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## **The effect of disrupting configural information on rats' performance in the Morris water maze**

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**Abstract**

Many experiments on spatial navigation suggest that a rat uses the configuration of extra-maze landmarks to guide its choice of arm or location to visit. In the present study, based on Chamizo Rodríguez, Espinet, and Mackintosh's, (2012) navigation paradigm, we conducted a series of experiments in which we focused on how changes to the configuration of stimuli surrounding the maze, implemented by transposing the location of both near and far landmarks, significantly affected rats' performance (Experiment 1, Test Phase 1). Subsequent tests demonstrated that it was the near landmarks that played the major role in this navigation task (Experiment 1, Test Phases 2 and 3). Experiment 2 provided evidence for a novel type of inversion effect in the water maze, by showing that rotation by 180 degrees of the location of one set of landmarks relative to a directional cue also strongly affected performance.

Keywords: spatial learning, associative learning, configural information.

## 1. Introduction

The use of visual cues to find a specific target has been demonstrated in numerous organisms including insects (Chittka, Geiger, and Kunze, 1995), turtles (Lopez et al., 2000), fish (Sovrano, Bisazza, and Vallortigara, 2003), rats (Suzuki, Augerinos, and Black, 1980), birds (Cheng, 1989), non-human primates (Sutton, Olthof, and Roberts, 2000) and humans (Spetch, 1995). These studies have revealed several ways in which spatial information from visual cues near a target may be encoded and used to remember the target location. Some findings on the use of landmarks by animals other than humans, such as the preference for landmarks near a target and competition between landmarks, have been demonstrated to be general across species from insects to humans (for reviews, read Cheng & Spetch, 1998, and MacDonald, Spetch, Kelly & Cheng, 2004).

Some of the most important findings on spatial navigation concern the use of landmark configurations. One design that has been deployed with several species involves training in which the target is hidden at a fixed location relative to a set of two or more identical landmarks all contained within a larger environment. Thus, the landmarks are located within a well-defined search space which provides directional cues, but the set of landmarks and the corresponding target are moved within the search space so that the landmarks must be used to localise the precise location of the target (MacDonald, Spetch, Kelly, & Cheng, 2004). There could be several ways in which spatial information about the landmarks is used in these experiments. For example, the subject could encode the entire set of landmarks as a configuration and learn the location of the target with respect to this configuration. Alternatively, a second strategy would be to encode the direction and the distance of the target from each landmark individually, but this is not so useful when the landmarks presented are visually identical. Another approach that could be used is to simply search for the target close to the landmark array, thus using the landmarks collectively as a beacon. This

could be very useful if the landmarks are extremely close to the target. Different manipulations of the landmarks have been implemented to investigate the various strategies used by animals and human in solving this type of problem. An example of such a manipulation is to enlarge the landmark array by moving all the landmarks farther apart. Interestingly, the results of these studies on various species have shown that honeybees and humans spontaneously use a fully configural representation of a landmark array (See Cartwright & Collett, 1982 for studies on training honeybees with three landmarks; Spetch, Cheng, & MacDonald, 1996 for studies on humans using both computer screen and table top tasks).

In this paper we investigate the effect that disruption and inversion of landmark configurations has on rats' navigation in a water maze. The classic study in the radial maze on this issue is perhaps that by Suzuki, Augerinos and Black (1980), in which they trained rats to run the radial maze using landmarks at the end of each arm, and then showed that random transposition of these landmarks severely disrupted performance, but rotation of the landmarks as a whole simply rotated the rats choices on test. Their interpretation of this result is that performance is based on the landmarks, but that they do not act as "beacons" either in isolation or collectively, but instead it is the configuration of landmarks that provides the information used for navigating in the radial maze. In other words, the spatial arrangement of the landmarks matters, and the whole configuration is more than just the sum of its parts.

We also know that rotation of landmarks in the Morris water maze will cause the animals to track the orientation of the configuration of landmarks and use this as their reference for navigation, not least because this is now standard procedure in training in such a maze for the type of experiment reported here. Studies in the Morris water maze have shown that animals trained with four (i.e., A, B, C and D) landmarks performed less accurately when tested with sets of two landmarks alone than animals initially trained with these two landmarks in

isolation. This could be because B and C, or D and A landmarks alone are perceived as different from A, B, C and D all together, and the response established to one stimulus configuration cannot be transferred perfectly to a different configuration, resulting in generalization decrement (Pearce, 1987, 1994; Chamizo, Rodríguez, Espinet, & Mackintosh, 2012). Another way of expressing this result would be to say that the four landmark case suffers from greater overshadowing of one landmark by the others than the two landmark case, but we note that Chamizo et al. (2012) demonstrated that the addition of two new landmarks, and the removal of two old ones, both disrupted performance. They argued that these results were consistent with the proposition that a change in the stimulus conditions from the training phase to the test phase led to generalization decrement. There is no doubt that Pearce's (1987) theory is one of those capable of providing both effects via one similarity-based mechanism (for other theories capable of generating this result, see McLaren, Forrest, & McLaren, 2012; and Honey, 2000). We also agree that a simple elemental theory employing something like the Rescorla-Wagner algorithm (Rescorla & Wagner, 1972) would have to appeal to a process such as external inhibition in combination with overshadowing to explain this result, making it a less plausible account of these results. More sophisticated theories of this type, such as the replaced elements model (Wagner & Brandon, 2001) and McLaren and Mackintosh's (2000, 2002) extension of McLaren, Kaye and Mackintosh (1989), do possess mechanisms that would produce external inhibition (elements active when A and D were presented together would decrease in activity or disappear altogether when C and D were added), however, and could explain this finding equally well. Putting any difficulty in pinpointing the precise mechanism generating this effect to one side, our point here is that the simple addition or deletion of landmarks seems to significantly influence performance in the water maze, once again suggesting that the configuration of landmarks is key.

In the same study, Chamizo et al. (2012) investigated whether rats learn about the identity of the landmarks in these experiments. Their results showed that rats knew about the identity of the landmarks learnt during the training phase, because a rat's performance was significantly disrupted by swapping the landmarks original positions. Given that there were only two landmarks, and that the platform was always between them, the fact that performance was impaired when the landmarks were swapped indicates that rats were distinguishing between them and not just treating them as a configuration of two identical features, but as specific landmarks at specific locations. And we are able to conclude that performance is not only affected by adding and removing landmarks, but is also affected by something akin to the type of transposition used by Suzuki et al. (1980). The weakness of this study, of course, is that only two landmarks were used, and we address this point in the experiments that follow.

