



Homebase behavior of zebrafish in novelty-based paradigms

Adam Stewart, Jonathan Cachat, Keith Wong, Siddharth Gaikwad, Thomas Gilder, John DiLeo, Katie Chang, Eli Utterback, Allan V. Kalueff*

Department of Pharmacology and Neuroscience Program, Zebrafish Neuroscience Research Consortium (ZNRC), Tulane University Medical School, 1430 Tulane Ave., New Orleans, LA 70112, USA

ARTICLE INFO

Article history:

Received 23 March 2010

Received in revised form 14 July 2010

Accepted 23 July 2010

Keywords:

Zebrafish

Homebase behavior

Exploration

Open field test

ABSTRACT

Zebrafish (*Danio rerio*) are emerging as a promising model species in neuroscience research. Many traditional rodent behavioral paradigms may be adapted for zebrafish testing. Exposing zebrafish to three different “open field” tanks for 30 min, we showed that fish display robust homebase behavior, in which one area of the tank is chosen as a preferred point of reference during the test, which the fish frequently return to and spend a longer duration in. This phenotype strikingly resembles rodent homebase behavior, confirming that both species use homebases as “reference points” for their exploration. Our study introduces a simple method for zebrafish homebase phenotyping, and further supports the utility of these fish in neurobehavioral and cognitive research.

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1. Introduction

Exploration is a key animal behavior in response to novelty (Kalueff and Zimbardo, 2007; Kim et al., 2005; Kliethermes and Crabbe, 2006). Traditional exploration-based paradigms include the elevated plus maze (Walf and Frye, 2007), light-dark box (Bourin and Hascoet, 2003) and the open field test (OFT) (Choleris et al., 2001; Kulikov et al., 2008; Prut and Belzung, 2003), widely used in neurobehavioral research. While extensively studied in rodents (Carola et al., 2002; Choleris et al., 2001; Koplik et al., 1995; Walsh and Cummins, 1976), the OFT has also been applied across many species, including humans (Perry et al., 2009), monkeys (Ferguson and Bowman, 1990a,b; Ferguson et al., 1996; Mothes et al., 1996), birds (Frederick, 1976; Gallup and Suarez, 1980; Kembro et al., 2008; Rodenburg et al., 2003; Spetch and Edwards, 1986), and fish (Budaev et al., 1999; Gerlai et al., 1990; Gerlai and Csányi, 1988; Miklosi et al., 1992; Warren and Callaghan, 2006). The rodent OFT is based on the thigmotaxis in an open novel environment, where animals freeze and stay closer to the walls (Choleris et al., 2001; Kulikov et al., 2008; Lamprea et al., 2008). Cognitive factors, such as habituation and learning, also play an important role in this paradigm (Comim et al., 2009; Dubovicky and Jezova, 2004; Tamasy et al., 1973).

Zebrafish show similar behaviors in novel environments (Bencan et al., 2009; Egan et al., 2009; Levin et al., 2007; Wong et al., 2010), suggesting common strategies of novelty exploration between rodents and zebrafish. Recently applied to zebrafish research, the OFT paradigm has provided important insights into fish motor and affective phenotypes (Guo, 2009; Levin et al., 2007).

Another behavior relevant to exploration and cognition is homebase formation, since animals tend to establish a “safe” home site to which they repeatedly return after exploring the environment (Eilam and Golani, 1989). This behavior is frequently observed in laboratory rodents, which use homebases as strategic “reference points” to orient and organize their exploration (Eilam and Golani, 1989; Golani et al., 1993; Tchernichovski et al., 1996). For example, animals visit many places in a novel environment, but typically choose one or two zones to spend most of their time, also showing highest grooming and rearing activity (Eilam and Golani, 1989) in this area.

While homebase formation represents an important aspect of animal exploration, the ability of fish species to establish homebases has not been examined previously. Here we demonstrate that adult zebrafish actively establish homebases when they explore novel environments.

