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

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Abstract

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 fluid transition across levels of analysis from genetics to physiology and complex behavioral phenotypes.

We induce stress-like behavior in zebrafish by exploiting natural tendencies (i.e. fear of novel environments, predator exposure and alarm pheromone contact) or with pharmacological treatment, such as EtOH or benzodiazepine withdrawal. Following chronic exposure to EtOH or Diazepam, withdrawal is induce by rapidly ceasing treatment prior to behavioral testing. Immediately after behavioral phenotyping, physiologic measure of stress and anxiety are examined via whole-body cortisol concentrations. Overall, EtOH and Diazepam withdrawal resulted in strong anxiety-like behaviors and increases in whole-body cortisol.

Introduction

A relatively simple vertebrae species, zebrafish (Danio rerio) is popular in biomedical research because its physiology is analogous to humans, permitting researchers to probe the mechanisms and pathways relevant to human disease and therapy.

The zebrafish nervous system possesses all of the "classical" vertebrate neurotransmitter systems, and contains a well-documented corticosteroid stress axis. Zebrafish are also an ideal animal model for laboratory research because they are low-maintenance and abundantly produce offspring. Together, this makes the zebrafish a premiere model to investigate principles of nervous system development, function, disease and behavior. Here we examine the zebrafish anxiety/fear-like behavior, and correlate these "affective" stress-evoked states with physiological phenotypes, such as the levels of stress hormone cortisol.

Zebrafish

Novel Tank

Methods

Novel Tank Exposure Test: Zebrafish were relocated from home-tanks to novel tanks. Latency to reach the upper portion of the tank, time spent in the upper portion of the tank, number of entries into the upper portion of the tank, erratic movements, freezing bouts, and freezing duration were recorded. Erratic movements were characterized as sharp changes in direction and velocity, or rapid darting behaviors. Anxiety response was measured as a significant decrease in exploration, including: longer latency to reach the top, shorter duration in upper half as a ratio to the number of entries to the top, an increase in freezing or erratic movements.

Ethanol withdrawal: Groups of zebrafish (n = 15) were chronically treated with .3% EtOH for 8 days. On the 8th day, fish were removed from the EtOH-treated tank and placed in a new tank with fresh water for 14 hours to induce withdrawal.

Diazepam withdrawal: Zebrafish were subjected to a model of benzodiazepine withdrawal syndrome. After chronic administration of the benzodiazepine diazepam (5 µM(, 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.

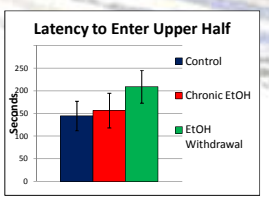
Cortisol extraction: After homogenizing each animal, samples were then transferred to glass tubes. Each sample was then extracted 2 times with 5mL of Ether. After the Ether had been allowed to evaporate (under a fume hood) the cortisol was reconstituted in 1mL of 1X PBS (Alsop and Vijayan, 2008).

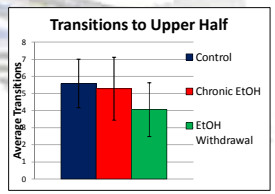
ELISA assay: ELISA was performed on cortisol samples using a cortisol assay kit (Salimetrics LLC, PA). Absolute cortisol levels (µg/dL) were assessed. Cortisol levels were then normalized based on the weight of the initial body sample.

Statistical analysis of behavioral data: The data were analyzed using the Wilcoxon Ranked-Sum test for significance between groups (control and experimental conditions). All data are expressed as Mean ± SEM. Significance was set at $p < 0.05$

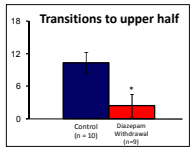
Results

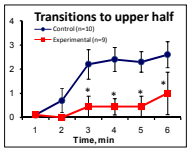
Ethanol Withdrawal: EtOH withdrawal precipitated an anxiogenic response in zebrafish. On average, EtOH withdrawal zebrafish took longer to enter the upper half of the tank and made less transitions to the top, these endpoint are indicative of elevated anxiety. Moreover, EtOH withdrawal fish had higher concentrations of whole-body cortisol.

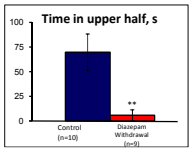


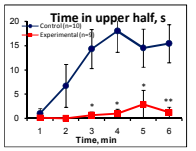


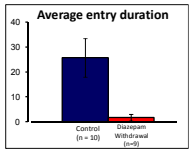
Diazepam Withdrawal: Diazepam withdrawal also induced an anxiogenic response in zebrafish. These fish made less transition to the upper half, spent less overall time in the upper half and when they did decide to cross over, spend significantly less time there. Additionally, the diazepam withdrawal cohort displayed significantly more erratic movements , a prime example of behavior in the novel tank corresponding to higher levels of stress and anxiety. Lastly, the withdrawal group also had higher levels of whole-body cortisol.

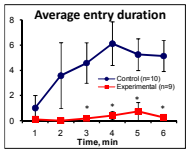


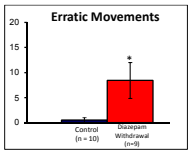


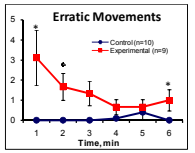


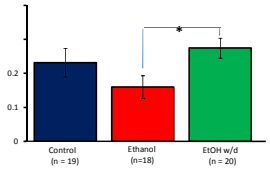


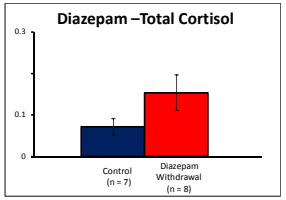












Conclusions

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.

We plan to use this preliminary data as the foundation for future studies to further explore the relationship between alcohol use/abuse and affective disorders such as anxiety and depression.

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