Taken together, these results strongly imply that rats use the spatial configuration of the landmarks present in order to find the platform location. In this study we used Chamizo et al.'s (2012) rat navigation paradigm by always employing a configuration of four landmarks during the acquisition phase and test phase. Our intention was to examine the extent to which the landmark configuration is important by means of various subtle (and not so subtle) changes to that configuration between training and test. As a secondary issue, we also examined the extent to which our manipulations differentially affected performance of male and female rats, i.e. whether any sex-based differences could be observed as a consequence of our manipulations. Recent research (Torres, Rodríguez, Chamizo, & Mackintosh, 2014; Chamizo, Rodríguez, Torres, Torres, & Mackintosh, in preparation) has shown that the appearance of landmarks can produce a substantial and reliable sex difference. For example, in the study by Torres et al. (in press, Experiment 2), male and female rats were trained in a triangular-shaped pool to find a hidden platform, whose location was defined in terms of two

sources of information, a landmark outside the pool and a particular corner of the pool. Two identical cylinders were used as landmarks, one plain white and the other divided into four vertical segments, each “patterned” differently. On the test trial where the two sources of information (landmark and pool geometry cues) were pitted against one another, female rats preferred the plain white cylinder to the geometrical cue, but this preference was reversed when the cylinder was divided into four different patterns. The implication is that the landmark would win out over the pool geometry cue for females only when it looked the same from all perspectives. Thus a previous sex difference observed in rats’ preference between a landmark and geometrical information from the pool (Rodríguez, Torres, Mackintosh, & Chamizo, 2010; Rodríguez, Chamizo, & Mackintosh, 2013) depended on the nature of the landmark. While exploring sex differences, similar inconsistencies related to the material used have also been found in humans (Heil & Jansen-Osmann, 2008; Jansen-Osmann & Heil, 2007) using mental rotation tasks and different stimuli.

Our intention in this paper is to explore exactly how landmarks are used in combination to guide navigation in the rat. As a subsidiary issue, we also investigate any sex differences that arise in the course of this investigation. In the present studies we first of all focused on how what we call “flipping” the location of both near and far landmarks (see later), affected rats’ performance (Experiment 1 Test Phase 1). To anticipate somewhat, this experiment establishes that this type of landmark transposition has the effect of reducing test performance in our paradigm. Following this, in Experiment 1 Test Phase 2, we investigated the effect that swapping only the nearer landmarks to the platform had on performance when leaving the far ones unaltered, and vice-versa. This experiment suggests that it is the nearer landmarks that are most important in aiding navigation to the target location (platform), in line with previous studies of this type. In Experiment 1 Test Phase 3, we investigated the effect of disrupting landmark configurations by swapping the location of one near landmark

with that of one far landmark and succeeded in demonstrating an effect (worse performance) even when only one of the near landmarks was manipulated. Experiment 1 established the parameters for Experiment 2 (as well as demonstrating effects found in other paradigms), where we employed a novel manipulation that tested the effect of disrupting the landmark configuration by rotating it relative to a strong directional cue.

## **2. Experiment 1**

### ***1.1 Method***

#### ***1.1.1 Subjects***

The subjects were 24 naïve Long Evans rats, 12 males and 12 females. They were approximately three months old at the beginning of the experiment. Rats 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.

#### ***1.1.2 Apparatus***

Following Chamizo et al. (2012), 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 0.49m with water that was made opaque by the addition of 1cl/L of latex. The temperature of the water was kept at 22 degrees C. The pool was placed in the middle of a large room, mounted on a wooden platform 0.43m above the floor. The pool was surrounded by black curtains from the ceiling to the base of the pool, and forming a circular enclosure 2.4m in diameter. Inside this enclosure, around the pool and hanging from a black false ceiling, four equally spaced landmarks were placed. They were suspended from the false ceiling, 23 cm above the surface of the water, and had their midline directly above the wall of the pool. These four objects were chosen with reference to Chamizo and Rodrigo's (2004) study in which they showed that the relative



distance of a single landmark from a hidden platform could contribute to the salience of the landmark. In a subsequent study, Chamizo, Rodrigo, Peris, and Grau (2006) demonstrated that the effects of two components of the salience of a landmark, such as its relative size and its relative distance from a goal (platform in our case), show additive properties. The consequence of this would be better control of the subjects' performance by the landmark as the sum of the salience components of that landmark increases. We were fortunate in that Chamizo et al. (2012) had already conducted a preliminary experiment with 32 rats to ensure that the four landmarks we used were of similar salience at the same distance. Our four landmarks were: -A: a white cardboard cube (20cm high) with a black circle at the center of each side of 9.5-cm diameter; -B: a green plastic plant approximately 35 cm in diameter and 30 cm in height; -C: a plastic beach ball 30 cm in diameter with alternate colored vertical segments; and -D: three mop-heads attached together forming a cylindrical figure 12 cm in diameter and 22 cm high. In the Chamizo et al. experiment, rats were trained with each of these four objects, one at a time, always placed in the same location, so that the centre of the landmark was approximately 50 cm away from the hidden platform. Following acquisition, a test trial without the platform revealed that the four landmarks acquired the same control of the rats' performance in males and in females ( $F_s < 2.5$ ). This null result notwithstanding, Chamizo et al. noted that, to the human eye, two of the landmarks, the cube and the mop-heads, looked more salient as they contrasted more sharply with the black curtains. Therefore, in all the experiments reported in that paper, these two were always the distal landmarks (A and D), while the plant and the ball were always the proximal landmarks (B and C), and we also adopted this convention. Thus, following Chamizo and Rodrigo (2004), we would expect landmarks B and C to be somewhat more salient than A and D by virtue of being nearer to the platform.

For all rats, the configuration of landmarks defined the location of the platform. In order to ensure that the animals used the landmarks rather than static room cues, the locations of the objects and platform were quasi-randomly rotated with respect to the room (90, 180, 270, 360 degrees), with the restriction that all four rotations were used equally each day. A closed-circuit video camera with a wide-angle lens was placed 1.75m above the center of the pool inside the false ceiling, and its picture was relayed to recording equipment in an adjacent room. A circular platform, 0.11m in diameter, made of Perspex was placed on a rod and base, and could be placed in one quadrant of the pool, 0.38m from the side, with its top 1cm below the surface of the water. The entire false ceiling with the landmarks could be rotated from trial to trial, and the platform always rotated with it. The platform was always placed midway between landmarks B and C. Hence the platform was approximately 0.58m from B and C, and 1.12m from A and D. For our purposes, the salient point is that the "near" landmarks, B and C, were roughly half the distance from the platform of the distance of the "far" landmarks A and D, from the platform.

### *2.1.3 Procedure*

*Pretraining.* This constituted five trials over 2 days, with two trials on day 1 and the rest on day 2, and consisted of placing a rat in the pool, without landmarks but with the hidden 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 120 s, it was picked up, placed on the platform and left it in 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 A, B, C, and D in Fig. 1, top). The same procedure was used in the *training phase*, but now the four landmarks were always present. The rats were given eight trials per day over 12 days in this phase, with the exception of the notional day 1, which was actually spread over two days with four trials on each of these days. The four landmarks were always located in such a way that

B and C were “near” to the platform and A and D were the “far” ones. Following escape training, all rats received 4 test days (Test Phase 1). Each test day started with eight escape training trials, followed by a single test trial, on which the rats were placed in the pool, with the four landmarks present, but no platform, and left for 30 s. The same four starting positions were used as in training. The test trials had the following order: Flipped, Normal, Normal, Flipped. The critical manipulation for the Flipped test was to swap the locations of the “near” landmarks and also the “far” landmarks: thus BC became CB and AD became DA. The normal condition was the control in which the landmarks were located as in the training phase. Following the first test phase, Test Phase 1, rats received 2 more days of escape training followed by another 3 test days (Test Phase 2). On day 1 and day 2 of this second test phase, subjects were tested in a counterbalanced order on flipped “near” landmarks (leaving the “far” landmarks in their original positions) or on flipped “far” landmarks that preserved the locations of the near landmarks. On Day 3, there was a control run for this test phase (landmarks in training positions). Finally, after Test Phase 2, rats were presented with another two days of escape training and then given two final test days (Test Phase 3). Day 1 of this phase involved a different “lateral” configural disruption of the landmark locations. This was achieved by swapping B (near) with A (far), or C (near) with D (far). Day 2 was a control run with the landmarks in their original training positions (see Figure 1 for a diagrammatic representation of these different phases). For the purposes of recording the rat’s behaviour, on test trials the pool was divided into four quadrants. Considering Fig. 1, top, they were: A-B, B-C (the platform quadrant), C-D, and D-A. The amount of time the rat spent in the platform quadrant was recorded automatically by the program.

