

Boundary primacy in spatial mapping: Evidence from zebrafish (*Danio rerio*)

Sang Ah Lee^{a,*}, Ambra Ferrari^a, Giorgio Vallortigara^a, Valeria Anna Sovrano^{a,b}

^a Center for Mind/Brain Sciences, University of Trento, Rovereto, Italy

^b Department of Psychology and Cognitive Sciences, University of Trento, Rovereto, Italy

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ABSTRACT

The ability to map locations in the surrounding environment is crucial for any navigating animal. Decades of research on mammalian spatial representations suggest that environmental boundaries play a major role in both navigation behavior and hippocampal place coding. Although the capacity for spatial mapping is shared among vertebrates, including birds and fish, it is not yet clear whether such similarities in competence reflect common underlying mechanisms. The present study tests cue specificity in spatial mapping in zebrafish, by probing their use of various visual cues to encode the location of a nearby conspecific. The results suggest that untrained zebrafish, like other vertebrates tested so far, rely primarily on environmental boundaries to compute spatial relationships and, at the same time, use other visible features such as surface markings and freestanding objects as local cues to goal locations. We propose that the pattern of specificity in spontaneous spatial mapping behavior across vertebrates reveals cross-species commonalities in its underlying neural representations.

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1. Introduction

Over half a century of research established that animals possess allocentric representations of locations and their spatial relationships (Tolman, 1948; O'Keefe and Nadel, 1978; Gallistel, 1990). This ability, commonly known as cognitive mapping, can be defined as a representation of locations within a coordinate system from which their mutual relationships can be derived (Leonard and McNaughton, 1990), and it can be observed in navigation behavior that cannot be explained by path integration or by the use of associative features, such as local cues or beacons (Jacobs and Schenk, 2003). While a detailed characterization of the neural basis of spatial mapping is currently limited to the mammalian hippocampus (Barry and Burgess, 2014; Burgess, 2008), studies of birds and fish have revealed that map-like hippocampal representations are shared far and wide across the phylogenetic tree (Vallortigara, 2009; Broglio et al., 2011; Herold et al., 2014).

Mammals rely heavily on environmental boundaries (i.e., 3D extended surface layouts) to encode locations (for review, see Cheng and Newcombe, 2005; Lee and Spelke, 2010a; Oliva,

2013). Studies showing selective activation of hippocampal and parahippocampal neurons in response to boundaries lend further support to the claim that boundary representations are indeed crucial for spatial mapping (Doeller et al., 2008; Solstad et al., 2008; Lever et al., 2009; Bird et al., 2010; Park et al., 2011). But do these boundary-based spatial representations exist also in other vertebrates? Can we observe their influence on navigation behavior?

The primacy and specificity of boundary representation in navigation behavior have been most clearly demonstrated in spontaneous, working-memory reorientation tasks (e.g., Cheng, 1986; Lee et al., 2015). In such tasks, animals reliably searched for hidden goals in accord with the shape of the testing arena (i.e., using the metric relationships to map locations), while using other features (e.g., visual and olfactory markings) only as local cues (i.e., using direct associations to the goal). For instance, while disoriented rats reoriented by the metric relationships of the walls of a rectangular arena to distinguish between a corner to the north-west of a long wall from a corner to the northeast of a long wall, they only used a visually distinctive corner panel as a local cue to identify that particular corner without using its spatial location to distinguish between any of the other corners (Cheng, 1986). This phenomenon has been tested in a variety of species (Cheng and Newcombe, 2005), but the characteristic specificity of boundary representations in humans has been described in detail in preschool children: Boundary layouts that are successfully used

* Corresponding author at: University of Trento, Corso Bettini, 31, Rovereto (TN) 38068, Italy.

E-mail address: sangah@gmail.com (S.A. Lee).

for mapping include any 3D, horizontally extended terrain structures, even those that are only a few centimeters in height and consist of curved, hill-like surfaces. In contrast, environmental features used as local cues (but not for mapping) include a variety of cues, such as freestanding objects, surface markings, color, and 2D forms (Lee et al., 2006; Lee and Spelke, 2008, 2010b, 2011). Furthermore, children rely selectively on distance relationships among boundaries for spatial mapping, as opposed to other geometric properties such as the lengths of surfaces or the angles at which they meet (Lee et al., 2012a). These characteristic “signature limits” (see Spelke and Kinzler, 2007) of boundary representations can be used to assess whether similar spatial behaviors across species stem from shared underlying processes or, alternatively, from unshared processes that have independently evolved to serve similar functions.

