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The effect of the number and size of animated conspecific images on shoaling responses of zebrafish

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ABSTRACT

The zebrafish is increasingly utilized in biomedical and psychopharmacological research aimed at modeling human brain disorders. Abnormal social behavior represents the core symptom of several neuropsychiatric and neurodevelopmental disorders. The zebrafish is a highly social species and has been proposed for modeling such disorders. Behavioral paradigms that can induce zebrafish social behavior are of importance. Here, we utilize a paradigm in which zebrafish are presented with computer animated images of conspecifics. We systematically varied the size of these images relative to the body size of the experimental fish and also investigated the potential effect of presenting different number of images in an attempt to optimize the paradigm. We report that images similar in size to the experimental fish induced a strong shoaling response (reduction of distance to the image presentation screen) both when the body size of the experimental fish was varied with the image size being held constant and when the image size was varied with the body size of the experimental fish being held constant. We also report that within the number range studied (from 1 to 8 conspecific stimulus fish), presentation of all animated shoals, but the image of a single conspecific stimulus fish, led to significant reduction of distance to the presentation screen. We conclude that the shoal image presentation paradigm induces robust social responses that are quantifiable in an automated manner, making the paradigm useful for screening of drugs and/or mutations.

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1. Introduction

Identification of mutations and drugs that influence brain function may require large scale behavioral screens especially if the target behavioral phenotype or modeled human brain disorder is complex. Social behavior and human brain disorders associated with abnormal social behavior are some of the most complex phenotypes with underlying mechanisms that are expected to involve a large number of molecular players. In the current paper, we attempt to explore the first steps towards the optimization of a simple social behavioral paradigm developed for the zebrafish, a vertebrate model organism that has been proposed to be among the most effective translational tools for large scale screening in the context of complex brain disorders (e.g. Gerlai, 2010a, 2010b). We argue that systematic analysis of methodological questions of behavior is a crucial endeavor, and the sooner the zebrafish field focuses on them, the faster we can integrate behavioral approaches into multidisciplinary analysis of the biological mechanisms of vertebrate brain function and dysfunction (Gerlai, 2002, 2014b; Crabbe et al., 1999).

Zebrafish are prolific (producing 200–300 eggs at each spawning), their eggs are fertilized externally, and their embryos are transparent (Kimmel, 1989). Adult fish are small (4 cm long) and cheap to maintain (Sison et al., 2006). These characteristics are among those that have made the zebrafish a favorite of developmental biologists (Vascotto et al., 1997). By now numerous forward and reverse genetic tools have been developed for this species (Grunwald and Eisen, 2002) to aid developmental biology research, but as a result the zebrafish has generated considerable interest in other subdisciplines of biology. One of these fields is behavioral neuroscience, which has seen a rapid accumulation of knowledge on zebrafish neurobiology and behavior (Gerlai, 2011; Kalueff et al., 2014). However, despite this rapid increase, the zebrafish is still considered a newcomer as our understanding of its behavioral features is rudimentary and the number of behavioral tasks with which to conduct our research is limited (Gerlai, 2012). This is a significant drawback as behavioral analysis could help us identify drugs or mutations that alter brain function and thus could aid the investigation of neurobiological mechanisms of diseases of the central nervous system (Gerlai, 2002). Briefly, the need for efficient and informative behavioral tests is clear (Sison et al., 2006).

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Recently, a simple behavioral test paradigm utilizing the natural tendency of the zebrafish to form groups (shoals) has started to be utilized (Saverino and Gerlai, 2008; Abaid et al., 2012). Shoaling behavior (Pitcher, 1983) of zebrafish can be observed in nature (Spence et al., 2008; Engeszer et al., 2007a, 2007b) and can also be easily induced in the laboratory (Saverino and Gerlai, 2008; Miller and Gerlai, 2011a). When a single zebrafish sees a group of conspecifics it will approach and attempt to join the group (Gerlai et al., 2000). Most of our understanding about shoaling in zebrafish has come from studies utilizing live shoals (Maaswinkel et al., 2013; Pagnussat et al., 2013; Green et al., 2012; Miller et al., 2012; Miller and Gerlai, 2007, 2008, 2011a, 2011b, 2012). While these studies have increased our ability to investigate dynamic features of shoaling behavior, the need for a simple and efficient social behavior test paradigm continues to exist (Miller and Gerlai, 2011a).

Recently, such a test paradigm has been developed. An alternative to measuring social behavior in live shoals is to provide computer generated conspecific images and measure the response of a single experimental subject to this stimulus (Saverino and Gerlai, 2008). Measuring the response of a single subject makes identification of mutants in mutagenesis studies easier and using computers to deliver the stimulus also has advantages (Sison et al., 2006; Gerlai, 2010a, 2010b). For example, one can precisely control the parameters of the stimulus, including numerous visual features, and the timing and location of its presentation. Computerized stimulus delivery eliminates experimental error variation inherent to studies using live shoals and also reduces the amount of experimenter interference. Furthermore, computerization of the stimulus presentation allows for the running of multiple trials simultaneously (Fernandes and Gerlai, 2009), characteristics that can significantly increase throughput to a level required for large scale mutation and/or drug screens (Gerlai, 2010a, 2010b).