Figure 1: about here please

## 2.2 Results

Figure 2 shows, in blocks of 8 daily trials, the mean escape latencies of male and female rats throughout the experiment. During the training phase (Days 1-12), all rats clearly improved their performance as days went by. During the rest of the experiment, the animals tended to either maintain the asymptotic level reached or to improve slightly, and, in general, males seemed to reach the platform faster than females (see Figure 2). In the analyses we report next, the statistical tests were two-tailed with an alpha of .05 unless otherwise noted. We give the relevant  $F$  ratios, mean square errors and the generalised eta squared measure of effect size for each result.

Figure 2: about here please

A repeated measures ANOVA conducted on the acquisition data (Days 1-12), taking into account the variables Sex and Days, showed that the variable Days was significant,  $F(11, 242) = 31.76$ ,  $MSE = 46.55$ ,  $\eta^2_G = 0.44$ ,  $p < .001$  as well as the interaction Sex x Days,  $F(11, 242) = 2.08$ ,  $MSE = 46.55$ ,  $\eta^2_G = 0.04$ ,  $p < .03$ . An analysis of the interaction Sex x Days revealed that males and females differed on days 10 and 11 [ $F(1, 22) = 5.55$ ,  $MSE = 32.61$ ,  $\eta^2_G = 0.12$ ,  $p < .03$  and  $F(1, 22) = 7.9$ ,  $MSE = 17.23$ ,  $\eta^2_G = 0.18$ ,  $p = .01$ , respectively (with males reaching the platform faster than females), and were close to differing significantly on days 1,  $F(1, 22) = 3.23$ ,  $MSE = 247.41$ ,  $\eta^2_G = 0.07$ ,  $p < .09$ , and 3,  $F(1, 22) = 3.542$ ,  $MSE = 101.24$ ,  $\eta^2_G = 0.08$ ,  $p < .08$ . A repeated measures ANOVA conducted on the escape trials during Test Phase 1 (days 13-16), taking into account the variables Sex and Days, revealed that the variable Sex was significant,  $F(1, 22) = 8.04$ ,  $MSE = 56.39$ ,  $\eta^2_G = 0.16$ ,  $p = .01$  (males reached the platform faster than females). Neither the variable Days nor the interaction Sex x Days were significant ( $F_s < 2.0$ ). A repeated measures ANOVA conducted on the first set of

retraining days (Days 17-18), taking into account the variables Sex and Days, showed that the variable Days was significant,  $F(1, 22) = 8.48$ ,  $MSE = 11.05$ ,  $\eta^2_G = 0.13$ ,  $p < .01$ , as well as the variable Sex,  $F(1, 22) = 8.06$ ,  $MSE = 16.25$ ,  $\eta^2_G = 0.17$ ,  $p = .01$ , but the interaction Sex x Days was not significant ( $F < .05$ ). The subjects took less time to reach the platform on day 18 than day 17, and males were faster than females. A repeated measures ANOVA conducted on the escape trials during Test Phase 2 (days 19-21), taking into account the variables Sex and Days, revealed that the variable Days was significant,  $F(2, 44) = 6.50$ ,  $MSE = 13.87$ ,  $\eta^2_G = 0.11$ ,  $p < .01$ , as well as the variable Sex,  $F(1, 22) = 10.39$ ,  $MSE = 34.17$ ,  $\eta^2_G = 0.20$ ,  $p < .001$  (males reached the platform faster than females), but the interaction Sex x Days was not significant ( $F < 2.5$ ). A repeated measures ANOVA conducted on the second set of retraining days (Days 22-23), taking into account the variables Sex and Days, revealed that the variable Days was significant,  $F(1, 22) = 9.20$ ,  $MSE = 12.48$ ,  $\eta^2_G = 0.09$ ,  $p < .01$ , as well as the variable Sex,  $F(1, 22) = 26.16$ ,  $MSE = 35.16$ ,  $\eta^2_G = 0.46$ ,  $p < .001$ , but the interaction Sex x Days was not significant ( $F < 2.5$ ). The rats took less time to reach the platform on day 23 than on day 22, and males were faster than females. A repeated measures ANOVA conducted on the escape trials during Test Phase 3 (days 24-25), taking into account the variables Sex and Days, revealed that the variable Sex was the only one close to significance  $F(1, 22) = 4.01$ ,  $MSE = 19.96$ ,  $\eta^2_G = 0.12$ ,  $p < .06$ . No other main effect or interaction was significant ( $Fs < 2.5$ ). Thus, we can conclude that, in general, the male rats were able to find the platform more quickly than the female rats during the course of our experiment, but that both sexes learned to find the platform.

### ***2.2.1 Results from the three test phases (see Figure 3)***

The time spent by the rats in the platform quadrant during the test trial was averaged across days for each of the two conditions.

*Figure 3, Test Phase 1* (left) shows the time spent in the platform quadrant by the two groups over the test trials (Control, Flipped) of Test Phase 1. As can be seen, one-way ANOVAs revealed that male rats differed from chance (7.5 sec) in the Control test (they spent longer in the platform quadrant),  $F(1, 11) = 24.37$ ,  $MSE = 4.70$ ,  $\eta^2_G = 0.52$ ,  $p < .001$ , while female rats showed a similar though weaker effect in the Flipped test,  $F(1, 11) = 5.60$ ,  $MSE = 1.51$ ,  $\eta^2_G = 0.20$ ,  $p < .04$ . An  $2 \times 2$  ANOVA conducted on these data taking into account the variables sex (Male vs. Female) and tests (Control vs. Flipped) revealed that the variable tests was significant,  $F(1, 22) = 7.61$ ,  $MSE = 4.40$ ,  $\eta^2_G = 0.09$ ,  $p = .011$  indicating that performance in the Control test was superior to that in the Flipped test, as well as the interaction sex x tests,  $F(1, 22) = 7.09$ ,  $MSE = 4.40$ ,  $\eta^2_G = 0.08$ ,  $p = .014$ . No other main effect or interaction was significant ( $F < 3.0$ ). The analysis of the interaction showed that males and females differed in the Control test,  $F(1, 22) = 5.78$ ,  $MSE = 10.14$ ,  $\eta^2_G = 0.11$ ,  $p = .025$ , with males outperforming females. In addition, the variable tests was significant for males alone,  $F(1, 11) = 16.38$ ,  $MSE = 3.95$ ,  $\eta^2_G = 0.27$ ,  $p = .002$ , with rats showing better performance in the Control test than in the Flipped test. No other main effect or interaction was significant ( $F < 0.5$ ). For completeness, we ran an ANOVA on the latencies to reach the platform quadrant, taking into account the variables sex and tests, which revealed that no main effect or interaction was significant ( $F_s < 0.5$ ). We can conclude on the basis of these analyses that both sexes showed evidence of having learned to find the platform location, that flipping the landmarks disrupted performance, and that this disruption was significantly more pronounced for the males than for the females.

