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

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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.

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 northwest 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

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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 Miklòsi 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.