

## Modeling Withdrawal Anxiety in Zebrafish (*Danio rerio*)

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Zebrafish (*Danio rerio*) are becoming a popular model in biological psychiatry and behavioral neuroscience. Their behavior is robustly observed and easily quantified, with the effects of environmental and pharmacological challenges emerging almost immediately. Following behavioral analysis in the Novel Tank (NT), physiological endpoints (i.e. cortisol concentrations) can also be obtained, making zebrafish a valuable model for high-throughput investigations of anxiolytic and anxiogenic manipulations. Here, we examined the behavioral and physiological endpoints of ethanol (0.3% EtOH), diazepam (74µg/mL), morphine (1.5mg/L), caffeine (50mg/L) and fluoxetine (100ng/L) withdrawal in zebrafish. All zebrafish were chronically treated with the drug for 1 week prior to withdrawal procedure (the SSRI cohort was treated for 3 weeks). Ethanol withdrawal (12 h prior NT) reduced exploratory behavior in addition to a robust increase in erratic movements and freezing behaviors throughout testing, compared to chronically-treated and EtOH-naïve controls. These endpoints indicate a strong anxiogenic effect caused by EtOH withdrawal in zebrafish. In subsequent experimental trials, this anxiogenic trend was further confirmed by repeated EtOH withdrawal paradigms (4 h twice/day, 1 week prior NT) and multiple cortisol measurements, in which withdrawal significantly increased whole-body concentration. In a similar fashion, diazepam withdrawal (72 h prior NT) induced a tendency to remain within the bottom half, committing to less top entries and significantly more erratic movements compared to diazepam-naïve and chronically exposed control fish. An assessment of cortisol levels did not yield significant results. Chronic morphine withdrawal (1 week prior NT) failed to produce statistically significant effects, although all behavioral measures favored an anxiogenic potential in morphine withdrawal compared to naïve and sustained control fish. However, the repeated withdrawal paradigm (4 h twice/day, 1 week prior NT), elicited strongly significant anxiogenic effects on behavior including a sharp increase in erratic movements and latency to enter the upper half as well as overall decreases in exploratory transitions to the upper half and time spent in the upper half. Fish experiencing caffeine withdrawal spent significantly less time in the upper half per entry, had more erratic movements but significantly less freezing bouts. Caffeine withdrawal fish also seemed to make more transitions to the upper half but spent less time in the upper half, results not significant. These trends were found in the repeated withdrawal paradigm but none were significant. Lastly, fluoxetine discontinuation did not appear to have anxiogenic effects on treated zebrafish. Overall, although some drugs did not evoke withdrawal phenotypes in this study, some other drugs produced robust anxiogenic responses in zebrafish, supporting the utility of zebrafish to study withdrawal-related anxiety and its potential as a model in translational neuroscience research.

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