How does early-life exposure to anti-depressants affect zebrafish (*Danio rerio*)?

Veterinary Research Scholars Program
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Introduction

Embryonic and early juvenile development is a critical time for the proper formation of a functioning organism, during which exposure to chemicals can lead to abnormalities. This study aims to examine the physiological and behavioral effects of administration of anti-depressant medications during embryogenesis in zebrafish (*Danio rerio)*. The widespread use of antidepressants, even during pregnancy, raises interesting questions in two different contexts. First, the zebrafish is used to model behavioral disorders in humans; it is unknown whether exposure to antidepressants in early life alters behavior and physiology in fish in a manner similar to mammals. This study will address that knowledge gap. Second, antidepressants are increasingly being found in trace amounts in environmental water sources. The effect this could have on native wildlife populations is also unknown.

Goal:

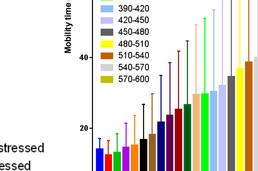
Determine the effects of anti-depressant medications on the development and behavior of juvenile zebrafish

Exposures

- **Control**
- **Desvenalfaxine**
  - Low = 10nM
  - Medium = 1μM
  - High = 100μM
- **Paroxetine**
  - Low = 100nM
  - Medium = 10μM
  - High= 100μM

Questions

Does early life exposure to anti-depressants affect:



1) Hatching
2) Larval Mortality
3) Locomotion
4) Stress responses

Methods

General Information

- Species: Zebrafish (*Danio rerio*)
- Antidepressants
  - Desvenlafaxine hydrochloride
  - Paroxetine hydrochloride hemihydrate (98%)

Husbandry:

- ~100 eggs per dish x 2 dishes per exposure
- Eggs were collected from breeding tanks and placed into a petri dish containing water, desvenalfaxine, or paroxetine at high, medium, and low doses
- The petri dishes were then placed into an incubator at 28.5 C
- Debris, egg remains, and dead animals were removed daily
- Tested 7 days later

Drug Dose Preparation:

- 1mM stock solutions of paroxetine and desvenlafaxine were prepared and placed into 1mL aliquots
- The doses below were added to 30 ml of water, then eggs were added

Paroxetine		
Low	100nm	3μL
Medium	10μM	300μL
High	100μM	3000μL

Desvenlafaxine		
Low	100nm	0.3μL
Medium	10μM	30μL
High	100μM	100μL

Mortality and Hatching Experiment

- Eggs were checked every 24 hours and the number of hatched individuals and number of deaths were recorded

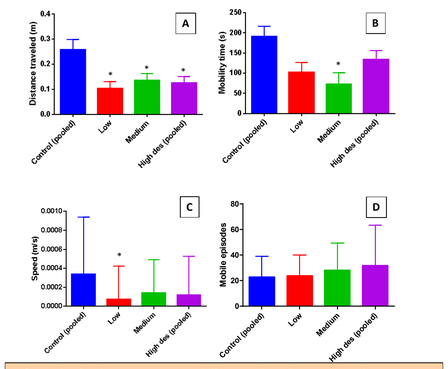
Locomotor Behavior Experiment

- 9 individuals at a time were placed into 24 well plates with 1 ml of aquarium water
- 1 minute acclimation period
- 10 minutes per trial
- ANY-maze software was used to record the following variables:
  - Distance traveled (m)
  - Speed (m/s)
  - Motility time (s)
  - Total # of mobile episodes

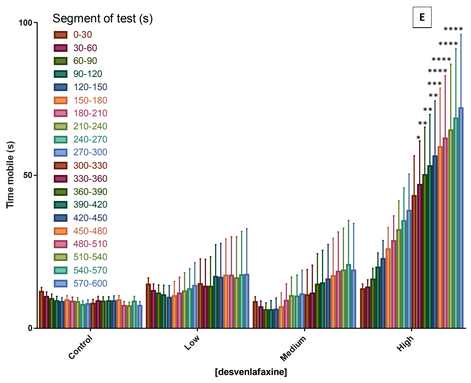
NaCl Stress Response Experiment

- Exposures: High dose desvenalfaxine and control
- 9 individuals at a time were placed into 30ml of 100mM NaCl for 10 minutes
- After 10 minutes the animals were removed and rinsed twice in aquarium water
- They were then placed into 24 well plates for testing
- 1 minute acclimation period
- 10 minutes per trial
- ANY-maze software was used to record same variables as the previous experiment

