Directional Cues and Landmark Configurations:

The effect of rotating one set of landmarks relative to another.

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Abstract

In this article we addressed the question whether rats can use distal landmarks as directional cues that are used in combination with other proximal landmark configurations. The animals were trained with an A, B, C, and D landmark configuration in the Morris pool, where B and C are the near (to platform) landmarks and A and D the far ones. We also added another more distal "directional" cue Z (a white strip attached to the black curtain surrounding the pool). Experiment 1 shows a robust detrimental effect on the time spent by the rats swimming in the platform quadrant when the location of all landmarks was "Inverted" (rotated by 180 degrees) with respect to Z. A similar detrimental effect was found when, after the inversion manipulation, the locations of the near and far landmarks were "Flipped" (B swapped with C and A with D). Rats in both Inverted and Flipped tests spent more time in the Z quadrant compared to the platform quadrant (BC). Experiment 1b provided evidence distinguishing between alternative explanations of how the directional cue Z acts in combination with the other landmarks. The results from both experiments show that Z operates differently to the standard landmarks. It can function as a beacon in its own right. It can also combine with the other landmarks to produce a high level of search performance, in a way that we hypothesize to be distinct from that described by the configural analysis often applied to multiple landmarks.

Keywords: Spatial navigation, Inversion Effect, Landmark Configurations

Rats placed in an environment where there is a concealed target in a certain location, can learn to find that location using the landmark configuration available to them at the time. There can be little doubt that landmarks can control search in the type of studies considered here. In the Morris water maze (which is used in our studies), the standard technique for training involves a transformation (rotation) between trials that ensures that the landmarks are the only reliable cue to the target (platform) location that the animal has available. And as we shall see and others have shown (Morris, 1981), rats have little difficulty in exploiting this information. Suzuki, Augerinos and Black (1980) were among the first to show that this was the case in a cylindrical chamber that could be rotated with respect to the arms of a maze that had a cue or landmark at the end of each arm. The rats would visit arms to receive food, and then go to the "unvisited" arms that matched the landmark configuration after rotation of the chamber, even if, in fact, those arms had been visited and the food at the end of them depleted. But if the landmarks were transposed (i.e. swapped with one another) in a way that disrupted the training configuration, then performance became less systematic and control by the landmarks was greatly reduced. These results suggest that the rats were not using single landmarks, because then the animal would just have tracked each landmark and used that to control performance. Instead, they appear to use knowledge of the way that the landmarks are arranged spatially with respect to one another, i.e. their configuration, to control their search (see MacDonald, Spetch, Kelly and Cheng, 2004 for more on this).

The question that we address in this paper is whether rats can use certain types of distal landmarks as directional cues; these would be cues with a different status to more proximal landmarks that are then used in combination with proximal landmark configurations. On the one hand, it could be that all landmarks/cues are equal, and any of them can play a role in a configuration that guides search. One example of such a state of affairs would be for the animal to learn a vector (i.e. distance and direction) from each landmark to the target location

(for an example see Cheng, 1989). To do this would require a very simple system that just used an egocentric reference frame, so that the animal codes the target location in terms of a given landmark being on its left and at a certain distance when it is looking directly at another landmark. This would allow it to guide its search, and the more of these "vectors" that were encoded and used in combination the better that search would be. Each landmark is treated equally in this scheme, and operates quasi-independently of the others. There would be no particular distinction between directional cues and landmarks as such, and this model of rats' spatial navigation would be of an elemental nature, with performance determined by the summed net effect of the elemental cues present. Such an analysis would fit well with certain versions of learning theory, and we note that spatial navigation in the rat has been shown to display similar phenomena to those found in Pavlovian conditioning, e.g. blocking (see Rodrigo, Chamizo, McLaren and Mackintosh, 1997).

But, as we have already noted, the evidence does seem to favor a more configural account of rat (and other species) spatial navigation skills. Quite apart from the evidence provided by Suzuki et al. (1980), Cartwright and Collett (1982) were able to show that honeybees used a configural representation of the available landmarks, by demonstrating that performance was relatively invariant under transformations that preserved that configuration (such as enlargement), and Spetch, Cheng and MacDonald (1996) made a similar case for humans. In Civile, Chamizo, Mackintosh and McLaren (2014) we also review the evidence provided by either deleting cues present during training (i.e. going from ABCD to AB) and adding cues not present during training (e.g. going from AB to ABCD) and note that both manipulations impair search performance in the water maze (see Chamizo, Rodríguez, Espinet, & Mackintosh, 2012). The effect of deletion of landmarks learned about in training can obviously be explained using an elemental type of account, but the similar effect of adding landmarks not present in training is not so easily dealt with (but see McLaren and

Mackintosh, 2000, 2002; and Wagner and Brandon, 2001 for the type of elemental theory that could accommodate these results). Our conclusion there was that this is the type of effect that would be expected if learning was about cue configurations rather than independent, separate landmarks, and that this conclusion fit well with the other evidence available.

But if this is the case, then what, if any role could a directional cue play in spatial navigation? Surely a configuration of landmarks is enough on its own? One answer is that this may well be the case in principle, but that in practice more distal landmarks will tend to find a role as directional cues that aid in the orientation of the more proximal landmark configurations. Our argument is that these distal cues are less able to provide any distance information themselves (because that judgement would be unreliable), but instead act as a reference direction from which to orient other cue configurations that can be used to find the target location. If this type of information is incorporated into learning during training, then its deletion should severely affect performance even if the cue configuration left is, in principle, entirely adequate to guide search. Equally, if the cue configuration is maintained, but rotated with respect to the directional cue, then this should also disrupt navigation.

The latter prediction was confirmed by Civile et al. (2014). After an initial experiment that demonstrated that the cues proximal to the platform location (B and C) gained control over search behavior at the expense of more distal cues (A and D), they showed that training with the four landmarks, ABCD and an additional cue Z that was both salient and further from the platform than B and C, produced chance levels of performance on test once ABCD were rotated by 180° with respect to Z. Figure 1 in this paper shows the training configuration used for this experiment in Panel a, and the rotation manipulation used for test in the "Inverted" condition shown in Panel b. What was striking about Civile et al's results was that there was no evidence of above chance levels of search in either the BC or Z quadrants, and no evidence that these two quadrants were collectively preferred to the other two, as might be

expected on an elemental account. This led us to suggest that Z was functioning as a directional cue used to orient the animal with respect to the ABCD configuration of landmarks, and the first set of experimental tests in this paper (Experiment 1a) seek to replicate and extend this finding in order to bring further evidence to bear on this hypothesis. The second set of tests done after re-training (Experiment 1b) are then designed to help us distinguish between alternative conceptualizations of how Z acts in combination with the other landmarks. In what follows we first describe the method and results for both sets of experiments, and provide an analysis of both separately, and then in combination in our General Discussion.

Experiments

Method

Subjects

The subjects were 12 naïve Long Evans male rats approximately five months old at the beginning of the experiment. 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 hrs of the light cycle.