*Figure 3, Test Phase 2* (middle) shows the time spent in the platform quadrant by the two groups over the test trials (Control, Flipped Near, Flipped Far) of Test Phase 2. As can be seen, male rats were significantly above chance (7.5 sec) in all three test trials [ $F(1, 11) =$

15.42,  $MSE = 14.51$ ,  $\eta^2_G = 0.41$ ,  $p < .01$ ;  $F(1, 11) = 5.08$ ,  $MSE = 15.37$ ,  $\eta^2_G = 0.18$ ;  $p < .05$ ,  $F(1, 11) = 30.75$ ,  $MSE = 8.75$ ,  $\eta^2_G = 0.58$ ,  $p < .001$ , Control, Flipped Near, and Flipped Far tests, respectively]. Female rats were significantly above chance in the Flipped Far test,  $F(1, 11) = 6.84$ ,  $MSE = 5.33$ ,  $\eta^2_G = 0.23$ ,  $p < .025$ , but there was a trend towards significance in the control condition as well,  $F(1, 11) = 3.62$ ,  $p < .09$ . Analysis conducted on these data taking into account the variables sex and tests (Control, Flipped Near, Flipped Far) revealed that the variable sex was significant,  $F(1, 22) = 9.91$ ,  $MSE = 33.58$ ,  $\eta^2_G = 0.22$ ,  $p = 0.005$ , with males producing better performance than females; the variable tests was also significant,  $F(2, 44) = 6.27$ ,  $MSE = 9.27$ ,  $\eta^2_G = 0.09$ ,  $p = 0.004$ , with performance on the Flipped Near test worse than that on the Control test (see next section). The interaction sex x tests was not significant ( $F < 1.0$ ).

Further analysis showed a significant effect on performance of flipping “near” landmarks compared to the control condition,  $F(1, 23) = 7.58$ ,  $MSE = 7.77$ ,  $\eta^2_G = 0.05$ ,  $p < .015$ . Breaking this analysis down by sex, there was a trend for flipping “near” landmarks to affect both male rats,  $F(1, 11) = 3.55$ ,  $MSE = 10.54$ ,  $\eta^2_G = 0.05$ ,  $p < .09$ , and female rats,  $F(1, 11) = 3.99$ ,  $MSE = 5.61$ ,  $\eta^2_G = 0.11$ ,  $p < .07$ . Finally performance in the control condition was significantly greater for male than for female rats  $F(2, 11) = 7.02$ ,  $MSE = 9.09$ ,  $\eta^2_G = 0.13$ ,  $p = .015$ . Analysis conducted on the latencies to reach the platform quadrant, taking into account the variables sex and tests (Control, Flipped Near, Flipped Far) revealed that no main effect or interaction was significant ( $F_s < 3.0$ ). We can summarize these results as showing that both sexes had learned the location of the platform, that performance in males was generally better than that in females, and that flipping the near (but not the far) landmarks resulted in worse performance for both sexes.

*Figure 3, Test Phase 3* (right) shows the time spent in the platform quadrant by the two groups over the test trials (Control, Flipped Laterally) of Test Phase 3. As can be seen, both males and females differed from chance in the Control test, [ $F(1, 11) = 20.65$ ,  $MSE = 13.56$ ,  $\eta^2_G = 0.48$ ,  $p < .001$ ;  $F(1, 11) = 36.54$ ,  $MSE = 4.34$ ,  $\eta^2_G = 0.32$ ,  $p < .001$ , respectively]. Analysis conducted on these data taking into account the variables sex and tests (Control vs. Flipped Laterally) revealed that the variable tests was significant, with Control performance superior to Flipped Laterally,  $F(1, 22) = 32.75$ ,  $MSE = 10.13$ ,  $\eta^2_G = 0.26$ ,  $p < 0.01$ , as well as the interaction between sex and tests,  $F(1, 22) = 6.87$ ,  $MSE = 10.13$ ,  $\eta^2_G = 0.07$ ,  $p = 0.016$ ). No other main effect or interaction was significant ( $F_s < 0.5$ ). Further analysis of the interaction showed that the variable tests was significant in males,  $F(1, 11) = 48.65$ ,  $MSE = 7.25$ ,  $\eta^2_G = 0.48$ ,  $p < 0.001$ ), with male rats producing better performance in the Control test than in the other test. This effect was smaller, but nearly significant in females as well ( $F(1, 11) = 3.74$ ,  $p = 0.08$ ). No other main effect or interaction was significant ( $F < 2.5$ ). Analysis conducted on the latencies to reach the platform quadrant, taking into account the variables sex and tests (Control, Flipped Laterally), revealed that that the variable tests was significant,  $F(1, 22) = 6.77$ ,  $MSE = 18.53$ ,  $\eta^2_G = 0.11$ ,  $p = 0.016$ , with rats reaching the platform quadrant in the Control test faster than in the Flipped Laterally test. No other main effect or interaction was significant ( $F_s < 1.5$ ). Thus, we can summarize these results as indicating that both sexes had learned the location of the platform, and that the Flipped Laterally manipulation disrupted the rats tendency to preferentially occupy the platform quadrant. This disruption was larger for the male rats.

Figure 3: about here please



### 2.3 Discussion

The results from the analysis show clear evidence of the link between training on a configuration and performance in the navigation task when that configuration is disrupted. The main finding is that disrupting this configural information caused the rats' performance to decline and become significantly worse than that seen in the control condition (Test Phases 1, 2 and 3). The supplementary finding is the pre-eminent role that the "near" landmarks play in rats' performance on this task. Thus, there was a significant reduction in performance when those landmarks were flipped, but there was no detectable effect of flipping "far" landmarks on their own (Test Phase 2). Finally, the results of our lateral manipulation in which we swapped the location of one "near" landmark with one "far" one, proved very disruptive, consistent with the hypothesis that the configuration of the two near landmarks is vital in guiding performance.

Additional findings come from our results on sex differences. Our analysis found a significant interaction in Test Phase 1. In Test Phase 1, the male rats were the ones most affected by the manipulation, and it would be possible to take the view that they were the only sex affected by it, though we recognize that this conclusion cannot be established by the data as they stand. Whilst male rats showed a clear effect of configural disruption in Test Phase 1 when analysed separately, unfortunately female rats' performance was not strongly above chance in this test, suggesting male rats may have learned the task faster than female rats, and complicating the interpretation of these interactions. Thus, it may simply be that a greater effect of disrupting configural information is seen in the performance of male rats on this test because there is more room, statistically speaking, to detect such an effect in male rats. If the female rats had not learned the task that well, disrupting the information on which they based their performance should have relatively little effect because they were not using it that effectively anyway. The presence of a main effect of the tests factor also argues against

there being statistical support for denying the existence of any disruption in the female rats as a consequence of the flipping manipulation.