2. Methods

2.1. Animals and housing

A total of 60 adult (6–8-month-old; ≈50:50 male:female ratio) wild type short-fin zebrafish were used in this study. The animals ($n = 20$ in each group) were obtained from a local commercial distributor (50 Fathoms, Metairie, LA) and given at least 20 days

* Corresponding author at: Department of Pharmacology, Room SL-83, Tulane University Medical School, 1430 Tulane Ave., New Orleans, LA 70112, USA. Tel.: +1 504 988 3354.

E-mail address: avkalueff@gmail.com (A.V. Kalueff).

to acclimate to the animal facility. The fish were housed in groups of approximately 20–30 fish per 40L tank. All tanks were filled with filtered/facility water, with room and water temperatures maintained at $\approx 25^{\circ}\text{C}$ and water pH at 7.0–8.0. Illumination (1170 ± 67 lux) was provided by ceiling-mounted fluorescent light tubes on a 12 h cycle (on 6:00 h; off 18:00 h), consistent with the zebrafish standard of care (Westerfield, 2007). All animals used in this study were experimentally naïve and fed Tetraamin Tropical Flakes (Tetra USA, VA) twice a day.

2.2. Apparatus and behavioral testing

Behavioral testing was performed using three different novel OFT tanks filled with aquarium water to the level of 12 cm. OFT1 represented a large rectangular plastic opaque tank (12.3 cm height \times 38.7 cm width \times 47.3 cm length) divided into 9 zones. OFT2 was a white plastic cylinder (23.6 cm height \times 22.8 cm diameter) divided into 8 sectors, and OFT3 was a white square tank (14.0 cm height \times 29.0 cm width \times 37.0 cm length) with textured