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 that was made opaque by the addition of 1cl/L of latex. The temperature of the water was kept at 22 degrees. The pool was placed in the middle of a large room, mounted on a wooden platform 0.43-m above the floor. The pool was surrounded by black curtains from the ceiling to the base of the pool and forming a circular enclosure 2.4-m 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. The four landmarks chosen were exactly the same ones used in Civile et al (2014)'s study and were: -A: a white cardboard cube (20 cm 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. Importantly, Chamizo et al (2012) had conducted an experiment to ensure the four landmarks had a similar salience at the same distance. Hence, following the acquisition phase, in the test trial (i.e. without the platform) the results revealed the four landmarks acquired the same control of the rats' performance. Following Civile et al (2014), in addition to the four landmarks we placed a directional cue Z (a strip of white curtain 30 cm wide going from top to bottom, attached to the black curtain surrounding the pool) that was 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, 0.97 m from the near landmarks B and C, and 1.85 m from the far landmarks A and D. It was thus further from the platform than the near landmarks, but closer than the far ones, 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.

In order to ensure that the animals used the landmarks rather than static room cues, the location of the platform, the landmarks, and the directional cue 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 1.75 m above center of the pool inside the false ceiling, and its picture was relayed to recording equipment in an adjacent room. A circular platform, 0.11 m in diameter

made of Perspex was placed on a rod and base, and could be placed in one quadrant of the pool, 0.38 m 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 0.58 m from B and C, and 1.12 m from A and D. For our purposes, the salient point is that the "near" landmarks, B and C, are roughly half the distance from the platform of the "far" landmarks A and D.

Procedure in Experiments 1a and 1b

Following Civile et al (2014) and Chamizo et al (2012) the *pre-training phase* constituted five trials over 2 days, with two trials on day 1 and the rest on day 2. This consisted of placing a rat in the pool, without landmarks but with the hidden platform present. Hence, the animal was given 120 sec to find the platform, and once it had found it was allowed to stay on it for 30 sec. If it had not found the platform within 120 sec, it was picked up, placed on the platform and left it in there for 30 sec. The platform was moved from one trial to the next, and the rat was placed in the pool at a different location on each trial in order to ensure that no residual inertial tracking or path integration could help it find the platform. The same procedure was used in the *acquisition phase,* but now the landmarks and the directional cue 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 (overall 13 days). As in Civile et al (2014) 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, in **Experiment 1a** all rats received 3 *test days*. 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 and the directional cue present, but no platform,

and left for 60 sec. The same four starting positions were used as in training. The animals were tested in a counterbalanced order during this phase of the experiment. For each *test day* four subjects were assigned to each of the following three test conditions. *Inverted*: All the four landmarks, ABCD, were rotated by 180 degrees, leaving the directional cue, Z, in the same location as in training. Thus, the location of the "near" and "far" landmarks were inverted with respect to the directional cue by this manipulation. *Normal*: Essentially our control condition, in which all four landmarks, ABCD as well as Z, were present and in the same locations as in training. *Flipped*: Here the near landmarks were swapped with the corresponding far landmarks (see Figure 1, Panel b) leaving B and C in their original orientation with respect to Z. The latter condition is different from that applied in Civile et al (2014)'s Experiment 2, where only the far landmarks (A and D) were swapped, which resulted in no effect on performance.
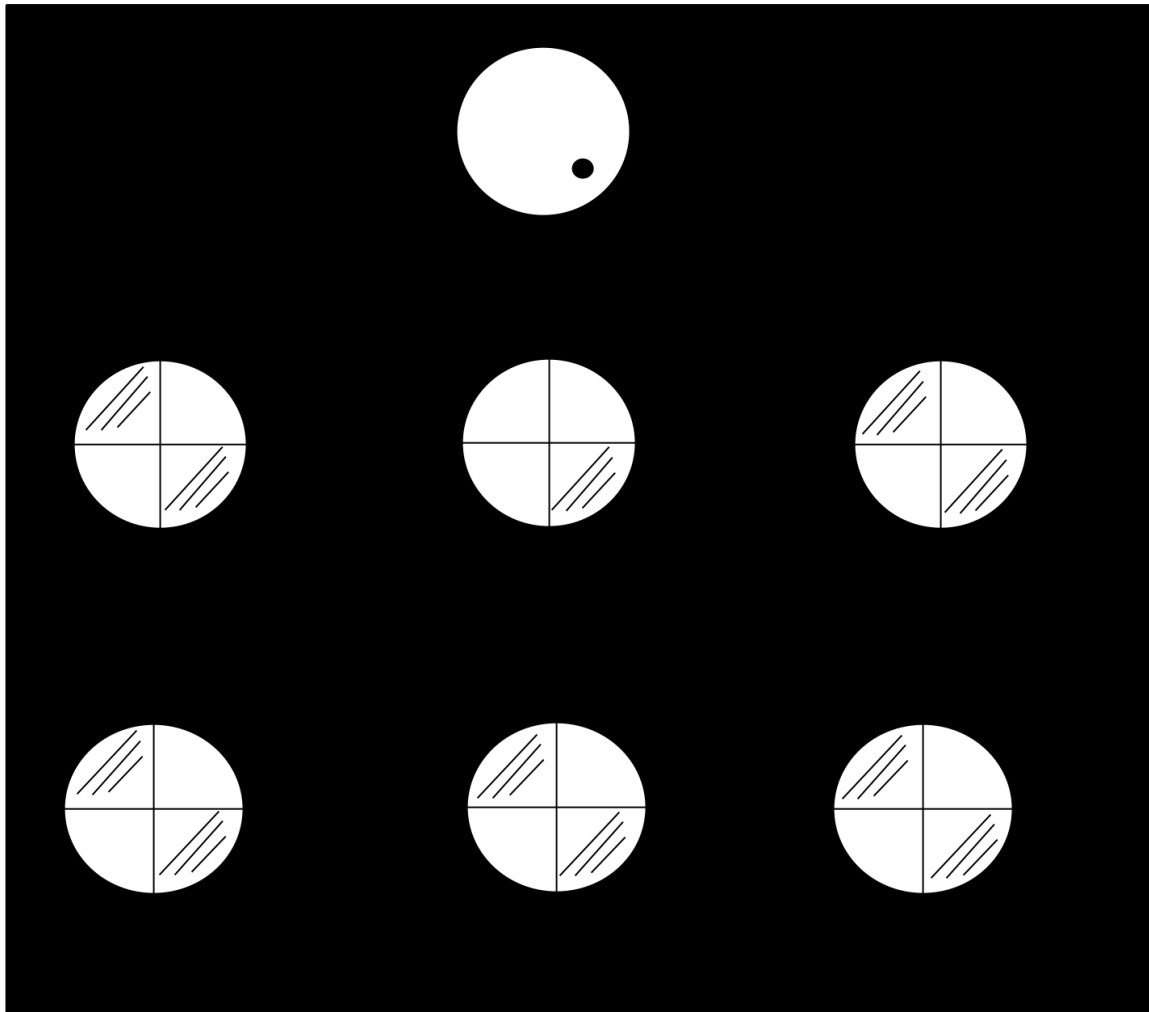
Figure 1. Panel a, shows a schematic representation of the pool and the position of the four landmarks (A, B, C and D) plus the directional cue (Z) as well as the hidden platform. Panel b shows the different test conditions in Experiment 1a. Panel c, shows a schematic representation of the test conditions in Experiment 1b. In both Experiments the hatched quadrant(s) were the analyzed quadrant(s) for the subjects on test.

