Social Affiliation in Zebrafish: From Synthetic Images to Biological Mechanisms

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The zebrafish is an increasingly popular species in behavioural neuroscience

Zebrafish are small freshwater fish that inhabit the tropical waters (small lakes, rivers and streams) of Southern and Eastern portions of Asia, e.g. India and Nepal. This species has been becoming increasingly popular among behavioural neuroscientists for many reasons (e.g. [4, 15]). First, due to the past four decades of extensive developmental biology research conducted with this species, powerful genetic tools have been developed for it that now may be utilized by several other disciplines of biology, including brain and behaviour research. Second, the zebrafish appears to strike an optimal compromise between system complexity (it is a typical vertebrate with basic neuroanatomy, neurophysiology, neurochemistry, and biochemistry similar to those of higher order vertebrates including mammals) and practical simplicity (it is small, easy and cheap to maintain in the laboratory, and it is highly prolific) [2]. Third, due to these above features and because numerous genes identified in zebrafish have been found to have nucleotide sequences highly homologous to mammalian counterparts, the zebrafish is believed to be an excellent translational tool, i.e. it may be utilized for modeling and analysing human diseases [5].

The zebrafish is a highly social species

Both in nature and in the laboratory zebrafish form tight groups, called shoals [7-10]. A shoal is defined as an aggregate of individuals in which group members remain in close proximity to each other. In nature, this behaviour is believed to serve several adaptive functions, including protection from predators, increased foraging efficiency and increased mating success. In the laboratory, this behaviour is also prevalent and may be quantified both in freely moving groups of fish [7, 8] as well as in situations where a single subject responds to the presence of social stimuli [6]. The prevalent and robustly inducible shoaling behaviour in zebrafish may serve as an excellent tool with which one can investigate the mechanisms of vertebrate social behaviour. For such investigation to be successful, however, the stimuli that trigger this affiliative response must be well known and their method of delivery must be standardized.

The zebrafish is a diurnal species that has excellent vision.

Zebrafish, similarly to our own species and unlike rodents, are diurnal and are active during the day and sleep at night. Although zebrafish use several modalities for perceiving stimuli in their environment, e.g. lateral line, olfaction and auditory perception, these fish also have excellent vision and respond to visual cues. The zebrafish visual system and its development and physiology have been well characterized and we know, for example, that this species has four different cones (tetrachromatic) to detect specific wavelength ranges of light, i.e. their colour vision is excellent. The practical aspect of good visual perception abilities of zebrafish is that the experimenter may be able to use visual cues to affect and manipulate the behaviour of these fish and, given that our own species also uses vision as one of the primary mode of perception, simple consumer grade "visual devices", such as cameras and TV screens are available for a reasonable cost for such research.

Visual stimuli to induce social responses

We have started the analysis of visual stimuli and explored if they induce social responses. We first investigated how zebrafish respond to live fish in a test where the zebrafish and the stimulus fish were allowed to swim freely [11]. We found that zebrafish prefer staying close to conspecifics and do not shoal with heterospecific fish irrespective of whether these heterospecific fish are social (shoaling) or non-social (not group forming) species. We also found that zebrafish shoal with (i.e. prefer staying close to) conspecifics even if the colour of the conspecific differed from their own (we used a pigment mutant "gold" variant of zebrafish to investigate this question). Subsequently, we examined if zebrafish may be sensitive to particular features of conspecifics and started modifying such features using computer animated images [11]. The results suggested that zebrafish respond to moving images of conspecifics just as they would respond to real live fish. The results also revealed that zebrafish are not very sensitive to the pattern of the conspecifics, i.e. in a binary choice test they preferred images that did not have the wild type horizontal stripe pattern equally to those that showed the normal wild type horizontal stripe pattern. Furthermore, the test subjects also showed equal preference to the wild type pattern and images that exhibited vertical stripes. Also, zebrafish responded normally to images that were coloured red. Interestingly, experimental zebrafish preferred yellow coloured images to the wild type colour (blue, green and yellow stripes). Last, zebrafish showed equal preference towards a shortened (and "fatter") image and the wild type body proportions, but did show a robust avoidance of the opposite, a lengthened and narrower fish image. These results suggest that computerized animated images may be utilized to induce social behaviour and that the decomposition of what zebrafish may regard as species specific stimuli representing their conspecifics will be possible.