The results of Test Phase 2 provide support for the claim that male rats' performance was generally better than that of females in this task (in line with the escape trial results reported earlier). In this test, however, both sexes were affected by the flipping of the near landmarks, and there was no interaction of this effect with the sex factor. Both male and female rats were also affected by the manipulation in Test Phase 3, but in this case males were affected significantly more. Once again, the interpretation of this interaction is complicated by numerically poorer performance for the female rats in the Control test. But, the above chance performance for both sexes in the control test and some evidence that the female rats were affected by the manipulation give us cause to speculate that this interaction may be trustworthy and indicates a genuine differential sensitivity to our manipulation between sexes.

These considerations notwithstanding, we have evidence that disruption of the landmark configuration by flipping the location of the landmarks has a clear effect on rats' navigation in the water maze if the landmarks nearer the platform are the ones affected. In the next experiment, we aimed to investigate a different type of configural disruption. The idea was that if rats are affected by configural transformations like flipping, perhaps something akin to inversion of the landmarks locations would affect navigation as well.

## **2. Experiment 2**

### ***3.1 Method***

#### ***3.1.1 Subjects***

The subjects were 24 Long Evans rats, 12 males and 12 females, that had previously participated in a perceptual learning experiment in which each of the landmarks used in Experiment 1 (i.e., A, B, C, and D –placed as in the previous experiment) was always

accompanied by another object, either X or Y, identical for all landmarks (for half of the animals in each sex X, and for the other half Y). The rats were approximately four months old at the beginning of Experiment 2 and were maintained under the same conditions as those in Experiment 1.

### *3.1.2 Apparatus*

This was exactly the same as in Experiment 1.

### *3.1.3 Procedure*

The *training phase* was the same as in Experiment 1, with two main exceptions. Firstly, the rats were given eight trials per day over three days (instead of over 12 days as in Experiment 1); and secondly, in addition to the four landmarks (i.e., A, B, C, and D –which were placed exactly the same as in Experiment 1), there was a directional cue Z (a strip of white curtain 30 cm wide going from the ceiling to the ground, attached to the black curtain surrounding the pool) always present and placed so as to be behind the midpoint of the “near” landmarks B and C. Hence, Z was approximately 0.79-m from the platform, 0.85-m from the near landmarks B and C, and 2.05-m from the far landmarks A and D. It was thus further from the platform than the near landmarks (recall these were approximately 0.58-m from the platform), but closer than the far ones (which were approximately 1.12-m from the platform), and was approximately twice as far away from the far landmarks as from the near landmarks. The aim was to give the rats a clear distal landmark behind where the platform would be placed. Following escape training, all rats received 3 test days (Test Phase). Each test day consisted of eight training trials followed by one test trial without the platform, exactly the same as in Experiment 1. Subjects were tested in a counterbalanced order during Test Trials 1 and 2 of this Experiment. Thus during Test Trial 1, all the four landmarks, ABCD, were rotated by 180 degrees, leaving the directional cue, Z in the same location as in training. Thus, the locations of the “near” and “far” landmarks were inverted with respect to

the directional cue by this manipulation. In Test Trial 2, all four landmarks, ABCD, as well as Z, were present and in the same locations as in training. Finally, in Test Trial 3 we only flipped the far landmarks A and D to see if this would have any effect on performance (see Figure 4).

Figure 4: about here please

### 3.2. Results

Figure 5 shows, in blocks of 8 daily trials, the mean escape latencies of male and female rats throughout the experiment. During the training phase (Days 1-3), the rats improved their performance as days went by, and males clearly reached the platform faster than females. On the escape trials during the Test Phase (Days 4-6), the difference between males and females was reduced.

Figure 5: about here please

A repeated measures ANOVA conducted on the acquisition data (Days 1-3), taking into account the variables Sex and Days, showed that the variable Sex was significant,  $F(1, 22) = 13.61$ ,  $MSE = 164.03$ ,  $\eta^2_G = 0.33$   $p = .001$ , with males showing lower latencies than females. The variable Days was close to significance  $F(1, 22) = 3.10$ ,  $MSE = 20.67$ ,  $\eta^2_G = 0.02$   $p < .06$ , and the interaction Sex x Days was not significant ( $F < 0.5$ ). Thus, males were faster than females, and both sexes tended to take less time to reach the platform as training progressed. A repeated measures ANOVA conducted on the escape trials during the Test Phase (days 4-6), taking into account the variables Sex and Days, revealed that the variable Days was significant,  $F(2, 44) = 4.09$ ,  $MSE = 17.08$ ,  $\eta^2_G = 0.06$   $p < .03$  [the rats were somewhat slower on Day 5, i.e.,  $Day\ 5 \neq (Day\ 4 = Day\ 6)$ ]. The variable Sex was close to

significance  $F(1, 22) = 3.52$ ,  $MSE = 62.50$ ,  $\eta^2_G = 0.09$   $p < .08$ ), and the interaction Sex x Days was not significant ( $F < 1.0$ ).

### 3.2.1 Results from the three Test Trials (Figure 6)

Figure 6 shows the time spent in the platform quadrant by the animals in the different test trials (Control, Inverted, Flipped Far). One-way ANOVAs revealed that performance was significantly above chance in the control condition (Test Trial 2),  $F(1, 22) = 25.60$ ,  $MSE = 8.23$ ,  $\eta^2_G = 0.35$ ,  $p < .001$ , and in the flipped far condition (Test Trial 3)  $F(1, 22) = 18.42$ ,  $MSE = 9.04$ ,  $\eta^2_G = 0.29$ ,  $p < .001$ , but not in the inverted condition (Test Trial 1,  $F < .2$ ). An  $2 \times 2$  ANOVA comparing Test Trials 1 and 2, taking into account the variables sex (Male vs. Female) and tests (Control vs Inverted), revealed a strong inversion effect,  $F(1, 22) = 13.41$ ,  $MSE = 14.28$ ,  $\eta^2_G = 0.25$ ,  $p < .002$ , but no sex differences emerged, ( $F < .05$ ). Additional analyses comparing Test Trial 3 (Flipped Far) and Test Trial 2 (Control) that included the sex variable (ANOVA, tests x sex) showed no effect of the variable test ( $F < .3$ ) and no sex differences ( $F < 1.7$ ). Finally, an additional  $2 \times 2$  ANOVA (Sex x Tests) of Test Trials 1 and 2 using the time spent in the directional cue quadrant during Test Trial 1 (i.e., the quadrant diametrically opposite to that shown in Figure 4 for Test Trial 1) showed a strong inversion effect  $F(1, 22) = 10.51$ ,  $MSE = 12.68$ ,  $\eta^2_G = 0.48$ ,  $p < .004$ , and no sex differences ( $F < .1$ ). An  $2 \times 2$  ANOVA conducted on the latencies to reach the platform quadrant, taking into account the variables sex and tests for Control and Inverted tests revealed that no main effect or interaction was significant ( $F_s < 1$ ). An analysis on the variables sex and tests for Control and Flipped Far tests showed no main effect ( $F < 3$ ,  $p = .10$ ) or interaction ( $F < 1$ ).

