed from chance,  $t(15) = 4.48$ ,  $p < .001$ ,  $d = 1.12$ ; and none of the groups differed from chance on Quadrant cQ, the quadrant controlled by Landmark c. At least at first glance, looking at quadrants MQ and BQ/bQ, these results suggest that the two groups, Bc and bc, seem to differ in their preferred strategies: Although Bc rats show no clear preference, the results of bc animals indicate a clear preference for Quadrant MQ; that is, a geometry preference. A final ANOVA was conducted to analyze these preferences, taking into account the variables Group (Bc, bc), Sex, Quadrants (MQ, BQ/bQ) and Days of tests (1, 2). This analysis revealed that the

variable Days was significant,  $F(1, 28) = 7.62$ ,  $p = .010$ ,  $\eta_p^2 = .21$  (reflecting that the performance of the rats was better on Day 1 than on Day 2; but days did not interact with any other variable), as well as the interaction Quadrant  $\times$  Group,  $F(1, 28) = 9.18$ ,  $p = .005$ ,  $\eta_p^2 = .25$ . No other main effect or interaction was significant ( $F_s < 3.0$ ). Simple effects analysis of the interaction Quadrant  $\times$  Group revealed that only bc subjects differed between both quadrants,  $F(1, 30) = 10.88$ ,  $p = .003$ ,  $\eta_p^2 = .27$ . Most importantly, the groups, Bc and bc, differed on both quadrants, MQ and BQ/bQ,  $F(1, 30) = 4.31$ ,  $p = .047$ ,  $\eta_p^2 = .13$ , and  $F(1, 30) = 8.81$ ,  $p = .006$ ,  $\eta_p^2 = .23$ , respectively. As expected, bc subjects outperformed Bc rats when testing the preference for the middle quadrant, MQ; that is, showing that the rats' performance was controlled by the geometry formed by the two landmarks, independently of their identity; but the reverse was true for quadrant BQ/bQ, where Bc subjects outperformed bc rats when testing elemental learning—that is, showing that the rats' performance was controlled by the most salient landmark, Landmark B.

## General Discussion

We began this article by wondering about what rats learn in the presence of two landmarks, and whether such learning would be like that of their close relatives, the gerbils (Collett et al., 1986), or more like that of pigeons (Cheng, 1988, 1989). There are many factors that influence what animals can learn (Mackintosh, 1974, 1983), and therefore the question has no easy answer. Under the specific conditions of the present work neither gerbils (at least, female gerbils) nor pigeons seem good models for rats. Experiment 1 established the relative salience of three landmarks and Experiment 2 revealed important sex differences. Namely, rats in Experiment 2 were trained to find a hidden platform in the presence of a configuration formed by two landmarks that varied in terms of their salience. Although the two groups (Bc and bc) did not statistically differ during acquisition, a strong sex difference emerged, with males reaching the platform faster than females. This sex difference, however, was not found in any of the subsequent test trials.

Spatial learning was found on all test trials of the Test Phases 1 and 2 (Figure 3, bottom left): Performance was always above chance, when the training configuration of landmarks was tested and when any single landmark was tested. Do these test trials show elemental instead of configural learning? Configural learning cannot be ruled out because it is possible that during acquisition the rats might have constructed a configural representation between the two landmarks, in addition to the wall of the pool, and the platform position. If that was the case, once this configural representation is formed it could be activated by any of the individual landmarks during a test trial (for the same argument, although using more traditional stimuli, see Sutherland & Rudy, 1989). Therefore, the rats' performance when any single landmark was tested would not be disrupted because this landmark will activate the representation of the missing one, thus persisting to swim in the correct quadrant. A clear distinction between elemental and configural associations is not an easy task (Pearce, 1987; Sutherland & Rudy, 1989).

