Approaches to analyse mood disorders in zebrafish

W.H.J. Norton[1], K. Webb[1], M. Harris[2], N. Rohner[2], C. Nüsslein-Volhard[2], J. Ninkovic[1], A. Folchert[1], and L. Bally-Cuif[1]
[1]*Department of Zebrafish Neurogenetics, Helmholtz Centre Munich, Munich, Germany, william.norton@helmholtz-muenchen.de*
[2]*Max Plank Institute for Developmental Biology, Tuebingen, Germany*

Mood disorders are debilitating diseases that show high prevalence and comorbidity in society. However, compared to other systemic diseases of the brain, the underlying genetic and neurological defects leading to mood disorders are not well understood. A lack of knowledge about their aetiology has hampered the development of drug treatments, which currently show varying efficacy and numerous side effects. Despite their poor efficiency, treatments for mood disorders still account for the largest percentage of pharmaceutical treatments prescribed, suggesting that a better understanding of the underlying pathology is crucial.

One of the aims of our research is to use zebrafish as a model organism to uncover and analyse the neural circuits that mediate mood disorder formation. The amenability of zebrafish for genetic manipulation, live imaging studies using fluorescent reporter proteins and the availability of a large mutant collection makes zebrafish an extremely appealing behavioural model. Work in our lab has already established several tests that model mood disorders in adult zebrafish, including drug addiction, boldness, and aggression. Using these protocols, we take a forward genetic approach to identify novel candidate genes which impact on mood disorder formation.

In a first set of experiments, we established a biased conditioned place preference (CPP) assay to measure drug addiction in zebrafish. Using this technique, we screened for and characterised novel zebrafish mutants that fail to change place preference following administration of the prototypical pyschostimulant amphetamine. This assay allows us to investigate the rewarding aspects of amphetamine addiction. As part of the Tuebingen screen 2005/6 we identified 6 novel mutant families that fail to become addicted to. We are in the process of further characterising one of these mutant families and are currently identifying the mutated locus by positional cloning. In parallel, we are using microarrays to examine the effect of amphetamine on the mutant and wild-type brains. A comparison of the brain transcriptome of wild-types and mutants with and without amphetamine allows us to specifically identify genes which mediate the rewarding effect of amphetamine administration. The specific aim of this set of experiments is to identify genes and pathways involved in the aetiology of addiction, with the hope of thereby identifying novel drugable targets.

In a parallel set of experiments, we have adapted an assay to measure aggression in adult zebrafish. In our assay, mirror induced stimulation is used to measure the amount of time spent attacking a perceived intruder fish in a mirror. The behaviour of single adult fish is recorded for ten minutes, and then films are replayed at a slow frame speed so that their behaviour can be analysed. Using this assay, we have identified a novel aggressive mutant, *spiegel*. *spiegel* mutants show high mortality and bite marks on the flank of conspecifics during mating. In our aggression test set-up, and in contrast to wild-type fish, *spiegel* fail to down-regulate aggression following initiation.

Using our conditioned place preference tanks, we have also measured the boldness of mutant fish. The place preference tank is divided into two separate areas: a brown side and a white side which contains two frightening black dots. Wild-type fish placed in the tank show a clear place preference, and spend approximately 85% of time on the brown side and 15% of time on the frightening white side. We reasoned that if *spiegel* is indeed bolder than wild-type, then they would modify their place preference and spend increasing amounts of time on the white side of the tank. Using this protocol we find that *spiegel* is indeed bolder than siblings.

spiegel harbours a hypomorphic mutation in *fgf receptor 1* and are both adult viable and morphologically normal. We are also dissecting the underlying neural defects leading to increased aggression through in situ hybridisation analysis. Expression of the Fgf targets Dusp6 and Phospho-ERK localises the Fgf signalling defect to a small nucleus in the inferior hypothalamus, the periventricular nucleus. This hypothalamic area has already been linked to the control of aggression in another teleost, the sunfish [1]. In parallel, the expression of the serotonin transporter gene, *serta*, is upregulated in the dorsal raphe nucleus. This suggests that a modification in 5-HT signalling underlies the behavioural changes in *spiegel*. In order to test this hypothesis, we are currently manipulating 5-HT activity within mutants, by applying the selective serotonin reuptake inhibitor Fluoxetine hydrochloride in tank water. Both wild-type and *spiegel* will be treated with a short pulse of Fluoxetine and then assayed for aggressive behaviour.

Taken together, our analysis of the *spiegel* mutant provides the first evidence that a reduction of Fgf signalling in the vertebrate brain can lead to increased aggression levels.

In summary, one of the major aims of work in our laboratory is to further develop behavioural tests for adult zebrafish. These projects will expand the number of species available for modelling the genetic and neurological changes leading to mood disorder formation in humans, and potentially identify new drug targets for their treatment.

References

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