For **Experiment 1b** the same subjects were retrained (*re-training phase*) for three days with the same landmarks and directional cue configuration as for the earlier *acquisition phase*. Following this, all rats received a further 3 *test days* using the same procedures as for Experiment 1a. The animals were tested in a counterbalanced order, such that on each *test day* four subjects were assigned to each of the following three test conditions. *Identity*: All the four landmarks, ABCD, were rotated by 180 degrees, and the directional cue, Z, was removed. Thus, this condition allows us to measure the influence of B and C in the absence of Z when the landmarks are in their original configuration. *Location*: The four landmarks,

ABCD, were rotated by 180 degrees, leaving the directional cue, Z, in the same location as in training. Then B and C were removed allowing us to measure the influence of Z under these conditions. Finally, *Flipped+*: the near landmarks were swapped with the corresponding far landmarks as before, and then A and D were also swapped to facilitate comparison with the other conditions.

Results

In the two experiments reported in this article, the statistical tests were two-tailed with an alpha of .05 unless otherwise noted. The results for the test trials in both Experiment 1a and 1b are from the full 60 sec., and are based on time spent in a certain specified quadrant or quadrants of the pool. We also provide heat maps based on these 60 sec test trials to enable a more detailed assessment of their performance in searching for the platform, but our numerical analyses are principally based on the quadrant data both for reasons of statistical power and to make contact with previous work. We do, however, go on to offer an analysis that is complementary (and orthogonal) to the quadrant-based analysis by looking at the time spent in four annular regions that cover the pool and are roughly equal in area. The equivalence is only approximate here because of the way the concentric circular rings fall on our square imaging grid, and for the inner most ring (really a smaller circle which we call Inner Centre) amounts to just under 23% of the pool, for the second and third rings out from this 25% of the pool, and for the outer ring (Outer Wall) it is 27% of the pool. These values could not be equal, and we chose our solution to this problem so as to very slightly disadvantage the centre location for reasons that will become obvious.

A repeated measures ANOVA conducted on the *acquisition phase* data (escape latencies for Days 1-12) showed all rats clearly improved their performance as days went by, $F(11, 121) = 23.54$, $p < .001$, $\eta_p^2 = .68$. During the rest of the study the animals tended to either maintain the asymptotic level reached or improve slightly but not significantly (see Figure 2).
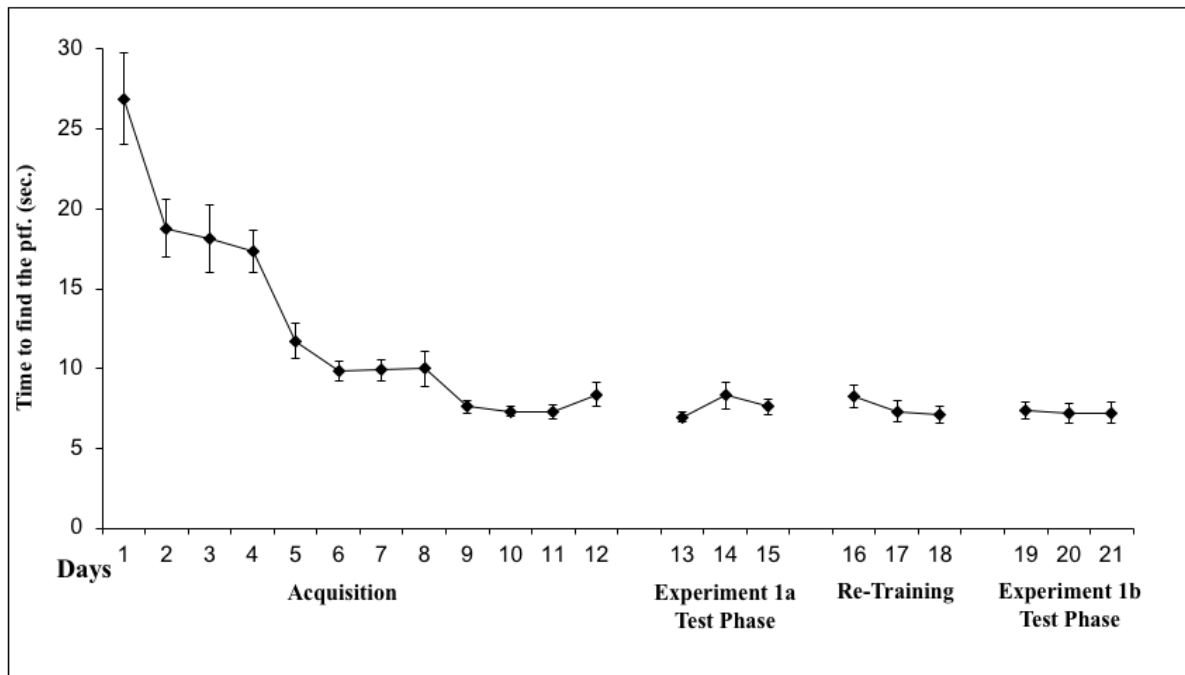
Figure 2. Mean escape latencies for the rats during training trials throughout the entire study.

Experiment 1a Test Phase

A repeated measure ANOVA using the factor *Test Conditions* (Inversion, Normal, Flipped) revealed a highly significant effect of the configuration manipulations when the rats were searching for the platform in the BC quadrant, $F(1,11) = 40.68$, $p < .001$, $\eta_p^2 = .78$. We followed this up with a planned comparison analysis that showed the rats spent significantly less time in the BC quadrant in the Inversion condition (M = 12.51 sec, SE = .87) than in the Normal (control) condition (M = 30.45 sec, SE = 2.12), $t(11) = 7.32$, $p < .001$, $\eta_p^2 = .83$. Similarly, the Flipped manipulation drastically reduced rats' time spent swimming in the BC quadrant (M = 10.92 sec, SE = 1.31) compared to Normal, $t(11) = 6.34$, $p < .001$, $\eta_p^2 = .78$. No significant difference was found between the Inversion and Flipped conditions, $t(11) = 1.16$, $p = .27$, $\eta_p^2 = .11$. Clearly, both these manipulations disrupted search behavior and severely reduced the influence of B and C.

As in Civile et al (2014), we then conducted another repeated measure ANOVA again using the factor *Test Condition*s, but this time looking at the time spent by the rats in the directional cue, Z, quadrant instead of the BC quadrant (in the Normal condition these are the same

quadrant). In this case as well, we found a highly significant effect of our manipulations, $F(1,11) = 21.53$, $p < .001$, $\eta_p^2 = .66$. There was a significant difference between the Inversion condition (M = 18.16 sec, SE = 1.61) which was lower than Normal, $t(11) = 5.75$, $p < .001$, $\eta_p^2 = .75$. Furthermore, a significant effect of the Flipped manipulation (M = 23.26 sec, SE = 2.06) was found compared to Normal, $t(11) = 4.98$, $p < .001$, $\eta_p^2 = .69$, with once again a reduction in the time spent in this quadrant. And this time a significant difference was also found between the Inversion and Flipped conditions, $t(11) = 2.55$, $p = .027$, $\eta_p^2 = .37$. It would appear that the influence of the directional cue has also been reduced by our manipulations of the landmark configuration, perhaps not by as much as in the case of the BC quadrant, and significantly less so by the Flipped manipulation than by Inversion.