Because in both groups (Bc and bc) the rats' best performance on test (Test Phases 1 and 2; see Figure 3, bottom left) was with the training landmarks when presented in compound [on Group



Bc, Test Bc > Test B > Test c; and on Group bc, Test bc > (Test b = Test c)], this result suggests a clear additive effect between the two landmarks of each configuration. Unfortunately this statement cannot be conclusive because the three test trials—the training landmarks in compound and one by one—were not counterbalanced (although it is worth mentioning that the escape latencies on the testing days did not differ). On Test B/b a clear salience effect was found: the best rats' performance was with Landmark B (the big ball), Group Bc, which differed from Landmark b (the small ball), Group bc. The reverse was true on Test c: Group bc performed better than Group Bc. These last results clearly show 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).

Finally, the test trials of the Inverted Position Test Phase (Figure 3, bottom right), pitted two sources of information against one another (i.e., the preference based on the individual Landmarks B/b vs. the spatial configuration or geometry formed by the two tested landmarks, independently of their identities). Although a good performance (i.e., above the level of chance) and no significant preference was found in Group Bc in the two target quadrants, MQ and BQ, a clear preference was found for the spatial configuration or 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, MQ, and their time swimming in quadrant bQ fell to chance). These results agree with the claim that only when the salience of the two landmarks is similar (like in Group bc), an alternative strategy to landmark preference, the geometry they form, can prevail. Interestingly, this does not seem to be the case in pigeons (Cheng, 1988, 1989), because after training to find food with two landmarks, when they were tested with the landmarks moved further away, the birds searched in the middle of the line defined by the two positions of the goal as indicated by the two landmarks, even though the two landmarks had, presumably, a very different salience. Noteworthy, by reversing the position of the two training landmarks on the Inverted test trials, Sutherland and Rudy's (1989) prediction would be performance at chance in the middle quadrant, MQ, because for these authors the identity of the landmarks within a configuration is crucial. In conclusion, here too the salience of the two landmarks seems to be a critical determinant of the kind of strategy preferred. When the two landmarks have a clear different salience, the strategy preferred will be elemental, based on the most salient landmark, and when the two landmarks have approximately the same salience, geometrical. These results agree with the claim that after extended training with a configuration of landmarks, the searching behavior of animals can come under the control of different strategies.

How can the total absence of sex differences in Experiment 1 in comparison with the clear sex difference found on escape trials in Experiment 2 be explained? We do not know, although several factors might have affected such a result. For example, it could be argued that Experiment 2 repeated Experiment 1 but made the task more difficult by presenting two landmarks, instead of a single one, to locate the platform (for a demonstration showing that male and female rats learn to swim to the platform equally rapidly when a swimming problem is made easier, see Forcano, Santamaría,

Mackintosh, & Chamizo, 2009). Furthermore, 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). We believe that such differences between males and females could have many important implications and deserve further research.

There are few experiments in the spatial domain in which the issue of salience of landmarks is addressed. In a previous study in our laboratory (Rodrigo, Sansa, Baradad, & Chamizo, 2006) rats (*Rattus norvegicus*) were trained to find a hidden platform which was signaled by a beacon in the presence of a second landmark located at a short distance (i.e., presumably, two landmarks of different salience). Then the rats received test trials, without the platform, in which the beacon was presented in different positions in relation to the second landmark. The test results showed a generalization gradient, which was a function of the relative distance of the two objects: more time searching in the beacon segment, where the platform should have been, when the beacon was in the original position, which decreased symmetrically with distance of the beacon from the second landmark. Similar results have been found in pigeons, *Columbia livia* (Cheng, Spetch, & Johnston, 1997), in honeybees (Cheng, 2000), and in humans (Artigas, Aznar-Casanova, & Chamizo, 2005; Cheng & Spetch, 2002). As Tommasi, Chiandetti, Pecchia, Sovrano, and Vallortigara (2012) claim, these studies suggest that often all objects in a configuration can be taken into account during spatial learning, even when they are not the most informative or salient.

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