

## **Zebrafish (*Danio rerio*) models of drug abuse and anxiety**

Jonathan Cachat<sup>1</sup>, Peter Canavello<sup>1</sup>, Peter Hart<sup>1,2</sup>, Carisa Bergner<sup>2</sup>, Rupert Egan<sup>2</sup>, Brett Bartels<sup>1</sup>, Esther Beeson<sup>1</sup>, Salem Elkhayat<sup>1</sup>, Marco Elegante<sup>1</sup>, Sopan Monhot<sup>1</sup>, David Tien<sup>1</sup>, Anna Tien<sup>1</sup>, Hakima Amri<sup>2</sup>, Eric Glasgow<sup>2</sup>, Zofia Zhukowska<sup>2</sup>, Allan Kalueff<sup>1,2</sup>

<sup>1</sup>Pharmacology Dept., Tulane University Medical School, New Orleans, LA,

<sup>2</sup>Physiology and Biophysics Dept., Georgetown University Medical School, Washington, DC

Zebrafish (*Danio rerio*) are becoming increasingly popular in genetic and behavioral neuroscience research. They represent a well-balanced compromise between throughput, neurobiological complexity and phenotypic robustness which permits multiple levels of analysis, from genetics to physiology and complex behavioral phenotypes. Our lab has recently adopted zebrafish as an experimental model of stress, anxiety and drug sensitivity. To induce stress-like behavior in zebrafish, we exploit natural tendencies (i.e. fear of novel environments, predator exposure and alarm pheromone contact) or introduce pharmacological treatment, such as ethanol or benzodiazepine withdrawal.

Behavioral observations in the novel tank test translate stress/anxiety into multiple quantifiable endpoints (e.g., latency to enter the top half of the tank, number of entries, time spent exploring the top half of the tank, fear-like erratic movements and freezing bouts). Following this behavioral phenotyping, whole-body cortisol levels are analyzed using ELISA, providing a sensitive and reliable physiological measure of the endocrine response to stress or anxiety. The juxtaposition of behavioral and physiological endpoints is particularly useful when evaluating pharmacological manipulation in zebrafish. For example, chronic administration of the SSRI antidepressant fluoxetine [for 2 weeks] markedly reduces anxiety-like behavior and lowers whole-body cortisol levels.

Currently, our lab is examining the biomarkers of affective disorders and ethanol use. Groups of zebrafish (n = 15) were chronically treated with 0.3% EtOH for 8 days. On the 8<sup>th</sup> day, fish were placed in a new tank with fresh water for 14 h to induce withdrawal. In both behavioral and physiological measures, we found that EtOH withdrawal had robust anxiogenic effects in zebrafish. To induce an anxiogenic response, zebrafish were subjected to a model of benzodiazepine withdrawal syndrome. After chronic administration of the benzodiazepine diazepam, drug treatment was halted for 3 days before novel tank exposure testing and subsequent cortisol assessment. While behavioral data signified a strong anxiety-like phenotype, cortisol levels also tended to rise in these fish.

Collectively, our experiments substantiate zebrafish as dependable and consistent subjects in anxiety and stress research, as well as in studies focusing on drug dependency and withdrawal. Based on the strong correlation of behavioral data and cortisol analysis, zebrafish prove to be an ideal model organism for experimental stress research.