Various studies of birds and fish (see Tommasi et al., 2012) have demonstrated that non-mammalian vertebrates also navigate using environmental boundaries and that this ability is unaffected by rearing conditions (Brown et al., 2007; Chiandetti and Vallortigara, 2008). However, while boundary-primacy in spatial mapping (alongside the use of other features as local cues) has been reported in avian species (Pecchia and Vallortigara, 2010; Lee et al., 2012b; Hurly et al., 2014), it has not yet been fully described in fish.

In the past decade, the zebrafish (*Danio rerio*) has emerged as a powerful non-mammalian animal model in neuroscience, behavioral genomics, and cognitive science (see Miklösi and Andrew, 2006; Lieschke and Currie, 2007; Levin and Cerutti, 2009; Kalueff et al., 2013; Bshary and Brown, 2014). The scientific advances made in the study of this animal model make the zebrafish an attractive species for investigating spatial mapping, particularly in comparison with mammals. To establish reliable behavioral measures of spontaneous spatial navigation in zebrafish, we recently developed an unrewarded task (Lee et al., 2012c; Lee et al., 2013) that harnesses their innate shoaling tendencies (Kerr, 1963) to motivate spatial behavior: when isolated zebrafish see a conspecific in one corner of an otherwise empty tank, they tend to approach that corner when released into the tank, even when the conspecific is no longer visible. Using this method, we found that zebrafish spontaneously reoriented by the shape of an opaque, rectangular arena (Lee et al., 2012c, 2013) and failed to reorient by a distinctively colored wall within a square arena or a rectangular array of four freestanding objects.

While the findings so far reveal some commonalities in spatial mapping behavior in fish and other vertebrates, it is still not yet clear whether the underlying representations of boundaries and landmarks are indeed the same. To gather evidence that would allow us to address this issue, we conducted the present study to characterize spatial mapping in zebrafish more rigorously, through a wide range of environmental manipulations.

In six experiments, we implemented the task described above (Lee et al.) to test the following: Experiments 1 and 2 replicated our past findings showing that zebrafish map the environment using a rectangular layout of opaque (but not transparent) boundaries. In Experiments 3, we tested zebrafish on a 2D rectangular form on the floor of the tank, like those tested in human children (Lee and Spelke, 2011) and domestic chicks (Lee et al., 2012b). In Experiment 4, we provided zebrafish with a visible landmark (a freestanding column) at one end of the transparent tank (outside of one of the short walls), along with a variation of the featural cue (a light bulb) in Experiment 4a. In Experiment 5, we place the column outside the long wall of the transparent tank and consequently distal to all corners (i.e., providing no local cues). Finally, in Experiment 6, we tested the use of the landmark within a rectangular array of opaque boundaries, to investigate potential interactions between cues. Table 1 provides a summary of experimental conditions and their main findings.

2. General methods

2.1. Subjects

Subjects were 112 (56 males) mature (average 6 months old) zebrafish (*D. rerio*, short-finned, heterogeneous wild types) obtained from local distributors in Trento, Italy. The fish were housed in two tanks (separated by sex) in temperature-controlled rooms and exposed to a 16-h light cycle. All subjects were naïve to the experimental procedure and tested only once.

2.2. Apparatus

The experiment was conducted in a transparent rectangular tank (23.5 cm × 38 cm; depth 25 cm), with a glass jar (diameter 5 cm; height 6 cm) in each corner. The floor of the tank was lined with light-colored gravel, and the tank was filled with water to a height of 6 cm. The entire apparatus was surrounded by a square array of black plastic panels (55 × 55 × 40.5 cm) to minimize the use of external visual cues. The experimental room was dark, except for a single lamp suspended over the center of the tank that uniformly illuminated the four corners. A video camera was placed directly above the apparatus to record behavioral responses.

2.3. Design

For each subject, we administered ten trials with a single, unrewarded goal location. An equal number of male and female subjects were tested at each corner. We recorded the first approaches and the total proportion of approaches to the four jars for 10 s following the release of the subject into the tank. We defined an approach to be any instance in which the fish swam to a distance of 1 cm from a glass jar.