Finally, we conducted a further analysis that directly compared the time spent in the BC quadrant vs the time spent in the Z quadrant in the Inverted test condition. This revealed a significant difference, $t(11) = 2.62$, $p = .024$, $\eta_p^2 = .38$, showing that the rats spent more time in the directional cue quadrant than in the platform quadrant. A significant difference of this type was also found in the Flipped test condition, $t(11) = 3.81$, $p = .003$, $\eta_p^2 = .57$, again showing that the rats spent more time in the directional cue quadrant than in the platform quadrant (see Figure 3). This strongly suggests that the influence of Z is greater than that of B and C for both Inverted and Flipped test conditions. But the fact that the difference between BC and Z quadrants is greater in the Flipped condition than in Inverted, as revealed by the significant Condition (Flipped vs. Inverted) by Quadrant (Z vs. BC) interaction, $F(1,11) = 4.94$, $p = .048$, $\eta_p^2 = .31$, tells us that the spatial arrangement of B and C is also important. We will return to this important result later.
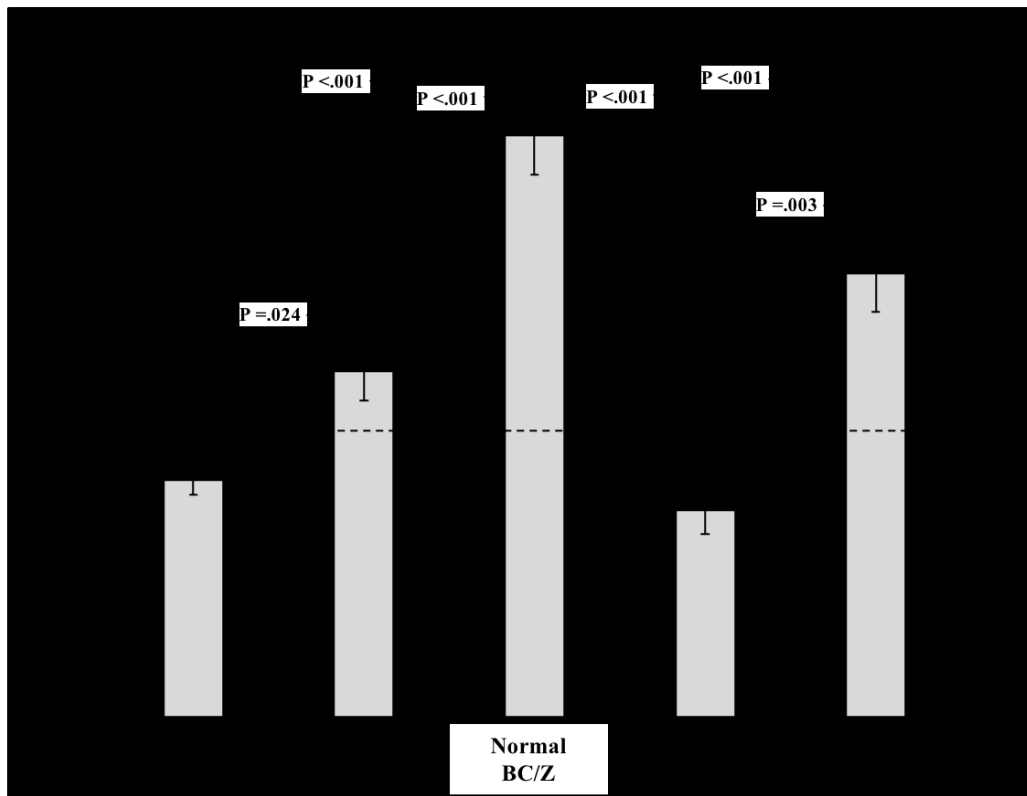
Figure 3. Mean time spent in the platform quadrant by the subjects in Experiment 1a during the test phases. Error bars denote standard error of means. A small asterisk above each bar indicates whether the rats' performance differed significantly from chance. This was found only in the Normal (control) condition [$t(11) = 7.27$, $p < .001$, $\eta_p^2 = .82$] and the Flipped condition for the Z quadrant (directional cue) [$t(11) = 4.03$, $p = .002$, $\eta_p^2 = .59$]. Also, the Inverted condition for the Z quadrant shows a trend towards being significantly above chance [$t(11) = 1.96$, $p = .076$, $\eta_p^2 = .26$], whereas Inverted BC [$t(11) = 2.83$, $p = .016$, $\eta_p^2 = .42$] and Flipped BC [$t(11) = 7.27$, $p < .001$, $\eta_p^2 = .46$] were both significantly below chance level.

Experiment 1b Test Phase

A repeated measure ANOVA using the factors *Test Conditions* (Identity, Location, Flipped+) x *Quadrant* (BC, Z) revealed a significant main effect of *Quadrant*, $F(1,11) = 6.97$, $p = .023$, $\eta_p^2 = .38$. No main effect of *Test Conditions* was found $F(1,11) = .84$, $p = .458$, $\eta_p^2 = .14$, but the interaction did approach significance, $F(1,11) = 3.29$, $p = .08$, $\eta_p^2 = .39$. A planned comparison analysis on the *Identity* condition showed no significant difference in the time spent by the rats searching for the platform in the BC quadrant (M = 16.60 sec, SE = 1.68) vs the AD quadrant (M = 14.17 sec, SE = .97), $t(11) = .94$, $p = .365$, $\eta_p^2 = .07$. But in the *Location* test a significant difference was found between the time spent by the rats in the

Z quadrant (M = 20.15 sec, SE = 1.77) vs the opposite quadrant (M = 12.25 sec, SE = 1.07), $t(11) = 2.89$, $p = .015$, $\eta_p^2 = .43$. And in the *Flipped+* test a significant difference was also found between the time spent by the rats in the Z quadrant (M = 19.19 sec, SE = 1.50) vs the BC quadrant (M = 13.31 sec, SE = 1.27), $t(11) = 2.22$, $p = .048$, $\eta_p^2 = .31$ (see Figure 4). These results suggest that the influence of Z was greater than that of B and C in these tests, and the similar results for Flipped+ compared to Location also suggest that having B and C "the wrong way around" with respect to A and D in the far locations did very little, and that performance in both conditions was largely controlled by Z.