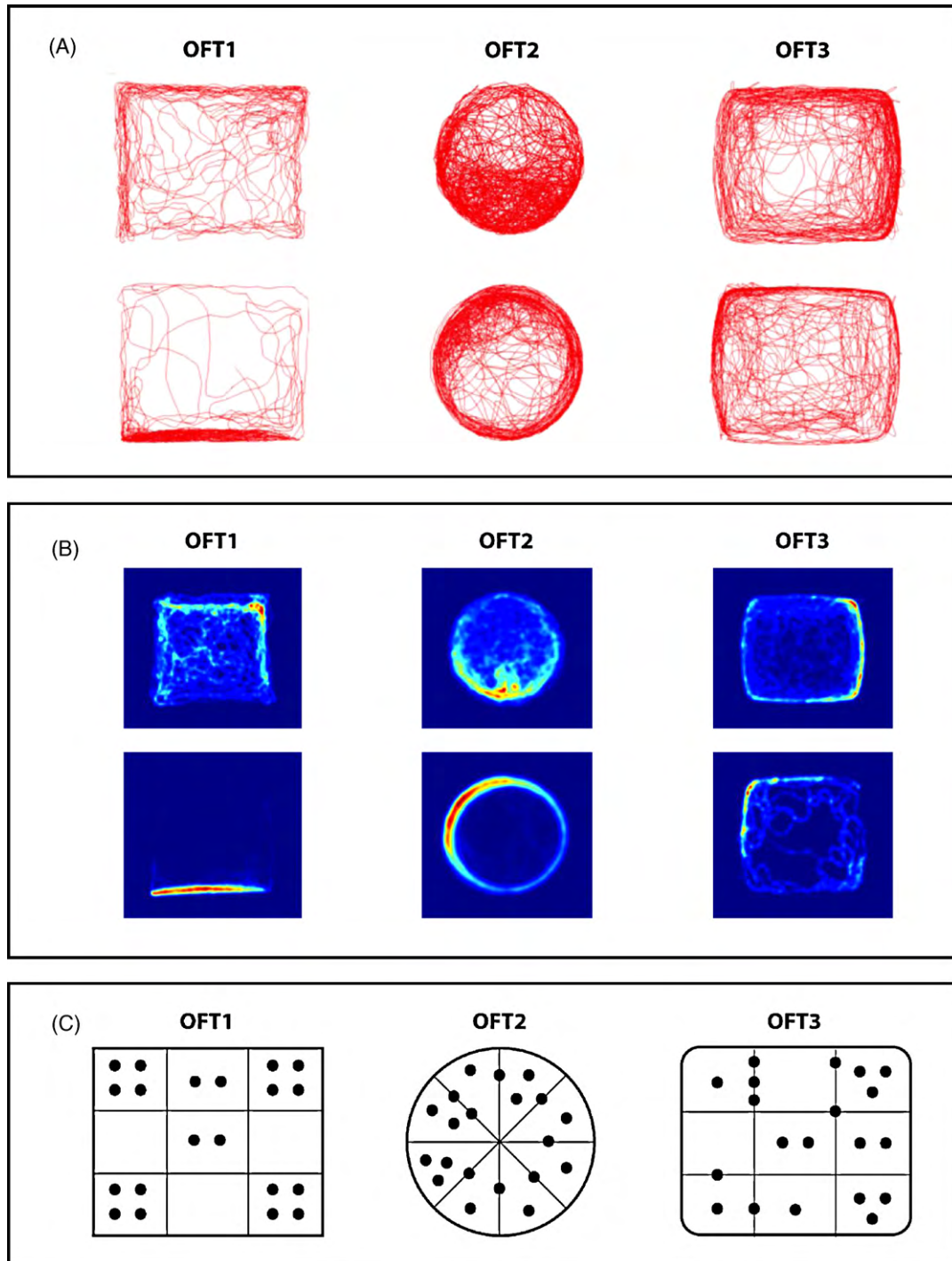


Fig. 1. Representative homebase behavior demonstrated in three different 30-min open field tests (OFT1–3). OFT1 was the large rectangular arena, OFT2 was the circular arena, and OFT3 was the small square arena (see Section 2 for details). (A) Traces generated by Noldus Ethovision XT7 software for three different zebrafish. Note clear spatial preferences of zebrafish OFT behaviors. (B) Density maps generated for three different fish (same as in panel A) by Noldus Ethovision XT7. (C) Summary of homebase topography for all fish ($n = 20$ per OFT) tested here. Each homebase is shown as a black dot. Note that each fish was able to establish a clear homebase, typically encompassing one or, less frequently, two sectors (2-sector homebases are shown as dots on the border between the two respective sectors).