2.4. Procedures

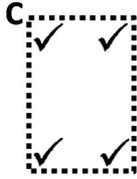
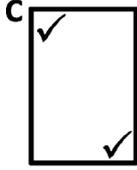
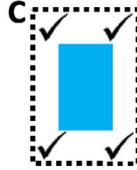
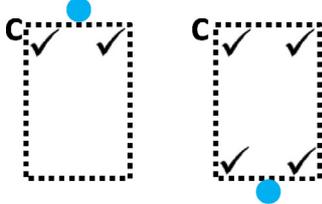
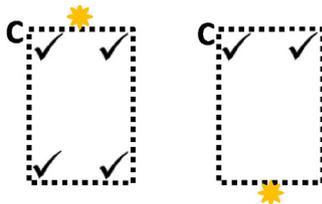
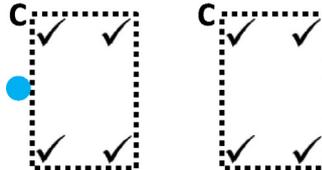
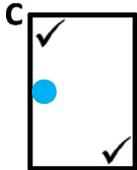
The subject was transferred from its home tank to the testing tank (with four empty jars at the corners) for 5 min of familiarization. The subject was captured and placed in a glass jar at the center of the tank, and a dark opaque cylinder (translucent at the top) was placed over the jar to occlude the subject's view of the tank. The empty jar at the target corner was replaced with one containing a female zebrafish (social attractor); the dark cylinder was then removed to give the subject visual access to the conspecific in the target corner. After 2 min, the jar containing the subject was covered again, gently removed from the tank and rotated slowly 360° clockwise, 360° counterclockwise, then an additional 90° along with the entire apparatus. The jar with the conspecific was replaced with an empty one, and the subject fish was finally released into the center of the tank. A video recording of each trial (first corner approached, and proportion of approaches made to each corner in 10 s) was analyzed offline. The subject was left to swim freely for 2 min in the empty tank, without any reinforcement for its responses, captured again, covered for about 20 s, and then placed back in the center of the tank with the conspecific in the target corner for the start of the next trial.

3. Experimental conditions

3.1. Experiment 1: Transparent boundary control

In Experiment 1 we tested 12 zebrafish (six males) in a rectangular tank with transparent acrylic (Plexiglas) surfaces (as in Lee et al., 2013) to ensure the effectiveness of the disorientation procedure and the symmetry of the testing environment.

Table 1
Summary of results. Behaviorally preferred corners in each condition are indicated by checkmarks.

Condition	Behavior	Interpretation
Exp. 1 Transparent		Failure
Exp. 2 Opaque boundary		Boundary mapping
Exp. 3 2D form		Failure
Exp. 4 Proximal landmark		Local cue use; no mapping
Exp. 4a Proximal light source		Local cue use; no mapping
Exp. 5 Distal landmark		Failure
Exp. 6 Boundary and distal landmark		Boundary mapping; landmark failure

3.2. Experiment 2: Opaque boundary control

In Experiment 2, we tested 16 zebrafish (eight males) to replicate the finding of spatial mapping by boundaries (as in Lee et al., 2013, 2012) using a rectangular tank made with opaque, white plastic panels (20 cm × 30 cm; 10 cm high).

3.3. Experiment 3: Two-dimensional form

Human children and young, domestic chicks encode environmental geometry specifically with respect to 3D surface layouts and fail to reorient using a 2D rectangular form (Lee and Spelke, 2011;

Lee et al., 2012a). To investigate whether untrained fish show the same limitations in their spatial coding, in Experiment 3 we tested 24 mature zebrafish (twelve males) using a flat (2D) blue plastic rectangle (30 cm × 10 cm) on the ground within the transparent rectangular tank used in Experiment 1.¹ The corners of the blue rectangle were directly pointed at the four jars at the corners.

¹ A preliminary finding showed that zebrafish were able to discriminate between the two sides of a 2D rectangle that was half white and half blue, providing evidence that they do pay attention to 2D visual stimuli at the bottom of the tank. The color blue was chosen to contrast with the light pebbles covering the bottom of the tank and because zebrafish have been shown to prefer it in various behavioral tasks (Arthur and Levin, 2001; Lee et al., 2012c; Oliveira et al., 2015).

3.4. Experiment 4 and 4a: Proximal landmark

Experiment 4 provided the fish with a visual landmark – a blue, cylindrical object (width 8 cm, height 25 cm) – centered directly outside of one of the short walls of the transparent tank (see Fig. 2). The cylindrical shape was chosen to provide 3D shape information, and the vertical orientation of the object was selected for its demonstrated effectiveness in navigation (Yaski and Eilam, 2007). We tested 24 subjects (12 males). In Experiment 4a, to test for directional navigation by light, we replaced the cylindrical object of Experiment 4 with an incandescent light bulb, creating a sharp gradient of illumination (see Fig. 3). We tested 24 subjects (12 males).