To further interpret these results, we conducted a planned comparison analysis directly testing the effect of BC in the *Identity* condition on time spent in the AD quadrant (AD minus BC) vs the effect of inserting Z in the *Location* condition on the same quadrant (the AD/Z quadrant minus the opposite quadrant), which gave a $F(1,11) = 6.54$, $p = .027$, $\eta_p^2 = .37$ confirming that these two manipulations produce rather different results as would be expected. The difference is driven both by the presence of the directional cue promoting search in the AD/Z quadrant for Location compared to Identity, $t(11) = 2.66$, $p = .022$, $\eta_p^2 = .39$, and by the reverse effect for the BC/opposite quadrant, as a similar analysis comparing performance spent in the BC quadrant in the *Identity* condition vs when BC was removed in the *Location* condition (using the quadrant opposite to Z), gave a $t(11) = 2.18$, $p = .052$, $\eta_p^2 = .30$. We cannot, however, say that the influence of Z in Location is significantly greater than that of BC in Identity, as that would require (Z – Opposite) in Location to be significantly greater than (BC – AD) in Identity and this test does not reach significance, $F(1,11) = 1.59$, $p = .14$, $\eta_p^2 = .18$. Nevertheless, there is considerable evidence in our data that Z is the stronger influence on performance, as even though this potentially decisive test fails to reach conventional levels of significance, the comparison between Z and BC quadrants in the Inverted condition for Experiment 1a does meet this criterion.
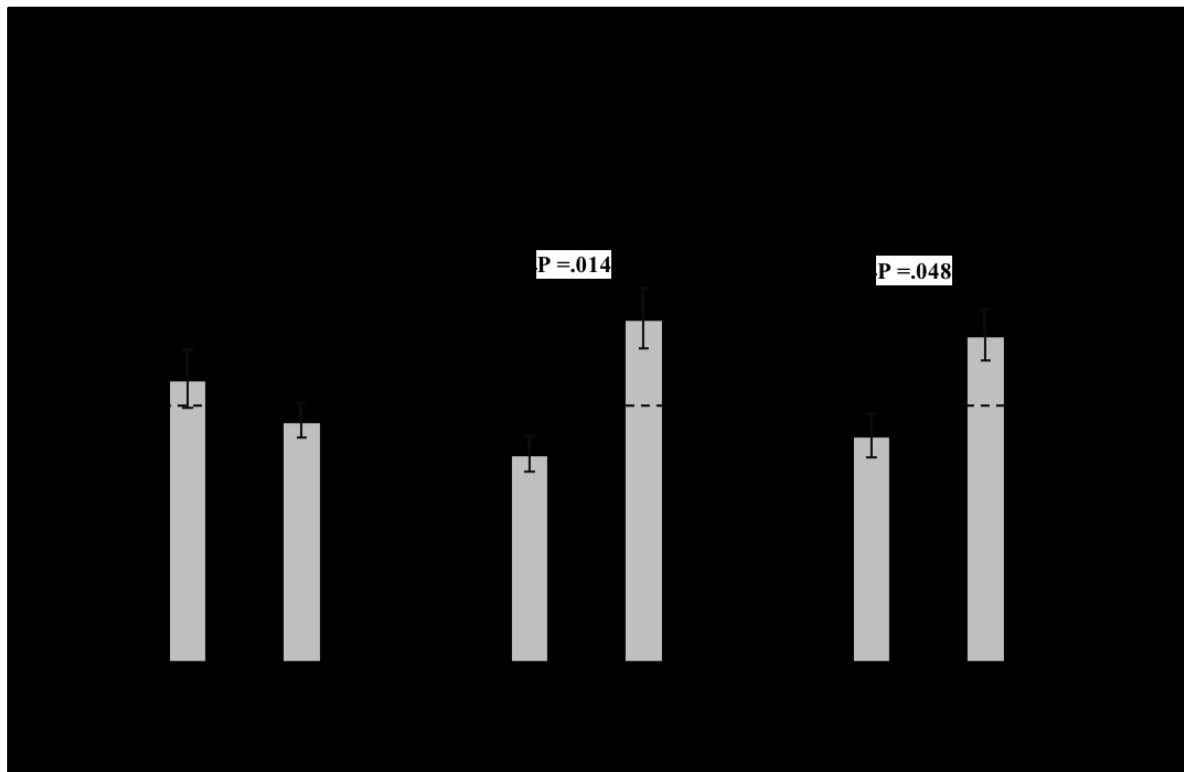
Figure 4. Mean time spent in the platform (BC) and directional cue (Z) quadrants by the subjects in Experiment 1b during the test phases. Error bars denote standard error of means. A small asterisk above each bar indicates whether the rats' performance differed significantly from chance. This was found to be the case in the *Flipped+* test for the Z quadrant [$t(11) = 2.27$, p = .017, $\eta_p^2 = .41$] and in the *Location* test for the Z quadrant as well [$t(11) = 2.90$, p < .014, $\eta_p^2 = .43$]. Whereas, in the *Location* test the Opposite quadrant was significantly below chance level [$t(11) = 2.54$, p = .027, $\eta_p^2 = .37$].

This concludes our analyses of the time spent by our rats in a given quadrant during the test phases of Experiments 1a and 1b. But we have a more molecular visualisation of the search behavior of the rats in these experiments that we can also offer. Figure 5 shows heat maps[1] produced by averaging the data for time spent in a given location in the pool across rats for each test condition. The temperature coding utilised in these maps means that blue indicates

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[1] We would like to thank an anonymous reviewer for suggesting that we offer this analysis.

that very little time was spent by any of the rats at these locations, with the time spent rising as we go through green to yellow, then orange and finally red.
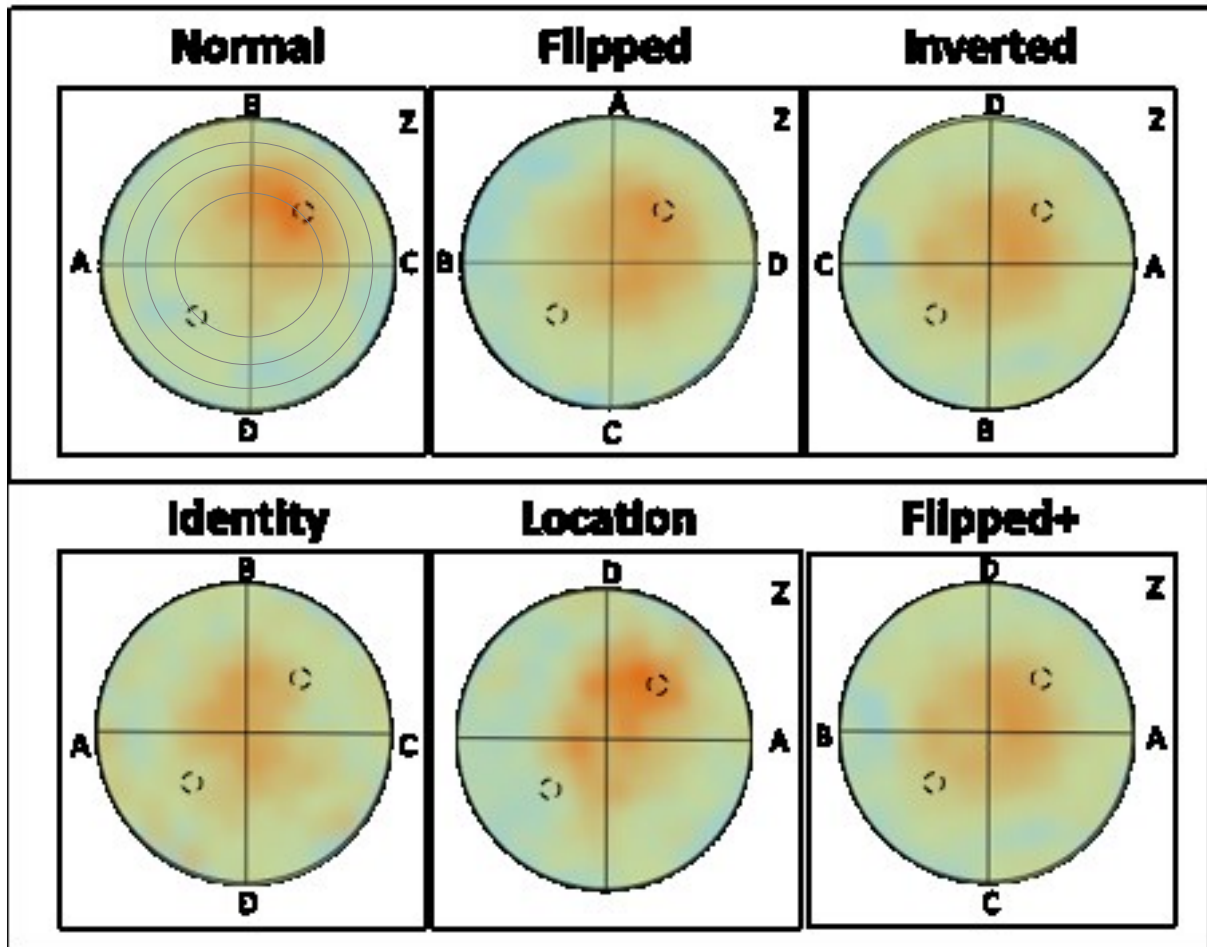

