Modeling Withdrawal Anxiety in Zebrafish

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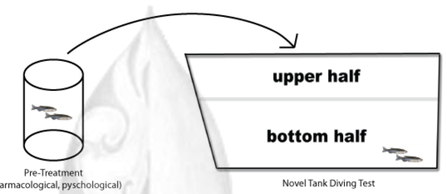
abstract

Zebrafish (*Danio rerio*) are becoming a popular model in behavioral neuroscience. Their behavior is robustly observed and easily quantified, with the effects of pharmacological challenges emerging almost immediately. Following behavioral analysis in the Novel Tank (NT), physiological endpoints (i.e. cortisol concentrations) can be obtained, making this a valuable model for high-throughput investigations of experimental manipulations. We examined the behavioral and physiological endpoints of ethanol, diazepam, morphine, caffeine and fluoxetine withdrawal in zebrafish.

methods

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



Ethanol & Caffeine withdrawal: Ethanol (0.3% EtOHvol/vol) and caffeine (1.5 mg/L) were administered for 1 week into respective home tanks, which were then filled with untreated water for 12 h before behavioral testing.

Diazepam withdrawal: The diazepam (72 mg/L) cohort was treated chronically for 2 weeks, then placed in drug-free water for 72 h prior to testing.

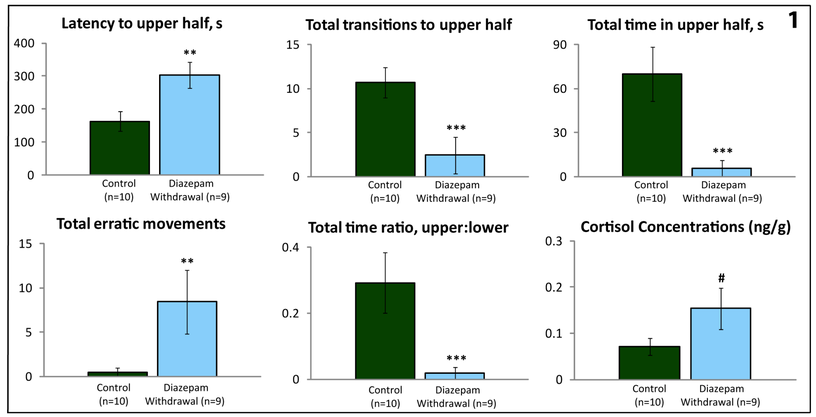
Morphine withdrawal: Zebrafish were exposed to morphine (1.5 mg/L) chronically for 1 week and then placed in drug-free water for 48 h, to elicit withdrawal.

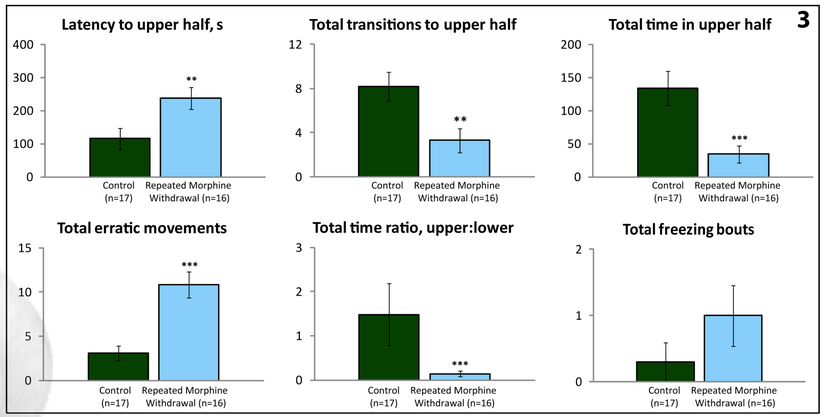
Fluoxetine withdrawal: Chronic administration of fluoxetine (100 µg/L for two weeks) was preformed prior to novel tank testing and subsequent cortisol assessment.

Repeated Ethanol & Morphine withdrawal: After 1 week of chronic treatment, zebrafish were placed into exposure tanks with drug-free water for 3h, 2x/day. After 1 week of repeated withdrawal, fish were taken from home tanks and placed in exposure tanks for a final 3-h withdrawal prior to behavioral testing.

Cortisol extraction: Performed using a human salivary cortisol assay kit (Salimetrics LLC, PA).

Statistical Analysis: All experimental data was analyzed with a two-sample Wilcoxon U-test for significance between control and experimental groups. Data is expressed as Mean ± S.E.M, *p<0.05, **p<0.005, ***p<0.0005.

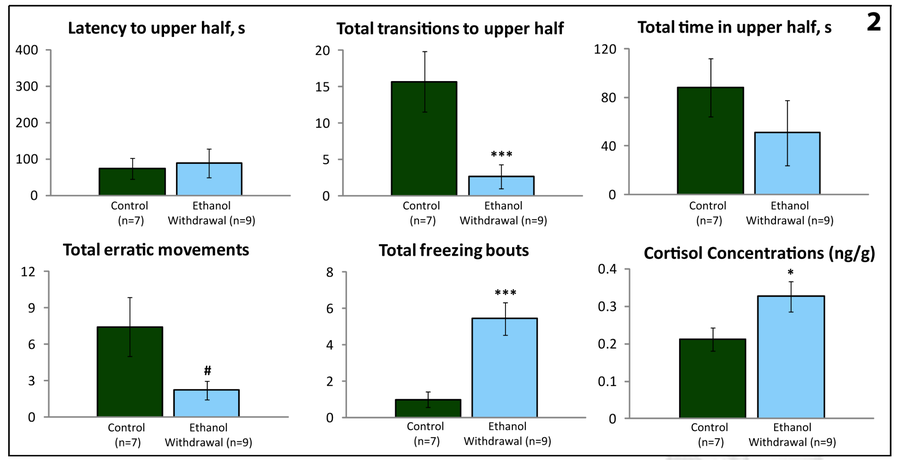




results

Diazepam withdrawal: Zebrafish showed a longer latency to the upper half, made fewer transitions and spent less time in top of the tank (Fig 1, shown to the left). Analysis of whole-body cortisol revealed a trend toward higher cortisol levels in the withdrawal cohorts. Indicating a strong anxiogenic response.

Ethanol withdrawal: Zebrafish experiencing ethanol withdrawal showed overall trends of anxiety-like behavior (Fig 2, shown to the right). Moreover, this general anxiety-like state was confirmed by physiological measures of average whole-body cortisol concentrations.



Morphine withdrawal: Although single morphine withdrawal did not evoke anxiety-like behaviors (data not shown), repeated morphine withdrawal produced robust anxiogenic responses (Fig 3, to the left).

Caffeine & Fluoxetine withdrawal: The caffeine withdrawal (data not shown) group showed a trend for fewer freezing bouts and displayed significantly more erratic movements, also showing (although not significantly) lower top:bottom ratio and time spent in the upper half. The fluoxetine withdrawal cohort (data not shown) displayed significant anxiolytic measures on almost all behavioral endpoints.

disscusion

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.

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