3.5. Experiment 5: Distal landmark

Placing a cue on the short side of the rectangular arena (as in Experiments 4 and 4a) could polarize the environment to corners proximal to vs. distal from the cue. Therefore, in Experiment 5 we placed the cylindrical object at the center of the long wall of the rectangular tank instead, such that it could not serve as a local cue to any of the corners. We tested 24 subjects (12 males).

3.6. Experiment 6: Boundary and distal landmark

Given that previous studies suggest a potentiating effect of features when presented within a geometrically distinctive boundary layout (Austen et al., 2013; Lee et al., 2012c; Timberlake et al., 2007), it may be possible to enhance the use of a distal landmark (as in Experiment 5) by embedding it within an array of boundaries. Therefore, in Experiment 6 we placed the blue cylindrical object (from Experiments 4 and 5) within an opaque rectangular tank (from Experiment 2), along one of the long walls. We tested 12 subjects (six males).

4. Results

The successful use of rectangular geometry requires spatial mapping (i.e., representation of relative locations with respect to the metric properties of the environment). The use of spatial mapping in Experiments 1–3 was measured by combining the proportions of approaches to the two geometrically correct corners (the correct corner plus the diagonal corner, C + D; see Fig. 1).

The use of a local cue directly associated with the goal location in Experiment 4–6 was measured by combining the proportions of approaches to the correct corner and the featurally symmetric corner (C + F; see Figs. 2–4). Because half of the target corners were along the same side of the tank as the landmark and half of the target corners were on the opposite side, we included the distance to the landmark as a between-subjects measure in our analysis. Furthermore, the use of a landmark as a mapping cue (in Experiments 4 and 5) or in combination with geometry (in Experiment 6) should result in a selective preference for the correct corner (C). Given our past studies showing success in working-memory tests of rectangular boundary geometry and local cue use (e.g., Lee et al., 2015, 2012), our one-tailed directional hypothesis was tested by comparing proportions of C + D (for geometry) or C + F (for features) against a chance value of 0.5. To provide a more valid test of the null hypothesis, we also provided Bayes Factors comparing the odds of a successful use of boundaries or local features (for a prior proportion greater than 0.5 but in the range of 0.5–0.7), compared to the odds of the null hypothesis (see Gallistel, 2009). Table 1 provides an overall summary of the experimental conditions and their results.

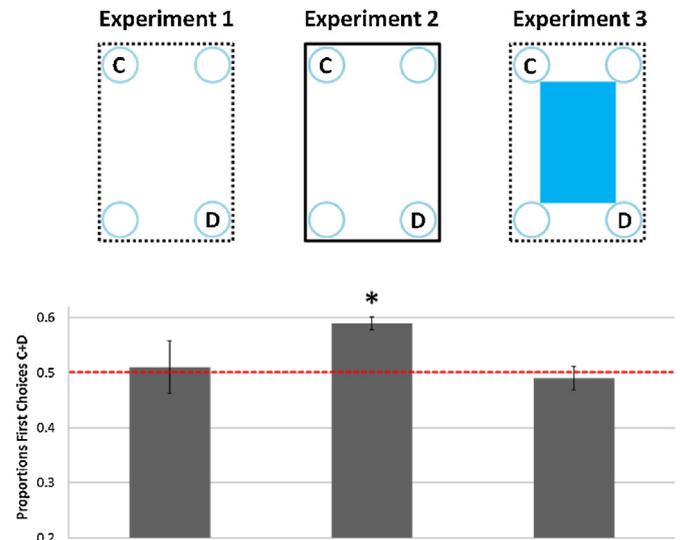


Fig. 1. Navigation by geometry. At the top are schematic representations of the experimental arenas used in Experiments 1–3. Dotted lines depict transparent boundary surfaces and solid lines depict opaque surfaces; geometrically correct corners are labeled as follows: C = correct, D = diagonal. The solid rectangle shown for Experiment 3 represents the blue 2D rectangle on the floor of the test arena. At the bottom are proportions of first approaches to the geometrically correct corners (C + D) for Experiments 1–3, from left to right. * $p < 0.05$ (t -tests against chance of 0.5). Error bars represent standard errors of the mean values (SEM).