Importantly, in a recent study the responses of zebrafish to computer animated conspecific images were found statistically indistinguishable from those induced by presentation of video-recordings of live stimulus fish, of live fish placed outside of the experimental tank and of live stimulus fish placed inside the experimental tank separated from the experimental fish by a perforated transparent Plexiglas barrier (Qin et al., 2014). These results suggest that induction of robust shoaling responses does not necessarily require live stimulus fish or even three dimensional appearance of movement of the presented stimuli.

Computerized delivery of social stimuli and the analysis of shoaling responses to these stimuli have been successfully employed in a variety of studies investigating a range of complex questions including the neurochemical correlates of social behavior (Gerlai, 2014a, 2014b; Saif et al., 2013; Scerbina et al., 2012), the effect of embryonic alcohol exposure (Fernandes and Gerlai, 2009), and the effects of acute and/or chronic alcohol exposure (Gerlai et al., 2009).

Given the potentially broad applicability of the shoaling paradigm, it is important to find optimal parameters of shoal image presentation, a method that would induce the most robust, maximal shoaling response in zebrafish. A robust shoaling paradigm may be utilized to investigate the biological (genetic) underpinnings of this complex behavior, and would also allow one to analyze pharmacological agents that modify this response. Furthermore, it would allow one to examine functional changes in the brain induced in zebrafish models of human brain disorders associated with abnormal social behavior (Gerlai, 2012). However, systematic analysis of the parameters of stimulus fish image presentation has not been conducted for zebrafish.

The present study represents the first step in this optimization process. Here, we examine the potential effect of the size of the animated conspecific images and also the number of the presented images on the strength of the shoaling response in zebrafish. Body size may be important in the context of dominance and mating competition. Larger fish are usually more dominant and can compete for food and mates more successfully (Huntingford et al., 1990). Body size may also be relevant from the perspective of the oddity effect (Landeau and Terborgh, 1986). Fish that stand out among other shoal members may be more easily detected by predators and this may be a disadvantage especially when the unique individual is larger than the rest of the shoal members. Thus, the size of the experimental fish relative to that of the stimulus fish may matter and the relative size difference may influence the strength of the shoaling response in the experimental fish. We predict that due to the conflict between the effect of body size in the context of mating versus predation risk (larger fish are more successful at mating but may be predated upon with higher probability), the experimental fish should show the strongest shoaling response towards conspecific images whose body size is similar to that of the experimental fish.

In addition, we also investigated whether the number of stimulus fish presented may alter the strength of the shoaling response. Larger shoals may allow better detection of predators by the shoal members (more eyes see more), but they may also lead to increased competition for resources such as food, and may also result in increased interference or stress among the shoal members. Zebrafish have been observed to form shoals in nature ranging in numerical size from only a few (2–3) to several hundred members per shoal (Spence et al., 2008). In several other fish species, larger, i.e. more numerous, shoals were shown to be preferred to smaller ones, at least when the number of shoal members tested ranged between 1 and 10 stimulus fish (Gómez-Laplaza and Gerlai, 2011a, 2011b, 2012, 2013a, 2013b; Piffer et al., 2013, 2012; Agrillo and Bisazza, 2014; Agrillo et al., 2012; Bisazza et al., 2014). Based on these results, we predict that zebrafish will show less strong shoaling responses towards shoals that contain fewer stimulus fish at least in the small number range studied in the current experiment.

To test the validity of the above predictions we systematically varied (a) the size of the experimental zebrafish while presenting stimulus fish of a constant size; (b) the size of the stimulus fish image while keeping the size of the experimental fish constant and (c) the number of stimulus fish images of a size identical to that of the experimental fish. As a measure of the strength of the shoaling response, we quantified the distance of the experimental zebrafish to the computer screen presenting the different stimuli.

2. Methods

2.1. Animals and housing

Adult sexually mature zebrafish (Danio rerio) between the ages of 12 and 18 months of the AB strain bred in our facility (University of Toronto Mississauga Vivarium, Mississauga, ON, Canada) were used in the current study. Progenitors of this population were obtained from the Zebrafish International Resource Center (ZIRC, Eugene, OR, USA). The experimental fish were sixth generation from the original founders. All fish were held in social groups in their home tanks, 3 liter plastic tanks that were part of a high density system rack (Aquaneering Inc., San Diego CA) that had a multistage filtration system equipped with mechanical (sponge), biological (fluidized glass bed), chemical (activated carbon) filtration and a UV light-based sterilizing unit as described before (e.g. Jia et al., 2014). The housing density was 10–13 fish per 3 liter tank. The water on the rack (system water) was identical to the water used in all experiments and was obtained using reverse osmosis purification. To obtain the desired water chemistry (300 microSiemens conductivity and neutral pH), the reverse osmosis purified water was supplemented by sea salt (Instant Ocean, Big Al's Aquarium Warehouse, Mississauga, Ontario, Canada).

2.2. Behavioral apparatus

The experimental setup was a 37-liter glass aquarium ($50 \times 25 \times 30$ cm, length × width × height) that had a flat LCD computer screen (17 inch Samsung SyncMaster 732N monitor) placed flush on its left and right side (Fig. 1). Each monitor was connected to a Dell Vostro 1510 Laptop running a custom made software application (Saverino

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