Figure 5: Heat maps for the experimental conditions in Experiment 1a (top) and 1b (bottom), blue/green denotes less time, yellow/orange/red more time spent in that cell, averaged over trials and rats. The positions of the landmarks present are shown for each condition. Training was to the configuration labelled "Normal" in Experiment 1a, and the platform was in the BC quadrant midway between B and C (shown), roughly corresponding to the hot spot in the heat map for that condition. A mirror image platform location in the opposite quadrant is also shown as an aid to interpretation, and we have given a rough indication of the four concentric regions used in our annular analysis on this condition. Note that we have oriented these maps differently to the schematics shown in Figure 1, and have shown the Identity condition oriented in the same way as the other maps to facilitate comparison.

These heat maps complement the bar charts we have offered in Figures 3 and 4 for time spent in quadrants of interest during test. They give a more global picture of the distribution of search behavior on test averaged across animals, and it is one that largely confirms the impression we have formed from the analysis of the quadrant data. Thus, performance in the Normal condition of Experiment 1a is highly localised in the platform quadrant. There is a similar, though weaker localisation in the Location condition of Experiment 1b. Perhaps most striking of all is the rather uniform and diffuse search in the Inverted condition of Experiment 1a, with search in Identity, Flipped and Flipped+ somewhere in between the distribution for Inverted and Location. We will comment further on these distributions and what they can tell us in the General Discussion, but first, we present the results of another analysis inspired by these heat maps that helps us better understand the pattern of search used by our rats.

This analysis uses the four concentric annular regions defined earlier (and see the Normal condition in Figure 5 to get a visual representation of how this was done) that are approximately equal in area and so should, if the animal is simply swimming around the pool at random, lead to a roughly equal distribution of time spent in each. In fact, based on the actual areas of these rings we can refine this prediction to state that a randomly moving animal would be expected to spend approximately 14 sec. in the Inner Centre region, 15 sec. in the 2nd and 3rd rings out from this, and 16 sec. in the Outer Wall region. Using these predictions, and noting that the platform position would be right on the boundary of the Inner Centre and 2nd Ring, we are able to see from the graph in Figure 6 that the rats are clearly avoiding the Outer Wall and 3rd Ring areas, as the time on average spent in these annuli is significantly below chance in every test condition bar one, which is the Outer Wall for the Identity Condition, and even here we have $t(11) = -1.86$, $p = .09$. Performance on the 2nd Ring is around chance, but significantly higher than that for the Normal Condition, $t(11) = 4.19$, $p = .002$, and significantly less in the Flipped+ Condition, $t(11) = -2.70$, $p = .02$. Intriguingly,

time spent in the Inner Centre circle is always greater than chance, and this result is significant for all conditions, smallest t(11) = 2.66, p = .022. So we have good support here for the notion that these rats, on test, avoid the edge of the pool and favor the center.
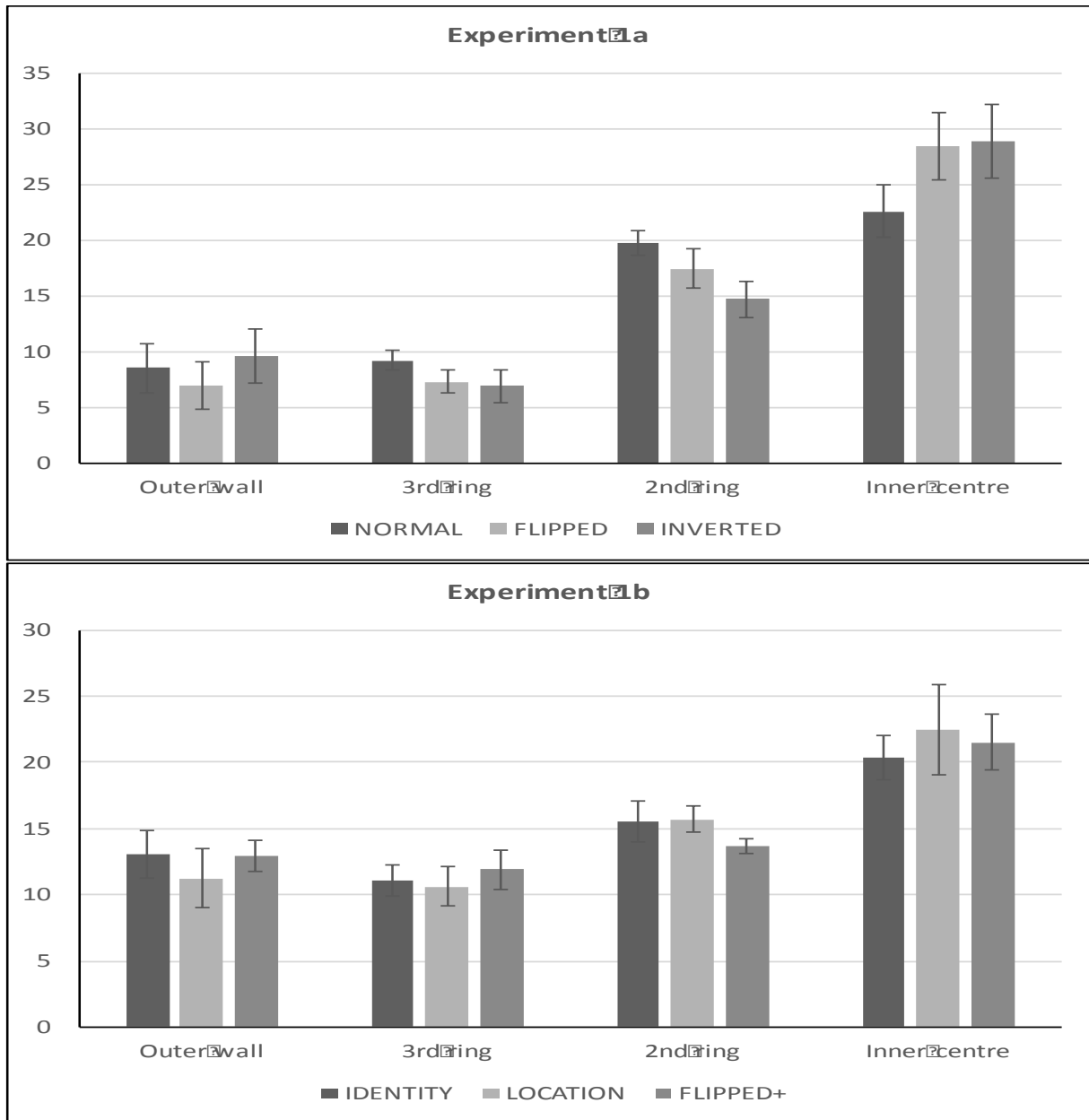

Figure 6: Graphs showing the average time spent in each of the four annular regions of the pool (central circle, then 2nd ring, 3rd ring then ring nearest the outer wall) by test condition. The top panel gives the results for Experiment 1a, the bottom panel for Experiment 1b. Error bars are SE of mean.

