Warsaw, October 20th, 2025

Review of the PhD thesis of Olga Doszyń

The PhD thesis of Olga Doszyń, prepared under the supervision of dr hab. Justyna Zmorzyńska, addresses the mechanisms underlying TSC-associated neuropsychiatric disorders (TAND) using the zebrafish model.

The thesis begins with a well-written and comprehensive introduction explaining the molecular consequences of *tsc1/2* mutations that lead to TAND. It discusses current knowledge derived from various animal models and provides an overview of available therapeutic strategies. The aims and scope of the research are clearly defined, ensuring a coherent framework for the experimental work that follows.

The main body of the thesis consists of several complementary components. It includes two review papers, two methodological chapters, and two experimental studies.

The first review, published in the *Handbook of Animal Models in Neurological Disorders*, describes zebrafish model of TSC developed for studying epilepsy. The second, published in *Frontiers in Molecular Neuroscience*, discusses the use of *Danio rerio* as a model organism in neuroscience, highlighting advanced methods for studying brain function, neural circuits, and behavior. It also carefully evaluates the advantages and limitations of zebrafish for investigating brain development and genetic disorders.

In addition, two methodological chapters, both published in *STAR Protocols*, provide valuable experimental procedures. One describes the visualization of pRps6-positive cells in larval zebrafish brains using immunofluorescence and lightsheet microscopy, while the other details the technique for microinjection of rapamycin into the zebrafish habenula.

Finally, the experimental part of the thesis comprises two studies: one published in *iScience* and the other available as a *bioRxiv* preprint. Together, these studies form the empirical core of the thesis.

Summary of experimental findings

The first study focused on **aberrant light processing in *tsc2* knockout zebrafish larvae**. The authors demonstrated that hyperactivation of mTORC1 in the left dorsal habenula leads to light hypersensitivity and avoidance behavior. This phenotype could be rescued by rapamycin treatment, administered either through medium exposure from two days post-fertilization (dpf) onward or via repeated habenular injections at three and four dpf. Interestingly, a single injection proved insufficient, suggesting the existence of critical developmental periods during which mTORC1 activity must be regulated for normal sensory processing.

The second study aimed at **mapping anxiety-related circuits in developing zebrafish**. It showed that TrkB is hyperactivated in the *tsc2* knockout line. Treatment with ANA-12, a TrkB antagonist, acting independently of mTORC1, normalized this hyperactivation and alleviated anxiety-like behaviors, as measured by the open-field and sudden light-change tests. The study also examined neuronal activity in the habenula, pallium, and subpallium, with the goal of identifying components of the amygdaloid complex in five-day-old larvae - structures previously defined only in adult zebrafish. This identification, based on dorsoventral localization and marker protein expression, was partially successful and provides a promising basis for further research on emotional circuitry in early development of zebrafish.

Evaluation

Overall, the thesis is well written, logically organized, and scientifically robust. It provides valuable insights into the neurodevelopmental mechanisms underlying sensory processing and anxiety-like behavior in tuberous sclerosis complex. The combination of genetic, behavioral, and imaging approaches is particularly noteworthy, demonstrating the author's ability to integrate multiple experimental levels to address complex biological questions.

The inclusion of the methodological chapters further enhances the thesis, providing useful tools for the research community. The zebrafish model is employed effectively to link molecular dysfunction to defined neural circuits, offering both mechanistic understanding and translational relevance.

Taken together, the work represents a significant contribution to the field of developmental neurobiology and supports the use of zebrafish as a versatile and informative model for studying neuropsychiatric disorders associated with *tsc2* mutations. Several scientific and methodological aspects of the work merit further discussion and clarification during the defense, as outlined below.

Points for discussion during the defense

1. Page 29 – It is stated that displaced cells in the white matter can act as ictal foci, suggesting that dysregulated migration may lead to mislocalized neurons driving seizures in TSC. How translatable are these findings from zebrafish to humans?
2. Page 44, Figure 1B – What do the relative numbers shown in this figure represent?
3. Page 84, Figure 1 – How do heterozygous fish respond to rapamycin? The light-preference index appears decreased when comparing DMSO and RAPA conditions - could this be clarified?
4. Evolutionary aspect – What could explain the observation that adult fish respond to light in the opposite way to larvae?
5. Pages 88–90, Figures 3–4 – Is there a difference between bath treatment and direct habenular injection of rapamycin? Is the observed effect driven primarily by the critical developmental period or by the duration of treatment?
6. Page 139, Figure 1B – The wild-type animals display greater variability in thigmotaxis behavior compared to the heterozygous animals, which appear to show an increased tendency toward this behavior. Could this indicate that heterozygous larvae exhibit heightened anxiety-like behavior relative to wild-type controls? Was this trend consistent across replicates?
7. Heterozygous animals are generally regarded as a more accurate model of tuberous sclerosis complex, as this genotype most closely reflects the condition observed in

patients. It would be helpful if the author could elaborate on how heterozygous animals were affected in the presented studies and whether their phenotypes correspond to clinical features of human TSC.

Final assessment

In summary, Olga Doszyń's thesis represents a high-quality body of work that combines thorough literature analysis, well-designed experiments, and innovative methodological development. The research is original, well executed, and contributes substantially to the understanding of TSC-associated neuropsychiatric disorders.

The thesis meets the expected academic standards for a doctoral dissertation and demonstrates the candidate's independence, creativity, and advanced methodological competence. The contribution of the candidate to the published papers is clearly defined and appropriate, reflecting her active involvement in all stages of the research. Therefore, I, the undersigned, hereby state that the doctoral dissertation of Olga Doszyń meets the requirements specified in Article 187 of the Act of July 20, 2018 – Law on Higher Education and Science (c.t., Journal of Laws of 2024, item 1571, as amended). I hereby recommend to the Doctoral Committee of the International Institute of Molecular and Cell Biology in Warsaw to admit Olga Doszyń to the subsequent stages of the procedure for the conferment of the doctoral degree in the field of natural sciences, in the discipline of biological sciences.

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Data: 2025.10.24 18:23:47 CEST

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