4.1. Experiment 1: Transparent boundary control

For both first choice and 10-s measures, the proportions of approaches to the two geometrically correct corners (C + D, see Fig. 1) were not different from chance of 0.5 (first choice: Mean = 0.51 ± 0.05 , $t(11) = 0.16$, $p = 0.44$, Cohen's $d = 0.10$; 10-s approaches: Mean = 0.51 ± 0.05 , $t(11) = 0.23$, $p = 0.41$, Cohen's $d = 0.14$). The Bayes Factor for geometrically correct choice in Experiment 1 was 0.22, with corresponding odds of the null hypothesis at 4.47, providing substantial support for the null hypothesis. For both dependent measures, there were no differences between males and females and no preference for the correct corner over the other corners (all $F_s \leq 1.3$, n.s.). These results replicate the findings of Lee et al. (2013) and show that there were no uncontrolled environmental cues that influenced navigation behavior in this task.

4.2. Experiment 2: Opaque boundary control

For both the first choice and 10-s measures, the preference for the geometrically correct corners was significantly higher than chance of 0.5 (see Fig. 1) (first choice: Mean = 0.59 ± 0.03 , $t(15) = 2.91$, $p = 0.005$, Cohen's $d = 1.50$; 10-s approaches: Mean = 0.55 ± 0.01 , $t(15) = 4.38$, $p = 0.0005$, Cohen's $d = 2.26$). The Bayes Factor in favor of geometrically correct first searches was 6.83, with corresponding odds of the null hypothesis at 0.15, providing substantial support for a successful use of geometry. For both dependent measures, there were no differences between males and females and no preference for the correct corner over the geometrically identical diagonal corner (all $F_s \leq 1.6$, n.s.). These results confirm that zebrafish successfully map space using a rectangular array of opaque surfaces.

4.3. Experiment 3: Two-dimensional form

For both the first choice and 10-s measures, approaches to the geometrically correct corners were not different from chance of 0.5 (see Fig. 1) (first choice: Mean = 0.49 ± 0.04 , $t(23) = 0.21$, $p = 0.42$, Cohen's $d = 0.09$; 10-s approaches: Mean = 0.48 ± 0.02 , $t(23) = 0.73$,

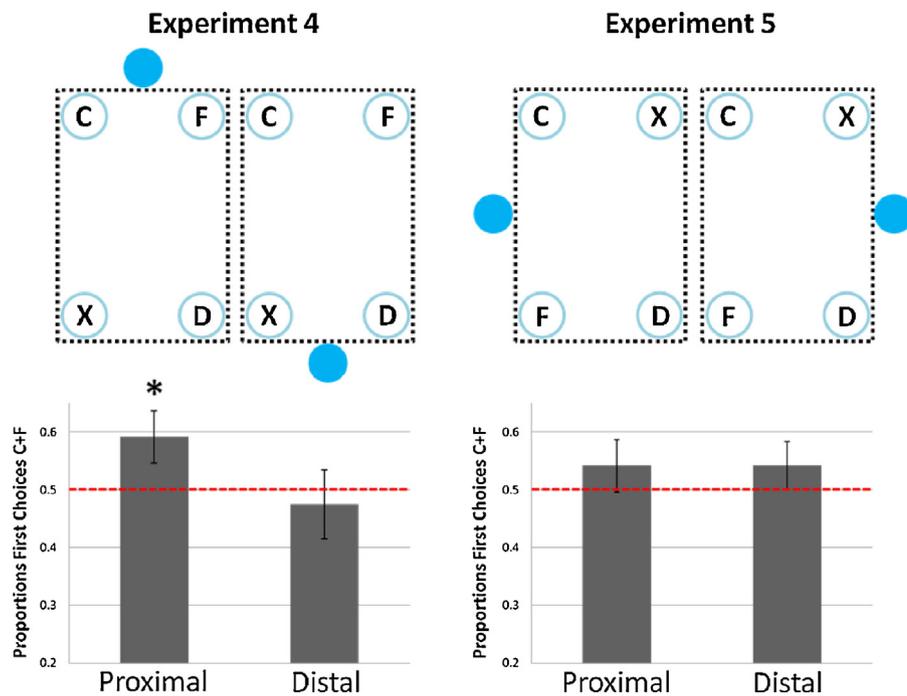


Fig. 2. Navigation by visible landmark. At the top are schematic representation of the experimental arenas used in Experiments 4 and 5, split for target locations proximal and distal with respect to the landmark (Corners: C = correct, F = featurally similar, D = diagonal, X = error). Dotted lines depict transparent boundary surfaces. The circles represent the freestanding 3D object. At the bottom are proportions of first approaches in accord with local feature use (C + F) for Experiments 4 and 5, respectively. Illustrations and results for are shown separately. * $p < 0.05$ (t -tests against chance of 0.5). Error bars represent SEM.

