Diving Deeper into Zebrafish Development of Social Behavior: Analyzing High Resolution Data

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Zebrafish as a behavioral neuroscience model of disease

The mechanisms and development of vertebrate social behavior are not fully understood. Numerous human clinical conditions exist in which abnormal social behavior is the core symptom, among these are alcoholism and fetal alcohol syndrome. Animal models may facilitate the understanding of the mechanisms of social behavior and the mechanisms underlying abnormal social behavior. Zebrafish are known to shoal, i.e. aggregate in groups. We have just completed a study that described, for the first time, the ontogenesis of shoaling, i.e. revealed significant increases in shoaling behavior with developmental stage in zebrafish [1].

The effect of embryonic alcohol exposure on social behavior development

In the current study, we aim to investigate how embryonic alcohol exposure affects the ontogenesis of shoaling. Although a large body of literature exists on the effects of fetal alcohol exposure, many studies have been completed using unrealistically high alcohol concentrations. To make our investigation more easily extrapolated to realistic clinical circumstances, we employed a modest alcohol dosing regimen of low doses and a short exposure time (2 hours). A previous study has demonstrated this dosing regiment to be sufficient to cause significant reduction in social preference (reduced response to images of zebrafish) when adult fish were tested [2]. The current study looks at this phenomenon from a group perspective. Zebrafish are a highly social species and studying them in groups, versus the individual, opens up the possibility to better understand complex behaviors for this species. As not sufficient information exists yet, and the only previous study looking in depth at group behavior was recently completed, we approach this in an explorative manner: starting from individual plots for each subject, for short time periods of 1-5minutes, we aim to visually observe potential patterns emerging from positional data. This serves as a starting point to investigate the possibility of modeling the observed social behavior. Previous studies from our research group has shown an oscillating pattern in shoal cohesion of zebrafish [3], here we attempt to identify additional patterns that can be observed from group interactions. Collective behavior is becoming a topic of interest and can be a direct link to better understanding how the brain works. Research suggests that animals act on environmental data using probabilistic estimation as a means of making behavioral decisions [4]. Work performed by Perez-Escudero and Plavieja (2011) supports patterns of choice of *Gasterosteus aculeatus* correspond very well to probabilistic estimation using the social information. The link found between these patterns of behavior and mathematical models can help in future experimental design using multi-subject testing. In particular, group cohesion or aggregation behavioral patterns might be influenced by pharmaceutical insults (such as embryonic alcohol exposure) and can provide us with an alternate or richer resource to test subtle effects of different compounds on the (developing) brain through behavioral research.

Quantification of social behavior

Zebrafish are a highly social species and aggregate in groups [1]. Previous research has shown that the sight of conspecifics is also rewarding to zebrafish [5]. Here we chose to observe zebrafish in groups of ten, mimicking a realistic shoaling situation (vs. exposure to either automated images of zebrafish or the sight of live conspecifics separated by a glass barrier.

Positional data were generated for each subject in a ten-member group during the testing period, at a frequency of 30 times per second. Shoaling trials were repeated regularly throughout development from 7 to 90 days post fertilization. From this data, it is possible to extract the distance between all members of the group, speed for

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each subject and the average for the group, as well as other parameters that can be deduced from positional data. For example, thigmotaxis of an individual or the group as a whole, the establishment of a home base, or potential dominance patterns. Distances between shoal members have been previously used as a measure of shoal cohesion, but this project aims to go beyond this and explore how high resolution positional data for each member can be used to gather more information on group dynamics in zebrafish social behavior, throughout development. A first step towards understanding behavioral changes throughout development, and the effect of embryonic alcohol exposure on these behaviors, is to use shoal cohesion as a measure (inter individual distances over time). The second step is to model high resolution positional data to determine if throughout development or as a result of a pharmaceutical insult (such as embryonic alcohol exposure), changes can be observed. For example, [3] found that zebrafish groups show an oscillating pattern in their shoaling behavior. One of our working questions is whether this changes over time, or due to embryonic alcohol exposure. Measuring this behavior can provide greater insights in subtle behavioral changes perhaps not captures purely with average inter individual distances.

The open field task

Ten member groups are observed while shoaling in an open field. The open field task is an established and frequently used tool in rodent studies, but still quite new in zebrafish research. While exploring patterns in social interactions between shoal members can be very informative, we also investigate exploratory patterns of a single fish in the open field task. Relative to other models, such as rodents, little is known with regards to the behavioral repertoire of zebrafish. Preliminary results show a difference in how zebrafish explore the open field compared to rodents. Our data suggests that zebrafish heavily explore the open field within the first 5 minutes of a 30 minute trial. The remaining time, the fish increasingly spends more time closer to the boundaries of the arena, and thus is showing stronger thigmotactic behavior. This is the opposite of what is observed in rodent behavior [6]. A noteworthy observation that can potentially be exploited in behavioral paradigms.

Behavioral patterns and data mining for these studies is performed using R: A language and environment for statistical computing.

Neurochemical analysis

To complement behavioral data obtained as described above, we have aimed to identify some neurochemical targets of interest. Dopamine and serotonin are implicated in several conditions with aberrant behavior as a component (such as depression and anxiety disorders), we use these targets to assess the effects of embryonic alcohol exposure on the brain. Our studies show that one time exposure of moderate to low doses of alcohol permanently disrupts normal dopamine and serotonin levels. Levels for both neurochemicals, as well as their metabolites DOPAC and 5-HIAA are significantly depressed in whole brain samples of 40, 70, and 102 day old zebrafish. These observations lay the foundation to further investigate the effects of embryonic alcohol exposure on the brain, over time.

Conclusions

Zebrafish are an increasingly popular model organism for human diseases. As zebrafish mature as a model in behavioral neuroscience, a better understanding of this species' behavioral repertoire is increasingly more important for future research. We aim not only to establish some new behavioral paradigms, but also investigate the long term effects of one time, low dose, alcohol exposure on the development of social behavior and neurochemistry in zebrafish.

References

1. Buske, C., Gerlai, R. (2010). Shoaling develops with age in Zebrafish (*Danio rerio*). *Prog Neuropsychopharmacol Biol. Psychiatry*. doi:10.1016/j.pnpbp.2010.09.003.

2. Fernandes, Y., Gerlai, R. (2009). Long-term behavioral changes in response to early developmental exposure to ethanol in zebrafish. *Alcohol Clin. Exp. Res.* **33**, 601-609: doi:10.1111/j.1530-0277.2008.00874.x.

3. Miller, N.Y., Gerlai, R. (2008). Oscillations in shoal cohesion in zebrafish (*Danio rerio*). *Behavioural Brain Research* **193**, 148-151. doi:10.1016/j.bbr.2008.05.004.

4. Pérez-Escudero, A, de Polavieja, G.G. (2011). Collective animal behavior from Bayesian estimation and probability matching. *PLoS Comput. Biol.* **7**, e1002282. doi:10.1371/journal.pcbi.1002282.

5. Al-Imari, L., Gerlai, R. (2008). Sight of conspecifics as reward in associative learning in zebrafish (Danio rerio). *Behavioural Brain Research* **189**, 216-219. doi:10.1016/j.bbr.2007.12.007.

6. Blaser, R.E., Chadwick, L., McGinnis, G.C. (2010). Behavioral measures of anxiety in zebrafish (*Danio rerio*). *Behavioural Brain Research* **208**, 56-62. doi:10.1016/j.bbr.2009.11.009.