Further support for this view comes from an analysis of each experiment using Condition (three levels) and Region (four levels corresponding to the four regions) as factors, and time spent in each region as the dependent variable. This analysis for Experiment 1a gave a highly significant effect for Region, $F(3,33) = 45.8$, $p < .001$, $\eta_p^2 = .81$ and no other significant or near significant effects. A follow-up analysis of pairwise comparisons revealed that the Outer Wall and 3rd Ring regions did not differ, but both of these regions differed (rats spent less time in them) from the other two. The central region (Inner Centre) also differed significantly from the 2nd Ring, in that rats spent more time here. This basic pattern was replicated for Experiment 1b. There was no significant or near significant effect for Condition, or for the interaction between Condition and Region, but there was a significant main effect of Region, $F(3,33) = 8.58$, $p = .003$, $\eta_p^2 = .44$. Once again the central region (Inner Centre) was the one that rats spent most time in, and this was significantly different to all the other regions. The time spent in the 2nd ring was significantly greater than in the 3rd, but did not differ from the Outer Wall (though numerically it was greater). The 3rd Ring and Outer Wall regions did not differ significantly. These results certainly confirm that the rats tended to stay away from the outer wall of the pool, and concentrated their search more towards central regions. We take this up again in the General Discussion that follows.

General Discussion

What can the results of Experiments 1a and 1b tell us about how rats learn to navigate in the water maze? We will consider the results of each experiment individually first, before using both in making our final conclusions. We will also, to a first approximation, assume that A and D do not contribute much to performance in our experiments. This is based on the results from Civile et al (2014), who found that the "far" cues, A and D, did not seem to play any

significant role in guiding rats search under the conditions used here in a manner similar to the finding reported by Cheng (1986).

But before we consider the theoretical analysis that can be obtained from a detailed consideration of the quadrant data, we will first continue our discussion of the heat maps in Figure 5. Taken as a whole, the six heat maps all have one thing quite strikingly in common. They indicate that search is more centrally, rather than peripherally distributed, and that this distribution is rather uniform in some of the conditions. The fact that this is the case lends itself very naturally to a class of theories that propose a significant role for the geometry of the pool, and in particular the pool wall, in guiding search (see Cheng, Huttenlocher and Newcombe, 2013 for a relatively recent review). There are a number of models that can make use of this type of information: Cheng et al (2006) consider an extended version of the vector sum model referenced earlier (Cheng, 1989) that allows for this, and scene matching models of the type proposed by Sturzl et al (2008) can also accommodate its influence. Whatever the exact mechanism by which pool geometry is taken to exert an influence, in our data can we simply posit that the animal has, in some way, learned that the platform is at a certain distance from the edge of the pool[2]? And that as a result, it tends to maintain that distance (or more) from the pool wall, which quite naturally leads it to spend more time in more central areas. It may be, for example, that it is sensitive to the degree of perceived curvature of the pool wall and uses this as a cue in guiding search. All this makes a great deal of sense in conjunction with our heat map data, and is something that would not be revealed by our quadrant-based analysis.

 That said, this description of our rats performance may not be entirely accurate. If they were swimming at a certain distance from the pool wall. Then we would certainly expect both the central region (Inner Centre) and the 2nd Ring to benefit (in terms of time spent in them) from

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[2] Again, we thank an anonymous reviewer for this suggestion.

this strategy, and the outer two rings to suffer by comparison, and that is indeed the pattern revealed by our analysis. The one feature of our results that does not sit well with this account, however, is the pre-eminent position occupied by the central region. It is much the favoured region, even though the platform is only half in it, and half in the 2nd Ring. And this statement applies to the Normal condition in Experiment 1a, when search is very good and centered on the platform location, as well as to the other conditions. In fact, the easiest way to generate this pattern of results is to argue that the rats are searching at the platform location in the Normal condition, not that they are swimming at a certain distance from the pool wall. Because of the geometry of the pool and the four concentric regions used in this analysis, that quite naturally leads to an advantage for the central region if we allow some random error in determining the platform location on the part of the rat. A lot of the 2nd Ring is not anywhere near the platform, and so will not benefit from this error. But the central region mostly is, and so picks up more search time. It may be that when the landmarks are transformed in some way this search breaks down, but even in this case they seem to search more in the central region of the pool which is, on average, closer to the platform location than the 2nd or outer rings. It is at least possible, then, that our results simply reflect knowledge of the platform location, subject to some amount of error dependent on the landmarks available to guide search. In any case, use of the pool wall would not generate our quadrant data, it is quite agnostic about which quadrant will be preferred once distance from the pool wall is determined, and it is to the quadrant data that we now turn to assess the role of the various landmarks present in guiding search.

Experiment 1a demonstrated the usual very strong performance in rats tested with the landmark configuration they were trained on. The fact that performance in the Normal condition was significantly higher than in either the Inverted or Flipped conditions strongly suggests that the rats were using all of B, C and Z to navigate to the platform quadrant. If

they were just using Z, performance in the Flipped condition would be similar to that in the Normal condition; and if they were just using B and C, the Inverted condition would produce similar performance to Normal. Hence, we conclude that all of B, C and Z are either being used separately or in some configuration(s) to guide search in this maze.

The results for Inverted and Normal partially replicate and extend the results obtained by Civile et al (2014). In that paper the inversion manipulation had a similar effect in terms of decreasing performance in the maze, but left search times in the BC and Z quadrants at chance levels (though numerically the effects were in the same direction as for the present results). Now, we have search in the BC quadrant significantly below what would be expected by chance in the Inverted condition, and in the Z quadrant significantly higher than for BC (though not quite significantly higher than chance). Given this, it would seem that we can be certain that our inversion manipulation compromises search, and fairly certain that Z is the more potent cue controlling behavior.

The comparison between Inverted and Flipped may help us to interpret the contribution made by these cues in controlling behavior. In both cases there is a tendency to search in the Z quadrant, but this tendency is significantly more pronounced in Flipped than in Inverted. If we were to assume that each landmark operates quasi-independently, then we could explain this result by noting that in the Inverted condition, B and C are in the correct orientation to promote search in the BC quadrant, whereas in the Flipped condition this is not the case. The animals will have learned to search to the right of B during training, and to the left of C, and these are now two different locations. Hence, all we have to do is assume that Z is a more salient cue that promotes search directly in front of it, and the results obtained can be generated. In essence, this explanation draws on the idea that in the Inverted condition BC and Z are in opposition, and so search in the Z quadrant is reduced relative to that observed in the Flipped condition where Z is largely unopposed. In the Normal condition all three

promote search in the same quadrant, and hence performance is best under these circumstances.