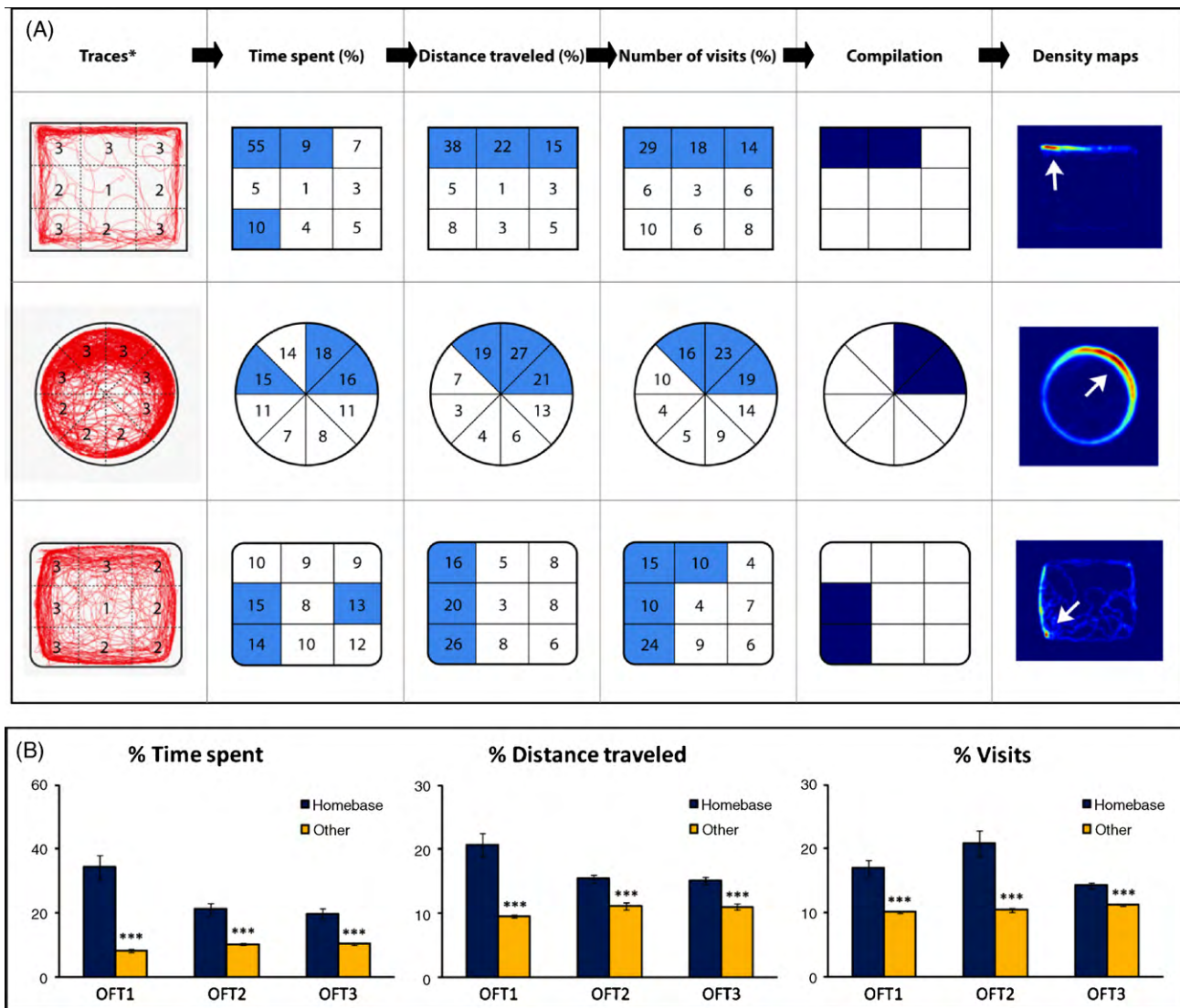


Fig. 2. Methodology of homebase identification in three representative zebrafish observed in three different open field test (OFT) tanks for 30 min (see details of the OFT tanks in legend to Fig. 1). (A) Summary of the algorithm used in this study to identify zebrafish homebases (see Section 2 for details). Briefly, the traces were generated by Noldus Ethovision XT7 and scored manually by two experienced observers, using a 0–3 scoring system. Time spent, distance traveled and number of visits (frequency in zone) were calculated using Noldus Ethovision XT7 for each zone of the OFT arenas, and expressed as % of total. Potential homebases were identified and mapped based on top three percentages. These maps were then compiled to establish the overlap of all four levels of analysis. Density maps were generated by video-tracking software (for time spent data) and used as an additional tool to visualize and reconfirm zebrafish homebase behaviors (indicated by white arrows). Note a good correlation between different homebase-related behaviors, and their spatial patterning that enables a precise identification of zebrafish homebases. (B) Confirmation of zebrafish homebases (identified using the method described above) based on calculation of average time spent, distance traveled, and the number of visits (expressed as % of total “arena” scores for each respective endpoint) per a homebase sector vs. a non-homebase sector of the OFT arena (*** $P < 0.00001$, U -test). Note striking and highly significant differences in zebrafish behavioral activity between homebase and non-homebase OFT zones.

surface and rounded corners divided into 9 zones (see Figs. 1 and 2 for details). These three apparatuses, differing in size, color, shape, and texture were selected to reveal differences in zebrafish homebase activity that may be potentially associated with distinct OFT environments. All apparatuses rested on level ground and maintained the same distance (114 cm) from the camera. The standardized 12-cm water level was enough for the fish to move freely in the OFT apparatus, yet shallow enough to minimize extensive vertical movements (which may be misdetected by video-tracking systems). Behavioral testing took place between 12:00 and 16:00 h. At the beginning of the trials each fish was gently placed in the center of the tank and video-recorded for 30 min, after which the water was changed, and the tank cleaned, prior to the next trial. The experimenters were not present in the room during the time of recording to prevent disturbances to the fish. One fish was tested per tank in this study.

