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Background:

Autism spectrum disorders (ASDs) are neurodevelopmental disorders characterized by deficits in social interaction, repetitive behaviors and difficulties with language and communication (Banerjee et al. 2014). Though we are able to identify the symptoms of ASDs, the etiology of the disorders remains largely unknown. Because of this, ASDs are extremely difficult to diagnose and treat. Determining the etiology of these disorders would allow for more accurate diagnosis and pave the way for new treatments. New evidence suggests that ASDs may be caused in part by abnormal microglia activity. Microglia are immune cells in the central nervous system that have shown to be responsible for typical neurogenesis and synaptic pruning. It was recently discovered that the transcription factor interferon regulatory factor-8 (IRF8) is required for the production of a reactive phenotype in microglia cells in mice and proper development of microglia cells in zebrafish (Masuda et al. 2012).

Methods:

It was previously determined that one week old zebrafish exhibit no interest in social interaction but at three weeks they begin to show social preference. Social behaviors among adult zebrafish include synchronized swimming and movements and a desire to be in close proximity with one another (Dreosti et al. 2015).

To test if *irf8* mutant fish show deficits in social behavior, we utilized the same behavioral setup and procedure previously described by Elena Dreosti and others. The setup consisted of six U-shaped wells. Single viewing chambers containing social cue fish were 1.5 cm square separated from the larger chamber. Fish were recorded for ten minutes then social cue

(SC) fish were added to one of the small chambers. These fish were then allowed to acclimate for five minutes and recorded for ten minutes. From these experiments we sought to determine if IRF8 mutant zebrafish exhibit the same social behavior as their wild type counterparts.

Results:

To determine if our assay was sensitive enough to detect changes in social behavior we tested fish at 7 days post fertilization (dpf) and 19 dpf and using tracking diagrams. We found that those at 7 dpf were not social while those at 19 dpf showed a preference for the region where they could view conspecifics. At 14 dpf fish began to show a trend that suggests social behavior was beginning to develop. Fish were then tested from 18 dpf and older. Upon analysis we found that wild type and *irf8* mutant fish did not exhibit a significant difference in social preference at 21 dpf. We then tested fish older than 21 dpf and while there seemed to be an increasing trend in social preference, data analysis showed no significant difference in social preference between *irf8* mutants and wild type fish. However, because the mutations generated were homozygous recessive we were unable to generate large numbers of mutants from our crosses. We therefore decided to pool together data from all experiments with fish 18dpf and older to get an increased sample size. Both wild type and IRF8 mutant fish showed a significant increase in social preference when placed with SC fish. There was no significant difference in social preference between wild type and mutant fish.

Discussion:

We determined that while wild type and IRF8 mutant fish showed significant social preference at approximately 21 dpf, there were no differences in social behavior between wild type and IRF8 mutant fish. This lead us to conclude that IRF8, and therefore, microglia are not responsible for the development of social preference in zebrafish. Several of the observer fish

showed negative SPIs suggesting that both social behavior and avoidance behavior may develop during this stage of development.

In the future we seek to perform more of the same social experiments, using three SC fish instead of one. Because these experiments rely solely on visual cues, the presence of more than one SC fish may alter the social behavior of the observer fish. Additionally, we would test a larger sample size of IRF8 mutants. We also aim to create and test more transgenic lines where we can induce expression of other behaviorally relevant neuropeptides, as well as Cocaine-and Amphetamine-Regulated Transcript (CART) mutants.

Bibliography:

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