Finally an ANOVA on the escape latencies with sex and tests (Control, Inverted by cue-Z) as factors revealed a trend towards significance for the variable tests,  $F(1, 22) = 4.09$ ,  $p = .055$ , with rats reaching the platform quadrant by cue Z faster in the Control condition than in the Inverted condition. No sex differences were found ( $F_s < 1.2$ ).

Figure 6: about here please

### **3.3. Discussion**

The results from Experiment 2 suggest that rats are affected by inversion of the landmark configuration when searching for the platform. Our manipulation of rotating the landmarks by 180 degrees disrupted the configural information and significantly reduced the rats' tendency to go to the quadrant defined by landmarks B and C, the "near" landmarks in training. Given that these landmarks controlled performance in Experiment 1, irrespective of the position of A and D, this is a somewhat surprising result. One potential issue with this finding, however, is that rats could have used the directional cue as a landmark and gone in that direction even when the near landmarks were rotated to their new positions. But analysis on the Z quadrant data showed that the time spent in that quadrant was simply what we would expect on a chance basis (Test Trial 1). Test Trial 3 also confirmed the fact that the "far" landmarks are not particularly important for navigation under these circumstances.

### **3. General Discussion**

In this paper we investigated the effect that transposition and inversion of a configuration of landmarks has on rats in a swimming pool navigation paradigm. Experiment 1 showed that rats' performance in finding the platform was significantly disrupted by different types of transposition manipulations, as long as these manipulations included the landmarks nearer the

platform. Experiment 2 provided evidence that a type of inversion effect could be obtained with this paradigm, as the addition of a salient directional cue resulted in the near landmarks no longer completely controlling responding. Instead, when the four landmarks were rotated through 180° with respect to this directional cue, performance fell to chance. This was not due to the directional cue simply acting as a pre-potent beacon, as the time spent in the quadrant defined by that cue was also at chance.

There are three main conclusions we can draw from these studies. First, our results demonstrate the negative effect that disruption of the landmark configuration can have on rats' navigation in this task. This effect is distinct from that in previous studies that added or deleted landmarks resulting in generalization decrement and goes beyond that obtained by Chamizo et al. (2012). Our results show that transposition of landmarks in the water maze can also have a detrimental effect even when more than two landmarks are available (cf. Suzuki, et al., 1980). If anything, the fact that transposition of the far landmarks has so little effect on performance is surprising, as they are still part of the overall landmark configuration, and any change in this configuration might be expected to impact on performance. Clearly, proximity to the target location is important in determining the effectiveness of a landmark in guiding navigation.

Given this analysis, we can make the case that it is the disruption of the "near" landmark configuration that has the major effect on performance. This finding is in agreement with previous studies like that of Chamizo and Rodrigo (2004; see also Chamizo, Artigas, Sansa & Banterla, 2011, working with humans and a virtual task), in which it was found that the control acquired by a single landmark depended on its relative distance from the platform, with closer landmarks acquiring better control than far ones. The finding goes somewhat against the idea of a configuration as some kind of unified "whole" such that a change to any of its elements disrupts the configuration and leads to a change in performance. Clearly the

two near landmarks, B and C, were sufficiently salient to, in effect, completely overshadow A and D, to the point where swapping the latter made little difference.

Finally, in Experiment 2, we demonstrated an effect that goes beyond the results from Experiment 1 and previous studies, in that it cannot easily be explained by an appeal to those results. Instead, we would argue that the results are consistent with the idea that the landmark configuration was oriented with respect to the directional cue, and when this orientation was disrupted the landmarks became ineffective. This last conclusion deserves closer examination.

We have already claimed that Experiment 1 indicates that, other things being equal, the landmarks nearer the platform control the rat's behavior in these experiments. If this is the case, then this should also be the case in Experiment 2, and there should be above chance performance in the target quadrant on test. Clearly this is not the result that we obtained. We might explain this loss of control by the B and C landmarks in terms of the new cue Z being very salient and overshadowing these other, nearer, cues. But, if this were true, we would expect it to act as a pre-potent landmark, a beacon, and thus lead above chance time spent in the Z quadrant, and this was not the case. How can we explain this pattern of results? If we assume that B, C and Z form a configuration that is used for navigation, then we might be able to explain these findings, but then we must explain why A and D did not form a configuration with B and C in Experiment 1. Our analysis will also make clear why we needed all three test phases in Experiment 1 to establish that it is the configuration of B and C that is important for navigation in that experiment. We start with the not unreasonable assumption that it is the distance between landmarks that governs the extent to which they participate in some configural representation that guides navigation. Consider the fact that A is exactly the same distance from B as B is from C, and so, on this basis, A might be expected to form a configuration with B just as easily as C does. But this clearly did not happen given



the results of Experiment 1. The fact that swapping A and B reduced performance to chance levels (Test Phase 3) could imply that the configuration of A and B was important for performance, or that some other configuration involving A or B was involved. The fact that swapping A and D had no effect (Test Phase 2) effectively rules out the idea that some configuration between A and B (or A and D) played any significant role in performance. The results of Test Phase 1, which demonstrates that swapping B and C does reduce performance to chance levels then leaves us with no alternative to the conclusion that the configuration that controls performance is that involving B and C. As a corollary to this conclusion, we must now accept that it is the distance from the platform that determines whether or not landmarks play a role in the configuration guiding navigation, not their distance from one another, and on this basis, as B and C are closer to the platform than Z, we would expect them to be the more important components of that configuration. An explanation in terms of simple configuration by B, C and Z is not ruled out by these considerations, but it does have its problems.

An explanation in terms of elemental control by B, C and Z also does not stand up to examination. If we simply assume that the nearer / more salient cues control performance individually, and that behavior is the net result of summing their contributions, then this explains why B and C might exert more control over navigation than A and D. When Z is added, let us assume that it joins B and C in controlling performance. But now the lack of any preference for either the quadrant defined by B and C, or by Z is puzzling. We would have to assume that the effects of these cues in some sense "cancel out" to obtain our results, when actually the elemental position would predict that they prefer both these quadrants to the other two (which is not the case because time spent in these quadrants is at chance).

Now imagine instead that the directional cue, Z, supplies orientation information that is used in conjunction with the other landmarks, ABCD. Z indicates "North" if you like. The

animal uses its stored representation of the configuration of landmarks to guide its search for the platform, but this representation also contains orientation information. Once the landmarks have been rotated to their new positions, there is a mismatch between the stored representation and actuality, and the information learned by the animal during training no longer applies, and navigation in the water maze suffers catastrophically. To put it another way, the animal expects, when facing Z, B to be to its left and C to its right, and now they are not, hence they are not recognized as being the landmarks used in training and do not control behavior. There is evidence for this "oriented configuration" hypothesis in previous studies, particularly in rats performance on the radial maze. Roberts (1981) looked at retroactive interference in memory for visited locations in the radial maze but also found evidence for a beneficial effect of allowing rats to visit the same configuration of locations as those experienced on the target trials if they were in the same room and oriented in the same direction (the "same" condition of Experiment 2). In a later set of studies, Olthof, Sutton, Slumskie, D'Addetta, and Roberts (1999) found no evidence for transfer if there was a 90° or 180° rotation between training and test (Experiment 1), and none for smaller rotations either (Experiment 2), or similar configurations in a different environment (Experiments 4 and 5). These results are consistent with the use of an oriented configuration by animals in the radial maze and predicts the type of result that we have obtained in the Morris water maze.

