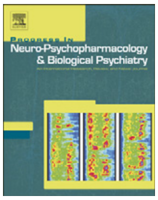
Progress in Neuro-Psychopharmacology & Biological Psychiatry 35 (2011) 1409–1415

Contents lists available at ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnp



Shoaling develops with age in Zebrafish (*Danio rerio*)☆

Christine Buske [a,1], Robert Gerlai [a,b,*]

[a] *Department of Cell and Systems Biology, Neuroscience, University of Toronto, Canada*
[b] *Psychology Department, University of Toronto, Mississauga, Canada*

A R T I C L E I N F O

Article history:
Received 8 July 2010
Received in revised form 3 September 2010
Accepted 4 September 2010
Available online 15 September 2010

Keywords:
Age-dependent behavioral changes
Social behavior
Shoaling
Zebra danio
Zebrafish

A B S T R A C T

The biological mechanisms of human social behavior are complex. Animal models may facilitate the understanding of these mechanisms and may help one to develop treatment strategies for abnormal human social behavior, a core symptom in numerous clinical conditions. The zebrafish is perhaps the most social vertebrate among commonly used laboratory species. Given its practical features and the numerous genetic tools developed for it, it should be a promising tool. Zebrafish shoal, i.e. from a tight multimember groups, but the ontogenesis of this behavior has not been described. Analyzing the development of shoaling is a step towards discovering the mechanisms of this behavior. Here we study age-dependent changes of shoaling in zebrafish from day 7 post fertilization to over 5 months of age by measuring the distance between all pairs of fish in freely swimming groups of ten subjects. Our longitudinal (repeated measure within subject) and cross sectional (non-repeated measure between subject) analyses both demonstrated a significant increase of shoaling with age (decreased distance between shoal members). Given the sophisticated genetic and developmental biology methods already available for zebrafish, we argue that our behavioral results open a new avenue towards the understanding of the development of vertebrate social behavior and of its mechanisms and abnormalities.

1. Introduction

The zebrafish has been enjoying much popularity in embryology for the past three decades (for examples see Schweitzer and Driever, 2009; Holder and Xu, 2008) thanks to this species' transparent embryo and its numerous other practical features that make it an ideal laboratory model organism (e.g. Lin et al., 2009). Indeed, the zebrafish has been employed as a model for a variety of human diseases including cancer (Stoletov and Klemke, 2008), movement (Flinn et al., 2008) and sleep disorders (Zimmerman et al., 2008) among other conditions (for a review see Lieschke and Currie, 2007).

As increasing number of genetic tools has become available for zebrafish (Patton and Zon, 2001; Keller and Murtha, 2004) the popularity of this species has grown in a variety of disciplines including behavioral neuroscience (Sison et al., 2006). However, unlike the body of knowledge available on embryonic development and genetics of zebrafish, the behavior of this species is still poorly characterized (Kato et al., 2004). This is a crucial drawback because behavioral analysis has the potential to reveal a variety of functional changes in the brain and has been argued to be an important screening method in forward genetics as well as pharmacology (Gerlai, 2002). Nevertheless, most recently numerous studies have appeared that demonstrated the utility of zebrafish behavioral analysis in the investigation of vertebrate brain function (Gerlai, 2010; Egan et al., 2009). Some of these recent papers utilized the analysis of zebrafish's social behavior (Gerlai et al., 2009; Miller and Gerlai, 2007, 2008; Saverino and Gerlai, 2008; Speedie and Gerlai, 2008).

Social behavior is a complex phenomenon whose biological mechanisms and development are not well understood in vertebrates. Abnormal social behavior is a defining characteristic of a variety of human psychiatric and neurodevelopmental conditions including depression (Bell-Dolan et al., 1993), anxiety disorders (Liebowitz et al., 1985), and the autism spectrum disorders (Reichow and Volkmar, 2010). The underlying mechanisms of the abnormal social behavior associated with these diseases are not well understood (Bartz and Hollander, 2006). Given the similarities between zebrafish and other vertebrates, including humans, in the layout of the brain (Tropepe and Sive, 2003), in the neurochemical properties of the brain (Zhdanova, 2006), and in characteristics of many other levels of biological organization (Egan et al., 2009), including nucleotide

---

Abbreviation: Dpf, days post fertilization.

