Zebrafish Assays to Measure ADHD Endophenotypes

M. Lange¹, W.H.J. Norton¹, M. Chaminade¹, P. Vernier², K.P. Lesch³, L. Bally-Cuif⁴

¹Zebrafish Neurogenetics, Neurobiology & Development, INAF, CNRS UPR3294, Gif-Sur-Yvette, France.
²“Development and Evolution of Neurotransmission”, Neurobiology and Development, CNRS UPR 3294, Gif-Sur-Yvette, France
³Department of Psychology, Biological Psychology, Clinical Psychology, and Psychotherapy, University of Würzburg, Würzburg, Germany

Attention Deficit Hyperactivity Disorder: Introduction

Attention-deficit hyperactivity disorder (ADHD) is a developmental disorder characterized by hyperactivity, impulsivity and inattention. It currently affects around 5% of children worldwide and frequently has long-term consequences. However, although there is a significant genetic component to ADHD, relatively few risk genes have been identified and characterized [1-3]. The drug treatments available for the disease are poor with variable efficacy and significant side effects. Furthermore the etiology of ADHD is poorly understood and there are relatively few animal models for the disease. Recently, Arcos-Burgos and colleagues [4] reported evidence for a risk haplotype in the gene encoding Latrophilin 3 (LPHN3), revealed by a linkage scan in a genetic isolate population and subsequent fine mapping. LPHN3 is a potential G protein-coupled receptor with the propensity to moderate cell-cell interaction (adhesion-GPCR). However the physiological function of LPHN3 is not well understood and endogenous ligands have yet to be identified.

ADHD endophenotypes

It is therefore essential to use animal models in order to investigate the genetics basis which leads to disease pathology. Unfortunately however, the complicated genetic basis of ADHD makes it difficult to fully recreate them in animal models. One way to simplify this problem is to measure endophenotypes, biological markers that correspond to a disease-gene’s activity [5]. Endophenotypes only model a few of the symptoms of a psychiatric disorder, they should also be connected to the symptoms of the disease, perhaps reflecting some of the underlying changes in neurobiology [6]. The creation of animal model for at least some endophenotypes of ADHD will allow in-vivo analysis and subsequently a better understanding of disease neurobiology.

Modeling ADHD in zebrafish

In recent years, the zebrafish (Danio rerio) was established as a valid animal model for probing the genetics and developmental bases of behavior, based on the combination of a well-characterized ontogeny, availability of numerous tools for genetic modification and a battery of tests for behavioral characterization [7-9]. By six days, larval fish swim continuously, search for food and are able to escape from predators, thus demonstrating a range of innate behaviors. Furthermore, the formation and function of neurotransmitter signaling pathways is conserved between zebrafish and other vertebrates, highlighting the usefulness of studies conducted in fish.

To validate LPHN3 as a potential ADHD risk factor, we analyzed the function of zebrafish Lphn3 in larvae. We used morpholino injection to achieve a transient reduction of Lphn3 activity. We next characterized the larval behavioral phenotype of lphn3 morphants.

Quantification of the lphn3 morphant endophenotypes: hyperactivity and motor impulsivity

We focus our attention on two behavioral endophenotypes of ADHD: hyperactivity and motor impulsivity. Locomotion can be easily measured in larval fish. We use automated videotracking software to measure fry
zebrafish. Automated software allows us to record and measure simultaneously multiple parameters. We can also readily modify the parameters of the assays (area size, detection threshold, integration time...). We first measured larval behavior at 6 days and found an increase in the distance swum by the morphants in a 5 mm experiment, as revealed by an increase in the mean swimming speed (the resting time was unchanged). Furthermore, this hyperactivity can be rescued by application of the pharmacological treatments for ADHD, Methylphenidate and Atomoxetine. ADHD patients exhibit constant hyperactivity [10]. To determine whether lphn3 morphant larvae were constantly hyperactive, we plotted the distance swum every three seconds during a 90 second experiment for several larvae. We found that the morphants displayed remarkably stable locomotion over time. ADHD-associated impulsivity can be subdivided into both motor and cognitive components [11]. We developed a method to analyse the motor impulsivity in 6dpf larvae by plotting the distance swum in short time windows for individual animals. We observed sharper acceleration peaks in the morphants’ curves, confirming a motor impulsivity endophenotype. Together these results suggested that lphn3.1 morphants show 2 main behavioral ADHD-like endophenotypes: a stable hyperactivity, and motor impulsivity.

**Neurobiological characterization of the morphants**

We currently aim to connect potential neuroanatomical defects with the observed behavioral endophenotypes. We focused on monoaminergic systems because of their known link with both ADHD and locomotor control [12]. While the serotonergic (5-HT) and noradrenergic systems remained unaffected, we found that lphn3 morphant larvae display a disorganization of dopaminergic (DA) neurons. In parallel, immunohistochemical, in situ hybridization and high pressure liquid chromatography (HPLC) studies of other neurotransmitter systems, (including NA, 5-HT, GABA and glutamate) failed to reveal obvious defects in these other neurotransmitter signals following loss of lphn3.1 function.

**Standardization of the ADHD-like endophenotypes assays in popular zebrafish strains**

As zebrafish neuroscience research becomes more and more popular, the definition of standard thresholds for measurable behaviors (such as sleep/wake activity, locomotion, predation, phototaxis, learning, etc.) and standardization of tests/techniques become crucial. Using the methods developed to study the lphn3 morphant, we characterized the locomotion activity/impulsivity throughout zebrafish life (6dpf/1month/3months). The zebrafish community uses several wild type strain of *D. rerio*, among which we chose AB, WIK, TU and EK strains to conduct a behavioral comparative study. We also decided to add to this study the transparent Casper line [13], which has become more popular with the emergence of optogenetics and live-imaging studies. We developed and conducted high-throughput analyses of the distance travelled, speed and resting time for 6dpf/1month/3months zebrafish with automated sorting of the data. We also created routine assays to record the stable activity and the motor impulsivity described above. We obtained the locomotion threshold profile for each strain according to their intrinsic polymorphisms. All this information will help to choose the best strain for a given experiment depending on the locomotion parameters to be investigated. Moreover the methods developed provide a robust and precise *modus-operandi* to study ADHD-like endophenotypes (as locomotion) in zebrafish.

**Summary and conclusion**

In the first part we provided evidence implicating Lphn3 in the control of locomotor activity and DA development. lphn3 morphants have the potential to become a new animal model for the locomotor endophenotypes associated with ADHD and will allow us to expand our knowledge of Lphn3 function. However, there is still a clear need to expand the number of endophenotypes that can be measured, in particular to include those that quantify cognitive impulsivity and attention. We also adapted the assays that measure ADHD-like endophenotypes to obtain a standardized *modus-operandi* to study the locomotion behavior in zebrafish at different stages. Moreover we used those automated solutions to describe the locomotion parameters of popular zebrafish strains. Finally the development of validated protocols to measure different ADHD-like endophenotypes in zebrafish suggest that zebrafish has the potential to become one of the primary model organisms for translational research of psychiatric disorders.
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References