Quantification of social responses

In this presentation I focus on quantification of shoaling behaviour, i.e. social affiliative responses. These responses may be induced and quantified in principally two different contexts. One, the behaviour of freely moving fish may be measured [8] or two, the behaviour of a single subject to social stimuli may be recorded [6]. In the former case, one can track all subjects of the freely moving shoal and measure numerous parameters that define shoal cohesion. For example, the average inter-individual distance measures the distance between a particular fish from all of its shoal members and calculates the average (mean) of these distances. Similarly, the variance of inter-individual distances may also be calculated for each shoal member. These measures are dependent upon the shoal size, i.e. how many members form the shoal, but they are highly informative as they take all distances between all possible pairs of fish in the shoal into account. Another measure often used in the quantification of group forming behaviour in fish, birds and other species that form aggregates, is the nearest neighbour distance. This measure is independent of shoal size, which is an advantage if one wants to compare social behaviour across a range of shoal sizes. However, it does not take into account all distances within the shoal and thus it may be less informative compared to the inter-individual distance measure. The second context in which one may quantify shoaling responses is with a single subject. In this case, a social stimulus, live stimulus fish or computer animated social stimuli, may be presented to the experimental subject adjacent to its test tank and, for example, the subject's distance from the stimulus is quantified [6].

The sight of conspecifics is rewarding

Using visual stimuli of conspecifics one may be able to trigger robust social responses [6]. These responses do not need to be trained and appear spontaneously in response to the social stimulus. It appears therefore that zebrafish are innately motivated to perform these responses. Briefly, social stimuli may be rewarding [3]. Indeed, this is what we found in a learning paradigm in which zebrafish were required to associate a visual cue (a red cue card) with the presence of conspecifics (in this case live stimulus fish) [1]. Zebrafish were found to be able to acquire the CS (cue card) US (stimulus fish) association within 20 trials and showed robust preference (conditioned response) to the CS alone when it was presented to them at a probe trial, a results that confirmed that the US (the conspecific stimulus fish) was a strong reinforcer. Given that this task was a typical appetitive conditioning task, we conclude that the sight of conspecifics is rewarding in zebrafish. A similar conclusion could be drawn from subsequent learning paradigms in which the mechanisms of associative learning were investigated with zebrafish [12-14]. In these studies, we found that the presence of conspecifics could provide sufficient drive (motivation) to learn both associative and spatial tasks and that learning performance in these tasks was NMDA-Receptor dependent, as a selective NMDA-R antagonist, MK801 could disrupt learning and memory at concentrations that did not affect motor function and/or vision.

The dopaminergic system, reward, and the sight of conspecifics

The dopaminergic system is known to mediate reward, among other functions, in mammals. Dopamine receptors (D1-R, D2-R D3-R and D4-R) have been identified in zebrafish and the nucleotide sequence of the corresponding genes has been found highly homologous to that of mammalian counterparts. We have started the mechanistic analysis of the whether the dopaminergic system is involved in social behaviour in zebrafish. First, we investigated whether presentation of conspecific images may induce changes in the levels of dopamine and DOPAC (a metabolite of dopamine). We also analyze potential changes in other neurochemicals. This analysis is still on-going but so far we have found unequivocal evidence showing that dopaminergic responses are enhanced by the sight of conspecifics, and in a manner that correlates with the length of the exposure to this visual stimulus. We also know that 6the stimulus must have certain features resembling the conspecifics as scrambled images or the testing procedure without conspecific images do not induce the dopamine responses. Interestingly, the serotoninergic system does not appear to be engaged by the presentation of social stimuli. Subsequently, we examined if disruption of the dopaminergic system may affect responses to social stimuli. We utilized a D1-R antagonist (SCH23390). D1-R is the most abundantly expressed post-synaptic dopamine receptor subtype in zebrafish. We found the antagonist to significantly impair social behavioural responses (to increase the distance of the treated experimental zebrafish from the stimulus screen on which animated images of conspecifics were shown) without affecting motor function and/or perception. Taken together the above results suggest that dopamine is likely to mediate the rewarding aspect of shoaling.

