Visualizing and Interpreting Drug-Evoked Anxiety Behaviors in Zebrafish

D. Tien[1], P. Canavello[1], J. Cachat[1], R. Egan[2], P. Hart[1,2], C. Bergner[2], M. Elegante[1], S. Mohnot[1], S. Elkhayat[1], B. Bartels[1], A. Tien[1], E. Beeson[1], D. Soignier[3], A. Kalueff[1,2]

[1]Pharmacology Dept., Tulane University Medical School, New Orleans, LA [2]Physiology and Biophysics Dept., Georgetown University Medical School, Washington, DC

[3]Department of Psychology, University of New Orleans, New Orleans, LA

Abstract

Zebrafish (*Danio rerio*) represent a rapidly emerging animal model for high-throughput behavioral analysis. Common in biomedical research, the zebrafish is favored due to physiological similarities to mammals. The corticosteroid axis in zebrafish is highly sensitive to environmental stress, providing opportunity to measure the effects of various anxiolytic and anxiogenic agents on cortisol fluctuation. As in other species, exposure to a novel environment induces robust anxious-like responses. When placed in a novel tank, fish dive to the bottom, maintaining maximum depth until ready to explore. The Novel Tank exploits diving and other behaviors as measures of anxiety, effectively quantifying behavioral parameters. To extend the reliability, endpoint coverage, and throughput of the Novel Tank model, we adopted a video tracking system for automated analysis. The results demonstrate the reliability of the video tracking system in producing behavioral data consistent with previously observed drug effects and physiological measures.

Methods

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

Chronic Ethanol: Group of zebrafish were chronically treated with 0.3% EtOH for 8 days. Fish were removed from the EtOH-treated tank and placed in a freshwater tank 12-hours prior observation to induce withdrawal.

Acute Ethanol: Zebrafish were exposed to 15 min of 0.3% EtOH treated tank prior observation.

SSRI Treatment: Chronic administration of SSRI fluoxetine (100 µg/L for two weeks) was performed prior to novel tank testing.

Acute Morphine: Zebrafish were exposed to 15 min of Morphine (2mg/L) treated tank prior observation.

Acute Caffeine: Zebrafish were exposed to caffeine (50mg/L) for 5 minutes prior to the Novel Tank.

Acute Kratom Extract (contains 7-OH-mitragynine): Zebrafish were exposed to Kratom extract (6.15g/L) for 10 min in darkness to control water color.

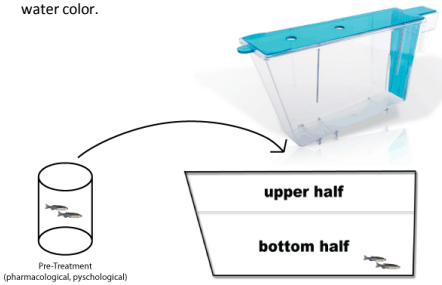

Figure 1: Novel Tank Test

Results

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 (Fig. 2). Moreover, the anxiolytic effects of fluoxetine are reflected by whole-body cortisol levels, in which experimental fish on average had significantly less.

Figure 2: Effects of SSRI fluoxetine exposure on zebrafish behavior

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 (Fig. 3)

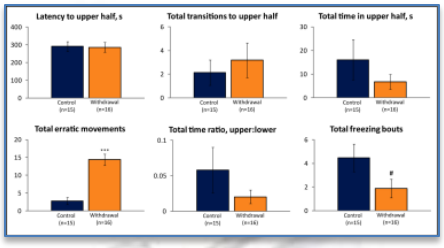

Figure 3: Effects of acute caffeine exposure on zebrafish behavior

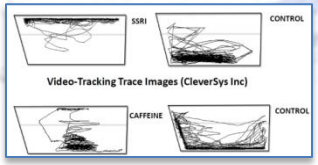

Figure 4: Traced-path for zebrafish

Acute Kratom: Acute administration of Kratom increased latency to enter the top half of the novel tank, reduced transitions to and time in the upper half, reduced erratic movements and significantly increased number and duration of freezing bouts. In a separate study comparing dosages of Kratom, a higher concentration (6.15 g/L) caused an anxiolytic trend in zebrafish behavior. Zebrafish treated with the greater dose demonstrated more transitions to the top, longer average duration in the top, decreased erratic movements, and decreased freezing bouts.

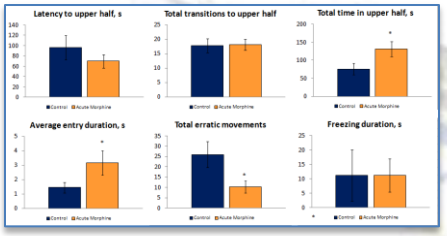

Figure 5: Effects of acute morphine exposure on zebrafish behavior

Acute Morphine: Acute morphine administration showed an increased in time spent in the upper half, an increased in average entry duration, and a decreased in erratic movement. Interestingly, the transitions to the upper half and the freezing duration of the zebrafish were very constant to the control, and did not varied much at all (Fig. 5)

Acute Ethanol: Acute treatment with 0.3% ethanol decreased latency to enter the upper portion of the tank, increased the number of transitions to the upper portion, and increased total time spent in that area. Anxiolytic effects were also observed in response to chronic ethanol treatment and included more time spent in top throughout trials (Fig. 6).

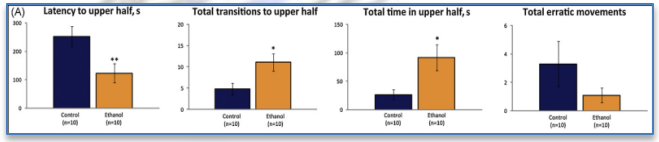

Figure 6: Effects of acute ethanol exposure on zebrafish behavior

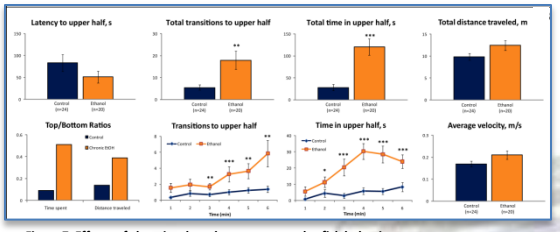

Figure 7: Effects of chronic ethanol exposure on zebrafish behavior

Chronic Ethanol: Zebrafish experiencing chronic ethanol withdrawal showed overall trends of anxiety-like behavior. 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 (Fig. 7). These behavioral endpoints are an indicative of elevated stress levels.

Discussion

These results demonstrate that zebrafish are sensitive to pharmacological modulation, and video-tracking system has illustrates great reliability, reduced inter-rater variability, and provides additional endpoints. The video-tracking system was able to produce behavioral data consistent with previously observed drug effects and physiological measures. 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. Zebrafish prove to be an ideal model organism for experimental stress research.

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