☆ Ethical statement: the research reported in our manuscript has been conducted in accordance with the local University, Provincial and Federal (Canadian Council for Animal Care) guidelines and has been reviewed and approved by the Local Animal Care Committee.

* Corresponding author. Psychology Department, University of Toronto, Mississauga, 3359 Mississauga Rd N., Mississauga, ON Canada L5L 1C6. Tel.: +1 905 569 4255.

E-mail addresses: Chr.buske@utoronto.ca (C. Buske), robert.gerlai@utoronto.ca (R. Gerlai).

[1] Department of Cell & Systems Biology, Neuroscience, 25 Harbord Street, Toronto, ON Canada M5S 3G5. Tel.: +1 647 339 2696.

0278-5846/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.pnpbp.2010.09.003

sequence of genes, the zebrafish is thought to be suitable as a model for investigating the biology and genetics of vertebrate brain functions (Gerlai, 2003). Briefly, findings from studies in zebrafish are expected to generalize well to humans and may shed insights on complex human conditions including the autism spectrum disorders (Tropepe and Sive, 2003).

The zebrafish is a shoaling fish, it aggregates, i.e. forms multi-member groups in nature and in the laboratory. Shoaling is thought to provide the individual fish with multiple benefits, including access to mates, efficient foraging, and defense against predators (Griffiths et al., 2004; Ledesma and McRobert, 2008; Morrell and James, 2008). Although the adaptive nature of shoaling is well documented, the biological mechanisms and the development of this behavior are far from understood. In the current paper, we focus on the latter: we investigate, for the first time, whether shoaling develops, i.e. whether it changes with age, in zebrafish. Our prior personal observations suggested that newly hatched zebrafish disperse while adult zebrafish have been documented to exhibit robust shoaling, a strong preference for staying close to conspecifics (Al-Imari and Gerlai, 2008; Miller and Gerlai, 2007, 2008; Saverino and Gerlai, 2008). A previous study investigating the effects of kin exposure on preference for conspecifics in zebrafish revealed an imprinting-like effect of olfactory cues at an early age of the fish (6 days post fertilization, dpf) and suggested that some preference for conspecifics already exists at this stage of development (Gerlach et al., 2007). Preference for conspecifics was also demonstrated to be based solely upon visual cues in another study (Engeszer et al., 2007), which found measurable preference for conspecifics at postflexion stage (about 12 dpf) of zebrafish. However, it is not known whether the preference responses quantified in the above studies represented shoaling or other type of responses (e.g. agonistic) because the subjects were tested singly and could not interact with the stimulus fish. Furthermore, the question whether shoaling changes with age, i.e. the developmental trajectory of shoaling itself, has not been investigated up till now.

To address the above questions, we analyzed age-dependent changes of shoaling behavior in freely moving groups of zebrafish. The main rationale for our study is as follows. If significant age-dependent (i.e. developmental) changes in shoaling behavior are identified, this discovery could open new research avenues for numerous investigations. For example, one could study the ecological/adaptive importance of developmental alteration of social behavior. Another important goal would be to investigate the mechanisms of the age-dependent changes in social behavior, a classical developmental biology question. Here we report behavioral findings showing a significant developmental change in shoaling in zebrafish and based on this we argue that zebrafish will be an excellent tool with which the mechanisms of vertebrate social behavior may be investigated.

