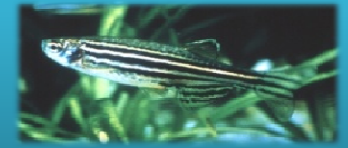# The Utility of Zebrafish in Stress Research

Hart, P.[1,2], Bergner, C.[2], Egan, R.[2], Cachat, J.[1], Canavello, P.[1], Bartels, B.[1], Beeson, E.[1], Elkhayat, S.[1], Elegante, M.[1], Mohnot, S.[1], Tien, D.[1], Tien, A.[1], Kalueff, A.[1,2]

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Introduction

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.

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.

Methods

Novel Tank Exposure Test: Observers record endpoints and sessions are also video taped for automated analysis (CleverSys Inc.).

Ethanol withdrawal: Groups of zebrafish 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 12 hrs to induce withdrawal.

SSRI Treatment: Chronic administration of the SSRI fluoxetine (100 µg/L for two weeks) was preformed prior to novel tank testing and subsequent cortisol assessment.

Acute Caffeine Treatment: Zebrafish were exposed to caffeine (50mg/L) for 5 minutes prior to the Novel Tank.
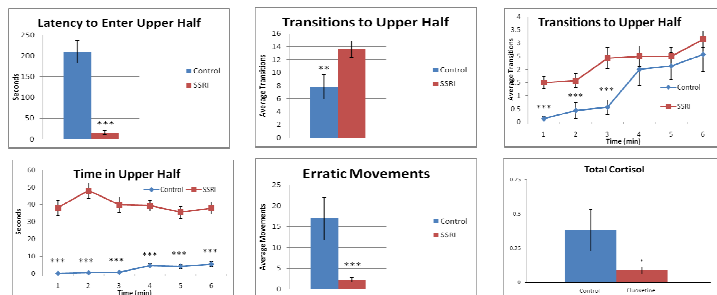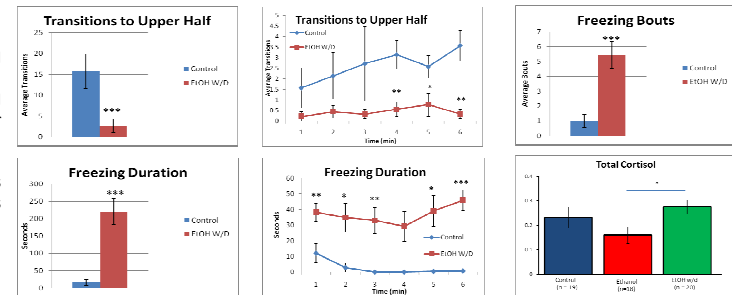
Cortisol extraction: Performed using a human salivary cortisol assay kit (Salimetrics LLC, PA).
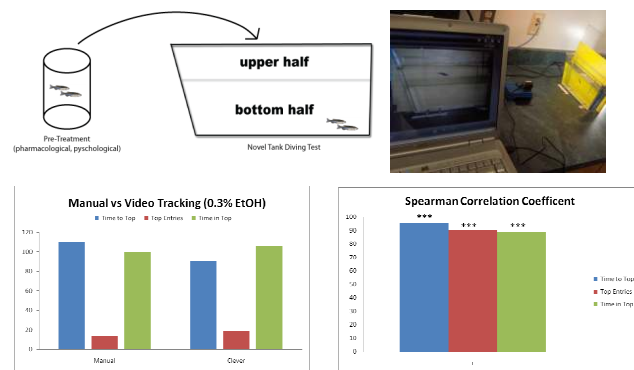
Results

Ethanol withdrawal: Zebrafish experiencing ethanol withdrawal showed overall trends of anxiety-like behavior (shown to the right). As compared to control fish, the withdrawal cohort displayed less transitions to the upper half of the novel tank, had significantly more freezing bouts and were frozen for a greater duration. These behavioral endpoints are an indicative of elevated stress levels.
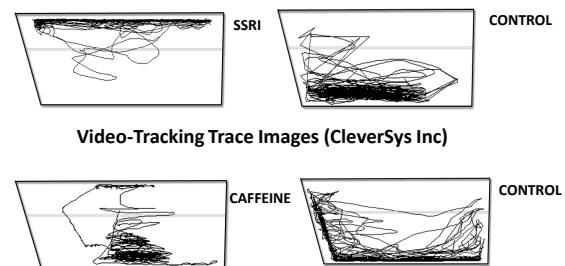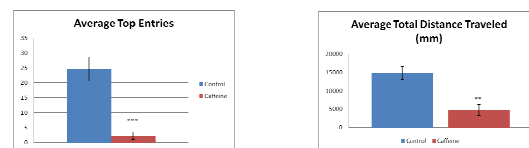
Moreover, this overall anxiety-like state is confirmed by physiological measures of average whole-body cortisol concentrations (p=.0135). Data is expressed as Mean ± S.E.M, *p<0.05, **p<0.005, ***p<0.0005.

Acute Caffeine: Zebrafish pre-treated with caffeine displayed significantly less top entries compared to control cohorts. Additionally, they traveled less overall total distance, perhaps indicating a stereotyped circling behavior common to stimulant drugs.

SSRI Treatment: Zebrafish pretreated with fluoxetine showed overall decreases in anxiety-like behavior. The experimental cohort took significantly less time to enter the upper half of the novel tank, in fact all fish almost immediately ventured into the top half. As compared to control fish, the SSRI treated fish also displayed significantly more transitions to the upper half of the novel tank, spend more time per upper entry and had significantly less erratic movements. Moreover, the anxiolytic effects of fluoxetine are reflected by whole-body cortisol levels, in which experimental fish on average had significantly less.

The video tracking software (CleverSys) was able to provide the trace images to the right, that eloquently reflect the general differences in swimming behavior between SSRI and control cohorts.

Video-Tracking Trace Images (CleverSys Inc)

Discussion

The attributes that give the zebrafish its power for genetics, drug discovery and developmental research, make this species an ideal subject for cost-effective models of disease pathogenesis, including stress and anxiety. By comparing the robust behavioral phenotypes seen here with the physiological evidence from cortisol assays, we were able to demonstrate the consistency and reliability of this species as an experimental model for stress and anxiety. Both manual and automatic registration of zebrafish behavior were able to distinguish differences in acute drug treatment and withdrawal paradigms. In future studies, we plan to further explore the effects of anxiolytics and anxiogenics on zebrafish behavior, physiology and eventually genetic expression.

Acknowledgements