$p = 0.24$, Cohen's $d = 0.30$). The Bayes Factor for the odds of a geometrically correct first search was 0.02, with corresponding odds of the null hypothesis at 66.62, providing strong support for the null hypothesis. For both dependent measures, there were no sex differences and no preference for the correct corner over the geometrically identical diagonal corner (all F 's ≤ 1.4 , n.s.). Zebrafish, like children and chicks, did not spontaneously map space according to a 2D rectangular form.

4.4. Experiment 4: Proximal landmark

A univariate ANOVA with *Sex* and *Distance* (goals near the landmark, along the same wall vs. goals far from the landmark, along the opposite wall) as between-subjects measures revealed no significant effects for the first choice to the goal (all F 's < 2.6 , n.s.). Subjects did not choose the correct corner C above a chance level of 0.25 (Mean = 0.26 ± 0.03 , $t(23) = 0.39$, $p = 0.70$, Cohen's $d = 0.16$). However, an analysis of local cue use (C + F) revealed a significant main effect of *Distance* for the 10-s approaches ($F(1,20) = 9.71$, $p = 0.005$, partial $\eta^2 = 0.38$). We found performance above chance level of 0.5 when the goal was near the landmark (Mean = 0.61 ± 0.03 ; $t(11) = 3.01$, $p = 0.003$, Cohen's $d = 1.26$) but not when the goal was across from it (Mean = 0.44 ± 0.05 ; $t(11) = 0.97$, $p = 0.17$, Cohen's $d = 0.40$; see Fig. 2). This was supported by the Bayesian analysis, which resulted in a Bayes Factor of 6.73 for goals near the landmark and 0.07 for goals far from the landmark, with corresponding odds of the null hypothesis of 0.15 for near-landmark goals and 14.76 for far-landmark goals. Nevertheless, even for goals near the landmark, the correct corner (Mean = 0.30 ± 0.03) was not distinguished from the featurally symmetric corner (Mean = 0.31 ± 0.03 , $t(11) = 0.09$, $p = 0.93$, Cohen's $d = 0.04$), indicating that the landmark did not serve as a spatial mapping cue and only served to provide a far/near distinction. There were no significant differences in performance between males and females.

4.5. Experiment 4a: Proximal light source

A univariate ANOVA with *Sex* and *Distance* (bright goal locations near the light, along the same wall vs. dim goal location far from the light, along the opposite wall) as between-subjects measures revealed no significant effects for both first approaches to C (all F 's < 1.6 , n.s.) and for 10-s approaches to C (all F 's < 0.90 , n.s.). Subjects did not exhibit a preference for the correct corner for both the first approaches (Mean = 0.30 ± 0.05 , $t(23) = 0.91$, $p = 0.37$, Cohen's $d = 0.38$) and the 10-s approaches (Mean = 0.27 ± 0.40 , $t(23) = 0.59$, $p = 0.58$, Cohen's $d = 0.25$). However, an analysis of local feature use (C + F) revealed a main effect of *Distance* for both the first approaches ($F(1,20) = 4.72$, $p = 0.042$, partial $\eta^2 = 0.19$) and the 10-s approaches ($F(1,20) = 7.42$, $p = 0.013$, partial $\eta^2 = 0.27$). We found that C + F was significantly above chance when the goal was far from the light, for both the first approaches (Mean = 0.73 ± 0.05 ; $t(11) = 4.55$, $p = 0.0005$, Cohen's $d = 2.74$) and the 10-s approaches (Mean = 0.73 ± 0.06 ; $t(11) = 3.82$, $p = 0.0015$, Cohen's $d = 2.30$). Interestingly, fish did not prefer the featurally correct corners above chance when the goal was near the light (first approaches: Mean = 0.53 ± 0.07 ; $t(11) = 0.34$, $p = 0.37$, Cohen's $d = 0.20$; 10-s approaches: Mean = 0.45 ± 0.08 ; $t(11) = 0.61$, $p = 0.27$, Cohen's $d = 0.37$). The Bayesian analysis on the first approaches confirmed these results (Bayes Factors for goals near light: 1.11 for local cue use vs. 0.90 for the null hypothesis; goals far from light: 6854.28 vs. 0.0001 for the null hypothesis). Nevertheless, even for the dimly lit corners, the correct corner was not distinguished from the featurally symmetric corner for both the first approaches (Mean = 0.33 ± 0.08 , $t(11) = 0.55$, $p = 0.59$, Cohen's $d = 0.33$) and the 10-s approaches (Mean = 0.32 ± 0.05 , $t(11) = 1.16$, $p = 0.27$, Cohen's $d = 0.70$), indicating that the light, like the freestanding object in Experiment 4, failed to serve as a directional cue. There were no significant differences between males and females.