Concluding remarks

Zebrafish behavioural neuroscience is in its infancy. The number of zebrafish behavioural tests is orders of magnitude smaller than what is available for laboratory rodents, the rat and the house mouse. Nevertheless, the past few years have seen an exponential increase in the number of zebrafish behavioural studies. Our knowledge of zebrafish behaviour is rapidly increasing and our tools with which we can investigate this species from a behavioural perspective are continuously improving. Given the translational relevance of this fish and its practical advantages, I believe zebrafish will become one of the primary model organisms of biomedical research. The above studies represent just one small subset of this field. But I hope they demonstrate the utility of how one may be able to employ zebrafish in the analysis of such complex functions of the brain, as for example, social behaviour.

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References

1. Al-Imari, L., Gerlai, R. (2008). Conspecifics as reward in associative learning tasks for zebrafish (*Danio rerio*) *Behavioural Brain Research* **189**, 216-219.

2. Chatterjee, D., Gerlai, R. (2009). High Precision Liquid Chromatography Analysis of Dopaminergic and Serotoninergic Responses to Acute Alcohol Exposure in Zebrafish. *Behavioural Brain Research* **200**, 208-213.

3. Gerlai, R. (2011) Associative Learning in Zebrafish (*Danio rerio*). *Methods in Cell Bioology* **101**, 249-270.

4. Gerlai, R. (2011). A small fish with a big future: Zebrafish in Behavioral Neuroscience. *Reviews in the Neurosciences* **22**, 3-4.

5. Gerlai, R. (2010). High-throughput Behavioral Screens: the First Step towards Finding Genes Involved in Vertebrate Brain Function Using Zebrafish. *Molecules* **15**, 2609-2622.

6. Gerlai, R., Chatterjee, D., Pereira, T., Sawashima, T,, Krishnannair, R. (2009). Acute and Chronic alcohol dose: Population differences in behavior and neurochemistry of zebrafish. *Genes, Brain and Behavior* **8**, 586-599.

7. Miller, N., Gerlai, R. (2011a). Redefining membership in animal groups. *Behavior Research Methods* **43**, 364-371.

8. Miller, N., Gerlai, R. (2011b). Shoaling in Zebrafish: What We Don't Know. *Reviews in the Neurosciences* **22**, 17-25

9. Miller, N., Gerlai, R. (2008). Oscillations in Shoal Cohesion in Zebrafish (*Danio rerio*) *Behavioural Brain Research* **193**, 148-151.

10. Miller, N., Gerlai, R. (2007). Quantification of Shoaling Behaviour in Zebrafish (*Danio rerio*). *Behavioural Brain Research* **184**, 157-166.

11. Saverino, C., Gerlai, R. (2008). The social zebrafish: Behavioral responses to conspecific, heterospecific, and computer animated fish. *Behavioural Brain Research* **191**, 77-87.

12. Sison, M., Gerlai, R. (2011a). Associative learning performance is impaired in zebrafish (*Danio rerio*) by the NMDA-R antagonist MK-801. *Neurobiology of Learning and Memory* **96**, 230-237.

13. Sison, M., Gerlai, R. (2011b). Behavioral performance altering effects of MK-801 in zebrafish (*Danio rerio*). *Behavioural Brain Research* **220**, 331-337.

14. Sison, M., Gerlai, R. (2010). Associative learning in zebrafish (*Danio rerio*) in the plus maze. *Behavioural Brain Research* **207**, 99-104.

15. Sison, M., Cawker, J., Buske, C., Gerlai, R. (2006). Fishing for genes of vertebrate behavior: Zebra fish as an upcoming model system. *Laboratory Animal* **35**, 33-39.