The trials were recorded via camera (Sony Handycam DCR-SR47, USA) and analyzed off-line using Ethovision XT 7 (Noldus Information Technology, Netherlands). OFT arenas were divided into various zones (Figs. 1C and 2A) and event rules were set to precisely register behavioral endpoints including time spent (s), distance traveled (m), and the number of visits to pre-defined zones. Fish tracks and density maps were also generated by video-tracking software to visualize zebrafish homebase behaviors based on swimming activity, location, and time spent (Figs. 1A and 2A).

2.3. Homebase analysis

The methodology of homebase identification is illustrated in Fig. 2. To identify zebrafish homebases, we first examined traces using a 0–3 rating system for each zone, with 0 denoting no traceable activity within that zone and 3 corresponding to very high

activity. This scoring was relative to each individual fish (see Fig. 2A for an example), and a zone qualified as a potential homebase based on tracing scores of 2 or higher. We next calculated three endpoints – distance traveled, the number of visits, and time spent – for each individual fish for each zone/sector of the OFT arenas. Total 30-min activity score for each individual fish for the entire OFT arena was expressed as 100%, and the percent of activity (of total) was then calculated for each zone of the OFT. A zone qualified as a potential homebase based on 3 maximal percentages of total distance traveled, time spent, and number of visits within that zone, as shown in Fig. 2A. All four criteria were superimposed for each tank in order to identify overlapping zones. Overlap of all homebase-specific loci was used to define that area as the final homebase for the particular trial. Density maps generated by Noldus Ethovision XT7 were used to visually reconfirm the homebase using the time spent in each zone, in which a color gradient ranging from yellow to red reflected time spent in location (Figs. 1B and 2A). Every step in this procedure was performed independently by two experienced observers (inter-rater reliability > 0.85), on a consensus basis. To further reconfirm the homebase behavior, an average *per zone* activity was assessed for homebase-specific (vs. non-homebase) areas, based on percentages of time spent, distance traveled and number of visits, calculated as described above.

2.3.1. Statistical comparison

Data is presented as mean \pm SEM, and was analyzed using the chi-square (χ^2) or Mann–Whitney *U*-test. The χ^2 test was performed to analyze the spatial distribution of homebase-related behaviors, comparing *actual* percentages of time spent, number of visits and distance traveled in each zone (of total 30-min scores) with *theoretical* random (by-chance) distribution of these indices (i.e., 11% per zone in 9-zone OFT1 and OFT3, and 13% in an 8-zone OFT2). χ^2 data was first calculated for each endpoint, each OFT tank and each individual fish. Once all homebases were identified (as described above), three combined homebase topographic maps were created for all three OFT tanks, with dots representing each individual homebase (Fig. 1C). The χ^2 test was again applied to compare actual spatial distribution of all homebases (established in the respective OFT) with random by-chance distribution (11 or 13% per zone, as described above).

3. Results

During the OFT trials, all fish tested here established clear-cut homebases—particular areas where they spent most of the time, traveled more, and visited most frequently (Figs. 1A and B and 2A and B). These homebases were almost always located near the walls of the tanks, and usually consisted of 1 (~60–80% of fish) or 2 (~20–40% of fish) zones (Fig. 1C).

While overt homebases were identified using our protocol in all fish tested, further analyses of their spatial distribution was applied to confirm that individual homebase-related behaviors (time spent, distance traveled and number of visits to zone) were not distributed randomly in the OFT arena. Using OFT1 as an example, we found that spatial distribution of time spent yielded significant χ^2 results (χ^2 ranged from 12.1 to 102.7, with *P* values from 0.00001 to 0.05, vs. random distribution) in 85% of fish. Spatial distribution of distance traveled showed statistically significant χ^2 results ($\chi^2 = 16.8$ –61.0 (range), *P* < 0.00001–0.05 (range)) in 40% of fish, and the number of visits showed significant χ^2 data ($\chi^2 = 13.8$ –48.0 (range), *P* < 0.00001–0.05 (range)) in 25% of fish. Overall, 85% of the fish showed statistically significant preference of spatial distribution of their homebase behaviors, based on χ^2 analyses of homebase locations. In contrast, analyses of topographical maps of zebrafish homebases in all three OFT tanks (Fig. 1C) showed that different OFT

zones were *at random* chosen by different zebrafish for their homebases, revealing no clear-cut spatial preference of their homebase location in relation to a particular OFT zone ($\chi^2 = 0.5$ –8.7 (range), NS). Subsequent examination of video tracks and density maps also confirmed that homebase areas were selected randomly at different OFT areas across trials (see Figs. 1A and B and 2A for details). Fig. 1C demonstrates that even though the χ^2 analyses lends statistical significance to 85% of the fish trials, overt homebase establishment, assessed using our approach, was observed in all fish (100%) tested here.