The results of Experiments 4 and 4a confirm past findings of spatial cue learning and visual beacon homing in fish (e.g., Warburton,

Experiment 4a

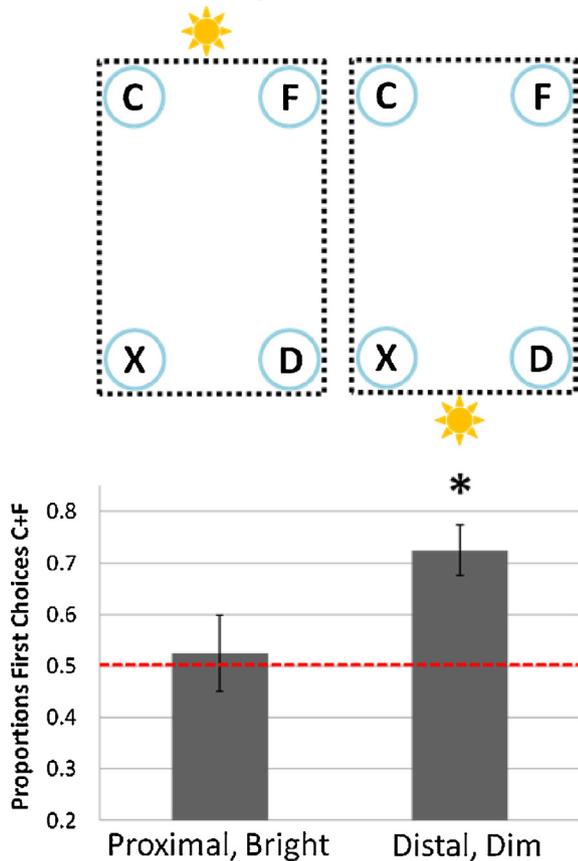


Fig. 3. Directional light source as a cue. At the top are schematic representations of the experimental arena used in Experiment 4a, for target corners proximal and distal from the light source (brightness measurements: corners near the light 56.7 cd/m²; corners far from the light 7.9 cd/m²). Dotted lines depict transparent boundary surfaces; corners are labeled as follows: C=correct, F=featurally similar, D=diagonal, X=error. The solar symbol represents the light bulb. At the bottom are proportions of first approaches according to local feature (brightness). * $p < 0.05$ (t -tests against chance of 0.5). Error bars represent SEM.

Experiment 6

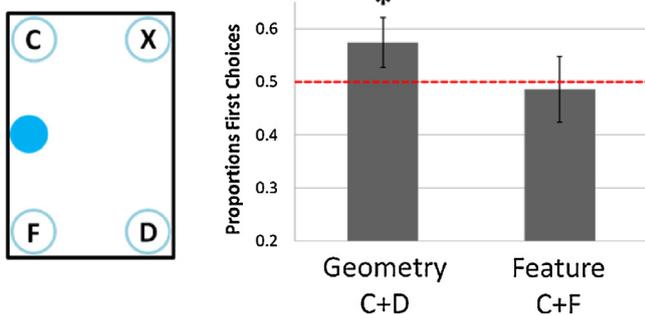


Fig. 4. Boundary geometry + distal landmark. On the left is a schematic representation of the experimental arena used in Experiment 6 (corners: C=correct, F=featurally similar, D=diagonal, X=error). Solid lines depict opaque boundary surfaces; the circle represents the freestanding 3D object within the tank. On the right are proportions of first approaches according to boundary geometry and local features. * $p < 0.05$ (t -tests against chance of 0.5). Error bars represent SEM.

1990; de Perera Burt and Macías García, 2003; Odling-Smee and Braithwaite, 2003) and extend them to include unrewarded, spontaneous use of a visible landmark and a light source. Furthermore, given that zebrafish have been shown to prefer the blue over other colors (Arthur and Levin, 2001; Oliveira et al., 2015) and to prefer dim environments over bright ones (Serra et al., 1999; Blaser

et al., 2010; Mansur et al., 2014), these findings demonstrate that the use of local cues can be modulated by pre-existing preferences or aversions.