The final issue to be considered in this general discussion regards the additional results we found with respect to sex differences. First, it is important to note that across these experiments male and female rats did show significant differences in speed of spatial learning (particularly near the end of training in Experiment 1). Thus, in Experiment 1 Test Phase 1, we should perhaps not be too surprised that the control performance for male rats was significantly greater than that for females. Also, on Test Phase 2, the analysis still shows

significantly greater performance for male rats in the control condition compared to female rats. These results would also seem to suggest that male rats might have learnt more than female rats. But a finding worth noting with respect to the sex difference results is the fact that in Experiment 1 Test Phase 3, both male and female rats were affected by our manipulation, even though males were significantly more affected, and this time performance on the control condition was not significantly different between the two subject groups. There are differences in males and females response to handling. Males tend to be more docile, whereas females are more active and more difficult to handle. It might be that the relatively poor performance by females in the earlier Test Phase 1 and 2 is in part due to this difference, and that they habituated to handling over the course of the experiment. This would explain why their performance improved in later Test Phase 3 in the control condition. Finally the sex difference found in this Test Phase 3 could mesh with studies on both humans and animals that show that males and females do not always use the same cues to solve a navigation task (Williams, Barnett, & Meck, 1990; Roof & Stein, 1999). In particular, males seem to rely on the geometrical configuration during navigation, i.e. on the arrangement of landmarks rather than on a specific single object (Rodríguez et al., 2010). If we assume this is the case, we might expect changes in this configuration to be more disruptive for males than females, which is essentially the result observed.

We realize that some of the arguments that we have made in this discussion of the results reported in this paper are speculative, in that they are consistent with our observations rather than established by them. But we feel that it is important to flag these possibilities as a guide for future research. Our results suggest that the systematic manipulation of one set of landmarks relative to another (or to a directional cue) may prove a fruitful methodology for investigating the mechanisms governing rats' spatial learning and memory.

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**Figure 1.** A Schematic representation of the pool and the position of the four landmarks (A, B, C and D) as well as the hidden platform and the different manipulations across Test Phase 1, Test Phase 2 and Test Phase 3. Oblique lines inside the pool define the platform area used for collecting and analyzing the data. Finally the manipulations in Test Phase 3 were counterbalanced; swapping C with D once, and B with A once.

**Figure 2.** Mean escape latencies for the rats of Experiment 1 during the initial training phase, and also during all the escape trials on test phases (1-3) and on retraining days.



**Figure 3.** Mean time spent in the platform quadrant by the subjects in Experiment 1 during the test phases. Error bars denote standard error of means. A small asterisk above each bar indicates whether the rats' performance differed significantly from chance (7.5 s searching in the platform quadrant).

**Figure 4.** A Schematic representation of the pool and the position of the four landmarks (A, B, C and D) plus the directional cue Z as well as the hidden platform and the different manipulations used for Test Trial 1, and Test Trial 3. The hatched quadrant was considered to be the target quadrant for the animals on test.

**Figure 5.** Mean escape latencies for the rats of Experiment 2 during the training phase (days 1-3), and also during the escape trials of the test phase (days 4-6).

**Figure 6.** Mean time spent in the platform quadrant by subjects in Experiment 2 during the test trials. Error bars denote standard error of means. A small asterisk above each bar indicates whether the rats' performance differed significantly from chance (i.e., 7.5 sec searching in the platform quadrant).