A similar analysis can be given if we postulate use of cue configurations. If we assume that B, C and Z form a configuration during training, then both Inverted and Flipped manipulations disrupt the configuration that is present in Normal. But it could be argued that the disruption is more severe in Inverted, where B and C are now in a different relationship spatially with Z. In Flipped, the spatial relationship is similar to that in training, but subject to an enlargement in that B and C are now further away from Z. Nevertheless, if the animal were in the BC quadrant, then seeing B on the left and C on the right might actually promote approach to Z, and result in more time spent in the Z quadrant. On this analysis, the reason for better performance in the Flipped condition is that its' configuration of the three critical cues more closely matches that encountered in training, rather than the effects of landmarks B and C counteracting the effects of Z in the Inverted condition.

The results of Experiment 1b can help us choose between these two alternative explanations for the results of Experiment 1a. The Location and Flipped+ conditions both show a preference for the Z quadrant over the opposite quadrant, and these preferences are strikingly similar. It is certainly not the case that adding B and C as shown in Figure 1 to the Location condition has enhanced the effect in Flipped+, but according to the configural hypothesis it should have. This is because by adding B and C in this way, we are effectively reinstating the configuration of B, C and Z encountered in training (though somewhat distorted). If it was this configuration that produced the enhanced preference for the Z quadrant in the Flipped condition in Experiment 1a, then we should expect to see a similar effect here. We do not. Thus it is unlikely that Z is forming some configural representation with landmarks B and C in this experiment.

The case against both an elemental account and a configural account involving only the four landmarks ABCD, at least in this experiment, is provided by the results for the Identity condition. This condition can be seen as simply the four landmarks, A, B, C and D in the correct orientation with respect to one another but without Z being present. The fact that they've been rotated relative to the curtain is something that should not, in itself, affect performance, as this is something routinely done during training. We do see a preference for the BC quadrant under these conditions, but it is weak, and not even as strong as the preference for the Z quadrant in the Location condition when Z is tested in the absence of B and C. The implication is that the control exerted by the configuration of landmarks is not that strong in these circumstances, and is not having a great impact on behavior. If B and C were acting relatively independently of Z, or as a configuration not involving Z, then we would expect better performance here. Note that in Civile et al (2014), rats had no difficulty in learning to find the platform based on exactly these landmarks in this configuration if they had been trained with them in the absence of Z. In fact, their performance was at a level similar to that of Normal in Experiment 1a. So it is certainly not the case that they cannot learn to use these landmarks to locate the platform, and the rather weak localisation shown in the Identity condition is something to be explained.

In summary, we have evidence that Z is capable of being an effective cue on its own (performance in the Location condition), and that it does not seem to play a part in configurations with other landmarks (comparison of Location and Flipped+ conditions). But we also know from our analysis of Experiment 1a that B and C are also influencing performance because of the significant difference between Flipped and Inverted conditions in terms of time spent in the Z and BC quadrants. How can we resolve this puzzle? There is another version of the configural explanation that is more in keeping with current theories that use landmark configurations to guide navigation. This simply says that Z is one kind of

cue, and B and C are another, and that for that reason they do not form an overall configuration. But B and C do form such a configuration, but this is a configuration oriented with respect to the directional cue Z, and the results of the Identity condition in Experiment 1b reveal the rather weak influence of that configuration in the absence of Z. The Flipped+ condition does not have the right configuration at all, which is why the BC cues are ineffective and have little or no impact on performance relative to the Location condition. Why should we prefer this last explanation? Following the argument in Civile et al (2014), a possible, more elemental explanation, in which the three landmarks operate independently and in some sense compete for control of search seems incoherent. It makes sense if we conceptualize each landmark as an attractive cue that the animal wants to approach, and perhaps we can refine this idea a little to say that the animal wishes to go straight towards Z, to the right of B and to the left of C. Then the effect of inversion can be understood as some kind of competition between these sometimes opposing tendencies. But surely the net effect would be for the animal to spend most of its time in one or other of these quadrants if this account is correct? It might be that Z would beat BC, as is the case here, but most likely the overall time spent in these two quadrants would be higher than that in the other two. But this is not the case. There was no significant effect of this kind in Civile et al (2014), and there is none here. The average time per quadrant spent in BC or Z quadrants for the Inversion condition is 15.34 sec against a chance expectation of 15. So to make this elemental explanation work one would need to invoke a more drastic form of the "cancelling out" hypothesis and it is difficult to see what the basis for this would be. Looking at the heat maps confirms <u>that</u> this <u>type of</u> <u>explanation would be difficult to sustain</u>, there is no sign of multiple "hot spots" for search in the Inverted condition, but rather a more uniform distribution of search in more central areas.

An advantage of adopting the landmark configuration approach for B and C is that it then gives us a basis for integrating that information with Z in a way that can explain the far stronger performance on the training cue configuration (i.e. the Normal test in Experiment 1a). If Z is a directional cue, and is used to orient the representation of the landmark configuration, then if B and C are oriented correctly with respect to the Z direction, all three cues control performance and performance is very good. If B and C are wrongly oriented with respect to that direction (inversion), then performance falls off drastically. B and C are not seen as counteracting Z on this account, they are now relatively ineffective. Which also explains why, in the Identity condition, a condition which should, on the basis of Civile et al (2014) results from Experiment 1 have no difficulty in supporting good performance when trained on its own, we have such weak control by the four landmarks present. The lack of the directional cue provided during training is severely impacting performance, though at least it is better than when the directional cue provided is inconsistent with the landmark configuration in the Inverted condition.

As we noted in Civile et al. (2014), there is evidence already extant for this "oriented configuration" hypothesis. Roberts (1981, and see also Olthof, Sutton, D'Addetta and Roberts, 1999) found transfer between configurations (this time of visited locations) only if they were in the same environment and had the same orientation with respect to that environment. These results can be understood if the animal is taken to maintain some representation of the spatial relationships between visited locations on the radial maze, but uses the environmental cues to orient these representations. If they fail to match up with actual experience, then there is very weak transfer, but if they do then memory for the visited locations is enhanced. In our experiments, we would argue that Z is the directional cue and the other landmarks provide the spatial configuration. The animals use this configuration (predominantly B and C) to guide their search, but only if it is correctly oriented with respect

to Z. Once the landmarks have been rotated to a new position there is a mismatch between the learned information and current reality, and the configural information is taken as no longer applying to that situation leading to poor performance in the inverted condition.

In summary, we have evidence in these experiments taken together, and in combination with the results reported in Civile et al (2014), that the directional cue Z operates rather differently to the other, more standard landmarks A, B, C and D. It can clearly function as something of a beacon in its own right, as the results of the Location condition show. And it also can combine with the other landmarks to produce a very high level of search performance, but in a way that we hypothesize to be quite distinct from that described by the usual configural analysis often applied to multiple landmarks. We hope, in future studies, to further develop the case for this hypothesis.

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