4.6. Experiment 5: Distal landmark

A univariate ANOVA with Sex and Distance (goals near the landmark, along the same wall vs. goals far from the landmark, along the opposite wall) as between-subjects measures revealed no significant effects, both for first approaches to C (all F 's < 2.7 , n.s.) and C+F (all F 's < 0.1) and for 10 s approaches to C (all F 's < 0.9 , n.s.) and C+F (all F 's < 0.9 n.s.). Subjects approached the four corners randomly (Fig. 3) and did not reorient toward the correct corner C (first approaches: Mean = 0.31 ± 0.04 , $t(23) = 1.57$, $p = 0.13$, Cohen's $d = 0.66$; 10 s approaches: Mean = 0.29 ± 0.03 , $t(23) = 1.27$, $p = 0.22$, Cohen's $d = 0.53$). Moreover, they did not use the distance to the landmark as a cue to location (C+F), both for first approaches (Mean = 0.54 ± 0.03 , $t(23) = 1.39$, $p = 0.09$, Cohen's $d = 0.58$) and 10 s approaches (Mean = 0.52 ± 0.03 , $t(23) = 0.55$, $p = 0.30$, Cohen's $d = 0.23$). The Bayes Factor in favor of successful cue use was 0.83, with corresponding odds of the null hypothesis at 1.21, providing no significant support for either hypothesis. Although a distal landmark in this condition was the only cue to spatial location, zebrafish did not spontaneously encode it to map the environment. They also did not use it to distinguish between the two corners that were relatively closer to it from the two farther from it. There were no differences in performance between males and females.

4.7. Experiment 6: Boundary and distal landmark combined

Zebrafish searched in accord with the boundary geometry (Fig. 4) significantly above chance for the first approaches (Mean = 0.59 ± 0.05 , $t(11) = 1.96$, $p = 0.04$, Cohen's $d = 1.18$) as well as for the 10-s approaches (Mean = 0.57 ± 0.03 , $t(11) = 2.54$, $p = 0.014$, Cohen's $d = 1.53$). The Bayes Factor in favor of successful boundary-based mapping was 7.27, with corresponding odds of the null hypothesis at 0.14, providing significant support for successful navigation according to boundary geometry. Nevertheless, they did not use the landmark to disambiguate between the correct corner and the geometrically identical corner (t 's < 1.2 , n.s.). There were no differences between males and females and no effects of goal location.

5. General discussion

The present study suggests that even in the absence of training, zebrafish, like other vertebrates, map spatial locations with respect to environmental boundaries and use non-boundary features as local cues to the target (see Table 1 for summary). Their failure to use a 2D rectangular form or a distal landmark, even when it was embedded within a layout of boundaries, replicate similar findings of boundary primacy and specificity found in mammals and birds (see Lee and Spelke, 2010a; Spelke and Lee, 2012).

These findings are consistent with the body of increasingly convergent evidence that environmental boundary structures are represented and processed independently from other features. In human adults, for instance, studies of scene perception (Greene and Oliva, 2009; Park et al., 2011) and virtual reality navigation in human adults (Doeller and Burgess, 2008; Doeller et al., 2008; Bird et al., 2010) show that boundary-like surfaces and landmarks activate distinctive hippocampal and striatal areas of the brain and follow different patterns of learning. Moreover, evidence from Williams Syndrome individuals who are specifically impaired at boundary-based navigation while relatively successful at feature-use has even raised the possibility of studying the genetic basis of boundary representations in humans (Lakusta et al., 2010). In

the rodent hippocampal formation, environmental boundaries are represented at the single-cell level in the entorhinal cortex and subiculum (Solstad et al., 2008; Lever et al., 2009) and are postulated to provide the principal input to hippocampal place mapping (O'Keefe and Burgess, 1996; Hartley et al., 2000; Lever et al., 2002).

While there is some evidence in avian species of specialized neural representation of boundary in the hippocampus (see Tommasi et al., 2012), no studies to date have reported such findings in fish. The present findings suggest that not only are fish capable of spatial mapping, they are guided by spatial representations of boundaries and features that may be common to all vertebrates. A combination of the behavioral tasks provided here with the techniques available in studying zebrafish neurobiology could provide powerful new insight into the neural underpinnings of spatial navigation in fish.

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