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Figure 1

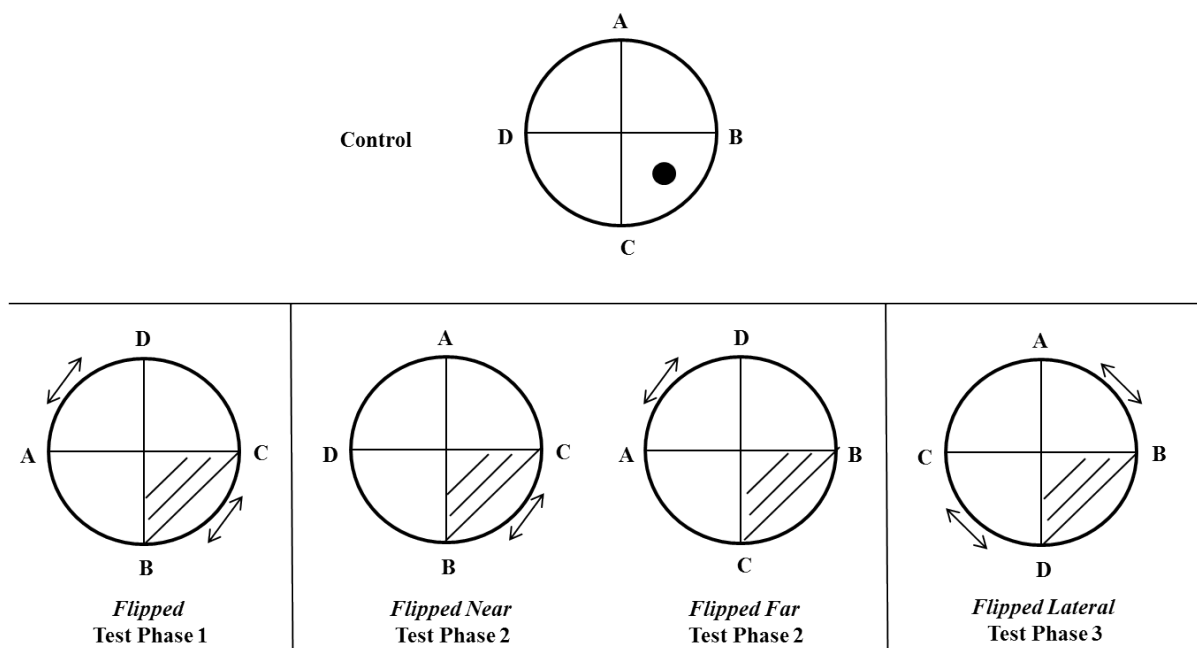


Figure 2

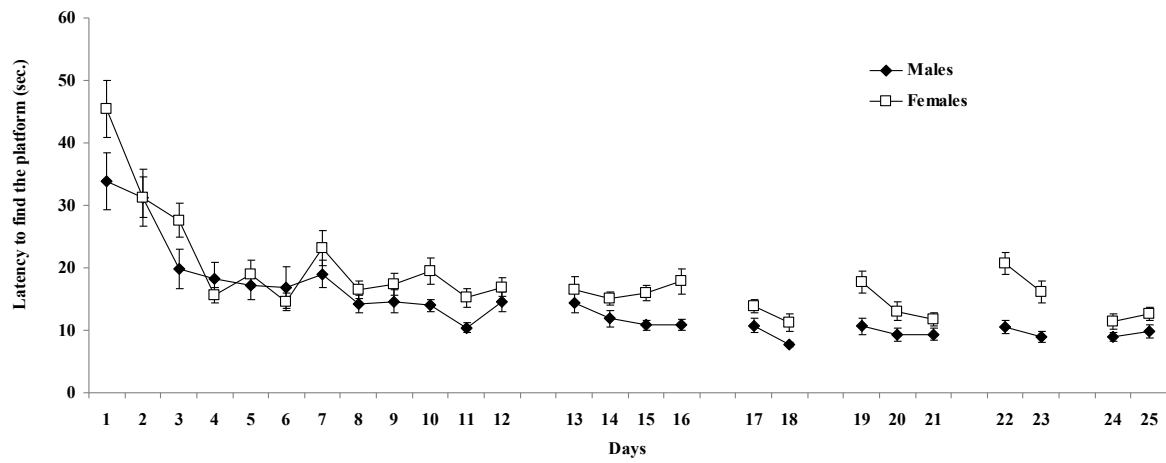


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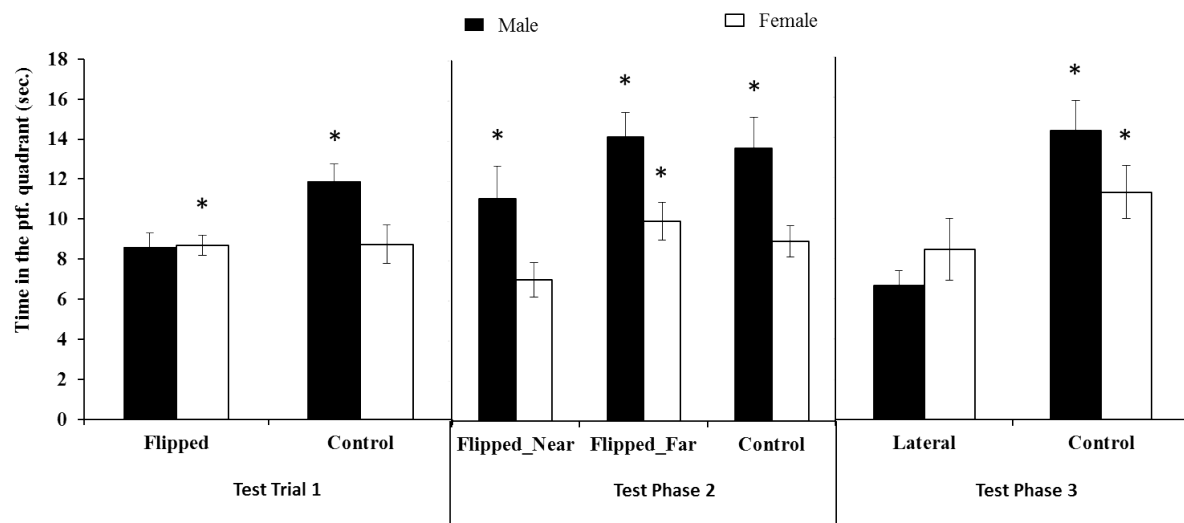


Figure 4

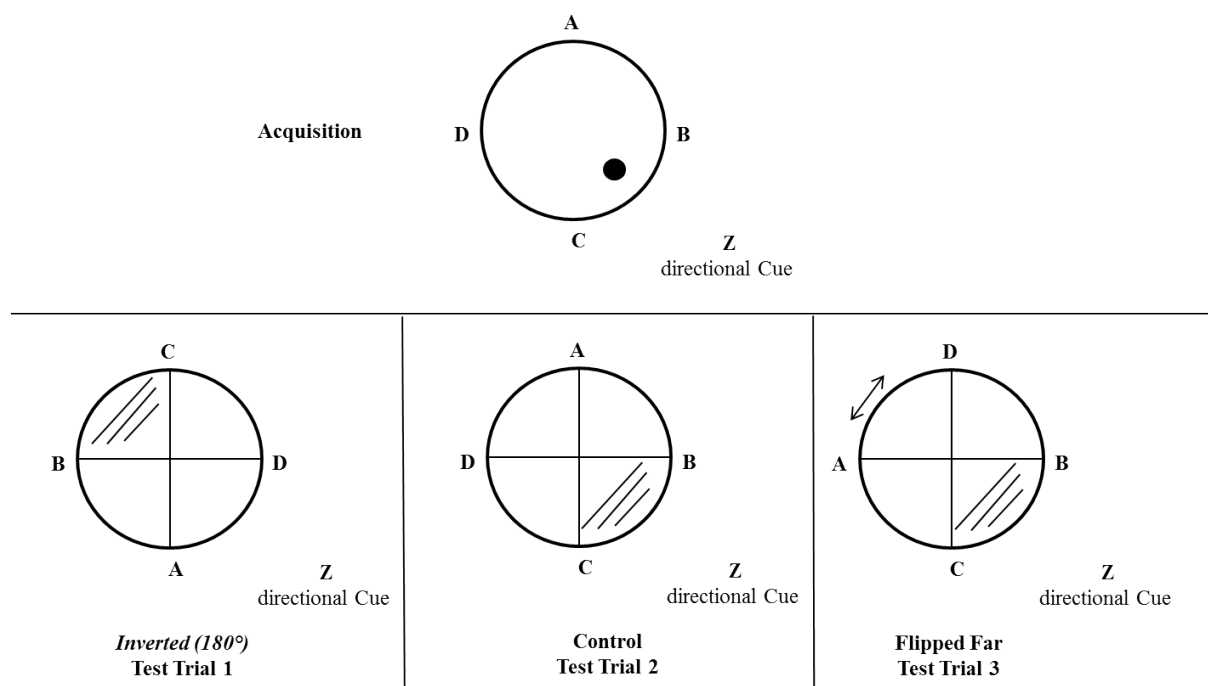


Figure 5

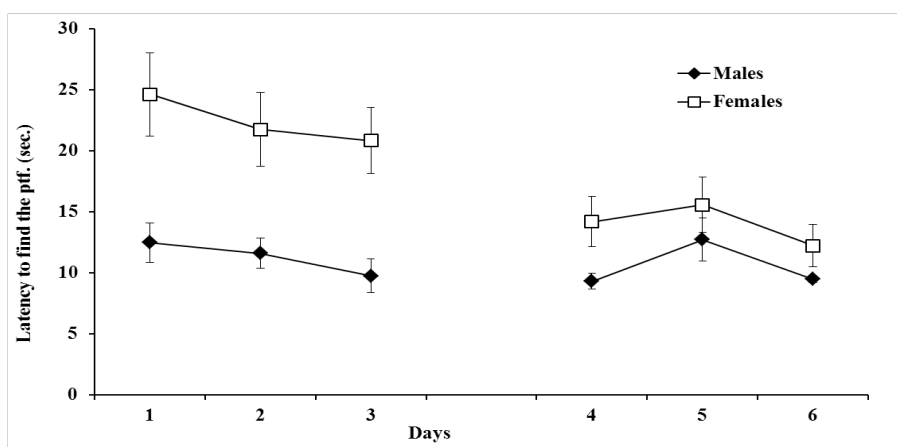


Figure 6