2. Methods

2.1. Animals and housing

In total, one thousand one hundred and ninety zebrafish (*Danio rerio*) of the AB strain were utilized for the three experiments outlined below. The fish were bred in-house and originated from progenitors obtained from the Zebrafish International Research Center (ZIRC) (Eugene, Oregon). All experiments described below were approved by the University of Toronto Animal Care Committee. All fish used in this study were bred, raised and housed in the same environment. Gender could not visually be determined when testing commenced (at 7 days post fertilization). After completion of the experiments and after the subjects had reached maturity, the gender distribution within the shoals tested was determined to be 50% male 50% female.

Upon hatching, the animals were housed in groups of ten in 1 l plastic aquaria. After 5 weeks post fertilization the animals were transferred to 2.8 l Plexiglas aquaria that were part of a recirculating filtration aquaculture rack system which had a mechanical, biological, and activated carbon (chemical) filter as well as a UV sterilizing unit (Aquaneering Inc. (San Diego, Ca, USA). Water was maintained at 27 °C. The system water used on the rack as well as during the development and testing of the fish was reverse osmosis purified and was supplemented with 60 mg/l Instant Ocean Sea Salt to achieve water chemistry appropriate for zebrafish.

Zebrafish were kept at a 12 h light/12 h dark cycle with lights on at 7 am and off at 7 pm. All fish were fed twice daily with Larval Artificial Plankton 100 (particle size below 100 μm, ZeiglerBros, Inc., Gardners, PA, USA) until 2 weeks post spawning, after which animals were fed twice daily with nauplii of brine shrimp (*Artemia salina*) until they were 4 weeks old. Older and adult fish were fed a 1:1 mixture of flake food (Tetramin Tropical fish flake food, Tetra Co, Melle, Germany) and powered spirulina (Jehmco Inc., Lambertville, NJ, USA).

2.2. Open field task

All zebrafish that were housed together were tested together, forming a given shoal. Each shoal was identified by an ID number and remained constant (same shoal members) throughout the experiments (the unit of statistical analysis here is the shoal, and the sample sizes (n) shown below represent the number of shoals tested). Each group (shoal) consisted of ten fish. The home tank was placed next to the testing arena for transfer. Fish were netted as a group (in most cases all 10 fish could be captured with one net, due to the size of the net and the holding tank) and immediately released in the center of the arena. Transfer (air time) from the holding tank to the arena was not more than 3 s. The fish were released simultaneously in the center of a square plexi-glass tank, the open field, and were allowed to explore the field freely. Each trial lasted 6 min and the behavior of fish during the trial was recorded with an overhead video camera (JVC Everio Hard Drive GZ-MG750BU). After the open field trial, the group was returned to its home tank. In experiments 1 and 2, the arena size was kept proportional to the body length of the growing fish (and thus constant for a particular age group), a practice recommended by others (Gallego and Heath, 1994; Masuda et al., 2003; Vogel, 2008). But in experiment 3, two separate age groups of fish (30 and 60 day old) were tested in six different arena sizes each, and the order of use of different arena sizes was randomized. Behavioral testing was always conducted between 0900 and 1600 h.

2.3. Experiment 1: longitudinal developmental analysis of shoaling

The purpose of this experiment was to investigate the trajectory of potential age-dependent changes of shoaling behavior in zebrafish. Nineteen groups, each consisting of ten fish, were utilized in this experiment. Each was tested at 7, 18, 26, 42, 49, 59, 66, 70, and 76 dpf. That is, the same groups of fish were followed throughout their development, a repeated measure design.

2.4. Apparatus

Distance traveled has been argued to be the function of the linear dimension of the fish, e.g. their body length, therefore arena sizes or movement parameters such as speed or total distance swum are usually normalized to the length of fish, i.e. expressed in body lengths (Hale, 1999). In zebrafish, speed has also been found to be positively and linearly correlated with body length (Kimmel et al., 1974) and thus we decided to keep the linear dimensions of our open field experimental tanks proportional to the body length of our developing fish. We employed open fields whose linear dimensions were 28x the average body length of the zebrafish tested in them. This ratio gave us sufficiently large tanks in which the fish were not physically forced to stay close to each other and thus any shoaling observed would be the result of social cohesion and not of the physical constraints of the