Finally, comparison of the distance traveled, frequency of visits, and time spent within the homebase zones revealed similar temporal dynamics of homebase behavior across different OFT arenas. In general, zebrafish maintained rather constant levels of their homebase behaviors (spending 10–40 s, visiting homebase zones 1–6 times and traveling 0.5–1 m there each minute; data not shown).

4. Discussion

Recent studies in fish (Budaev et al., 1999; Gerlai et al., 1990; Gerlai and Csányi, 1988; Miklosi et al., 1992; Warren and Callaghan, 2006) (including zebrafish (Blaser and Gerlai, 2006; Echevarria et al., 2008)) show a substantial similarity with rodent OFT responses, suggesting that novelty exploration in different species is driven by shared evolutionarily conserved rules. Homebase formation is an important adaptive behavioral strategy used by animals (Eilam, 2003; Whishaw et al., 2006). Although frequently reported in rodent literature (Clark et al., 2006; Draï and Golani, 2001; Dvorkin et al., 2010; Eilam and Golani, 1989), this phenomenon has not been previously examined in fish.

Our study is the first report on homebase behavior in zebrafish. Overall, the time spent, distance traveled and the number of visits to the selected homebase were significantly higher than other areas of the apparatus, and were seen in all three tanks in all fish tested here (Figs. 1 and 2). The zebrafish did not prefer to establish a homebase along a shorter (vs. longer) wall, as demonstrated in rectangular OFT1 and square OFT3 (e.g., see Fig. 2 for details). Their homebase activity also was not hindered by a lack of corners, as shown in the circular OFT2 and corner-less OFT3. As already mentioned, the choice of homebase location was statistically random, with no area being preferred over another between different experiments (Figs. 1 and 2). Taken together, these findings imply that observed homebase behavior is not determined by innate features of the OFT novelty, but rather actively established by zebrafish exploring their environment.

Importantly, homebases were typically established close to the OFT walls. Such topography of homebase formation is also commonly observed in rodents usually establishing homebases close to walls (Eilam, 2003; Eilam and Golani, 1989; Horev et al., 2007). It has been suggested that rodents use vertical surfaces as spatial clues for their navigation (Dvorkin et al., 2008) and therefore place their homebases close to OFT walls accordingly. Our present observations (Figs. 1 and 2) suggest that zebrafish may employ similar behavioral strategies in novel environments. For instance, walls serve as guiding and attractive forces on mouse locomotion, with mice modulating their heading and speed in reference to walls during OFT navigation (Horev et al., 2007). Likewise, a homebase also provides an attractive influence in rodents, exerting a gradually increasing attraction for the animal to return to it (Dvorkin et al., 2008). These forms of attraction, based on recognition and locational memory, may also underlie the zebrafish locomotion observed in this study.

Notably, while rodent homebase behavior is not identical to that in zebrafish, they are comparable in many ways. For example, rodents spend substantial time grooming, rearing, and crouching in

the homebase (Eilam and Golani, 1989). Although zebrafish behavior is less complex, the fish also spend a large amount of time in their respective homebases. Also importantly, this behavior was maintained at a constant level since zebrafish, like rodents, leave and frequently return to the homebase (rather than merely remaining frozen there throughout the test).

In general, the interpretation of animal homebase activity is complicated because rodents are nocturnal and were assessed during the daylight hours. Therefore, their activity in homebase (e.g., exploring briefly, grooming, and then nesting) may reflect a preparation for rest, rather than setting up a homebase *per se* (e.g., Eilam and Golani, 1989, 1990). Using the diurnal zebrafish (whose behavior cannot be misconstrued as nesting or grooming), our study is in line with rodent evidence that homebase behavior is an intrinsic animal response to novelty (Eilam, 2003; Whishaw et al., 2006). Specifically, we found that zebrafish behavior is quite active within the homebase (Figs. 1A and 2B), where in comparison to outlying zones, the fish were significantly more active, meandering and swimming greater distances and for longer durations.

There are several methodological limitations of this study. For example, while it provided a general methodology for quantification of zebrafish homebases, other criteria may be developed to further characterize this phenotype. Furthermore, our study was limited to relatively small-to-medium size OFT arenas (in contrast with some rodent OFT studies, where arena sizes ranged from a small box to a big yard (Eilam and Golani, 1989; Whishaw et al., 2006)). Therefore, further studies may examine the impact on zebrafish behavior produced by a significantly larger variation of arena size (also see Fonio et al., 2009; Kalueff et al., 2006). Likewise, the 30-min OFT trials used here were sufficient for zebrafish to establish visible homebases, and were similar to 1-h observation time in early rodent studies reporting homebase formation (Eilam and Golani, 1989). However, longer OFT exposure time (e.g., 24–48 h, as in recent rodent reports (Fonio et al., 2009)) may be used, to better characterized homebase behavior and its spatiotemporal dynamics in zebrafish. Notably, we used a top view to video-record zebrafish locomotion in this study. While this method provides an accurate detection and quantification of zebrafish behavior, further studies may utilize multiple cameras to generate three-dimensional traces of zebrafish locomotion and homebase behaviors. Our pilot observations suggest that zebrafish display robust habituation of their vertical behavior in novel environments (Wong et al., 2010), and do not use “preferred” vertical locations in novel tanks.

Additional applications of zebrafish homebase analysis may include screening pharmacological agents known to affect homebase behavior in rodents, as well as testing various inbred and mutant zebrafish strains which may display altered homebase phenotypes. For instance, while rats generally establish a single homebase if given a low dose of amphetamine, higher doses lead to the establishment of two homebases (Eilam and Golani, 1990). Prior rodent studies have also shown that dopaminergic drugs and strain differences may influence the probability of stopping in specific locations (Dvorkin et al., 2008). Therefore, the sensitivity of zebrafish homebase behavior to pharmacological and genetic manipulations may merit further investigation.

In summary, in-depth analyses of animal behavior in novelty tests may provide important insights into their affective (Kazlauckas et al., 2005; Luksys et al., 2009; Ricceri et al., 2007) and cognitive (Bolivar et al., 2000; Kalueff et al., 2006) phenotypes. Mounting evidence confirms that many rodent models can easily be adapted to study zebrafish emotionality (Egan et al., 2009; Levin et al., 2007) and cognitions (Levin et al., 2006; Wong et al., 2010). Recently, novelty tests were applied to assess more complex cognitive functions in rodents, including creating spatial representations and networks of points of attraction, such as homebases and knots

(e.g., Dvorkin et al., 2008, 2010; Fonio et al., 2009; Horev et al., 2007). Therefore, this represents an interesting avenue to explore in zebrafish-based behavioral models. For example, homebase formation and path tortuosity could be examined in the presence of additional object placed in the novel arena.

Moreover, prior rodent literature has revealed a remarkable stability in their behavioral patterning despite varying the size and shape of an arena (Kalueff et al., 2006). This spatiotemporal patterning may also be examined in the zebrafish models. Additionally, as prior research suggests that fish display good learning and memory (Spence et al., 2008), the role of memory in homebase location could be examined by subjecting each fish to repeated trials. Our present study demonstrates that zebrafish form homebases in novel environments just as rodents do, suggesting that spatial representations and key loci can also be created by fish. Collectively, these findings support the utility of zebrafish to study the fundamental and evolutionarily conserved factors that drive animal exploration and cognition.

Acknowledgements

The study was supported by Tulane University Intramural funds, Zebrafish Neuroscience Research Consortium (ZNRC), the Gordon and the G. Lurcy Fellowships, Provost's Scholarly Enrichment Fund, Newcomb Fellows Grant, LA Board of Regents Pfund and NARSAD YI awards.

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