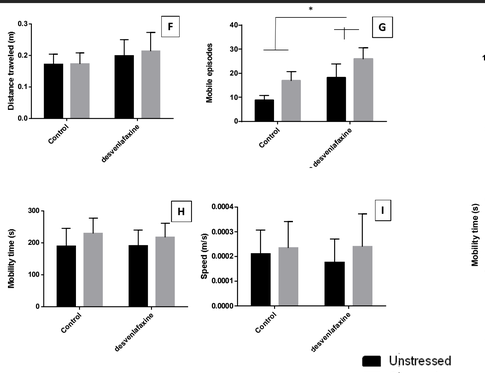
Desvenlafaxine Results



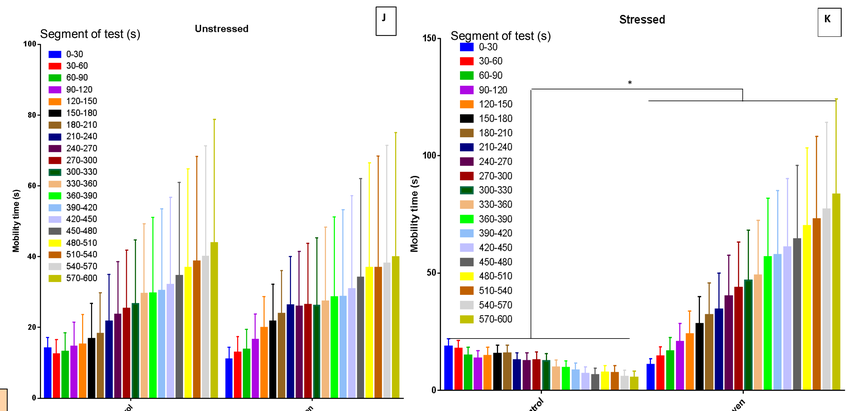
- Control animals move more total distance than those exposed to desvenlafaxine at any dose; One-way ANOVA, p =0.0016 (Fig A)
- Animals exposed to a medium dose of desvenlafaxine were mobile less total time than control animal; One-way ANOVA, p = 0.0278(Fig B)
- Control animals move faster than animals exposed to a low dose of desvenlafaxine; One-way ANOVA, p = 0.0209 (Fig C)
- Total number of mobile episodes was not statistically different in any exposure; p = One-way ANOVA, 0.1061 (Fig D)



- Animals that were exposed to high doses of desvenlafaxine moved more than control animals from 330s-600s; One-way ANOVA, p = 0.0001 (Fig E)
- There appears to be a trend that drug treated animals are moving more later in trials; One-way ANOVA, p = 0.0697

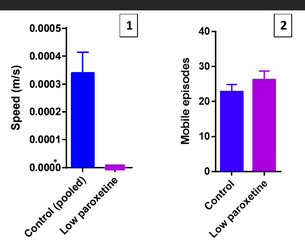


- There is no effect of stress (p =0.8736) or drug treatment (p = 0.4955) on distance traveled (Fig F)
- Desvenlaxafine increased the number of mobile episodes; Two-way ANOVA, p = 0. 0335 (Fig G)
- There is no affect of antidepressant or NaCl stress on mobility time (Fig H) or average speed (Fig I)

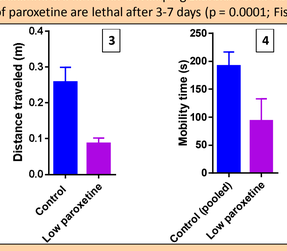


- In unstressed animals there is no difference in mobility time between control and drug treated animals; Two-way ANOVA, p = 0.9999 (Fig J)
- When mobility time was analyzed by time segment, desvenlafaxine was found to have a significant effect on animals stressed with NaCl; Two-way ANOVA, p = 0.0001 (Fig K)

Paroxetine Results

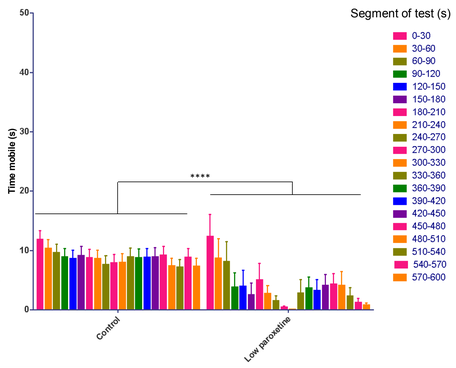


- Control animals move faster than paroxetine treated animals; Unpaired t-test, one tailed p = 0.0332 (Fig. 1)
- There were no differences in mobile episodes between control and drug-treated animals; unpaired t-test, p = 0.9422 (Fig. 2)
- High doses (100μM) of Paroxetine are lethal for developing zebrafish after hatching (p = 0.0001; Fisher's Exact Test)
- Low and medium doses of paroxetine are lethal after 3-7 days (p = 0.0001; Fisher's Exact Test)



Trends:

- Control animals travel farther during trials than paroxetine treated animals Unpaired T-Test, p = 0.0599 (Fig 3)
- Paroxetine treated animals were mobile less total time than controls; Unpaired T- Test, one-tailed p = 0.0880 (Fig 4)



- Paroxetine treated animals spend less time being mobile than control animals; Two-way ANOVA, P = 0.0001 (Fig 5)

Answers

Does early life exposure to anti-depressants affect:

1) Hatching- No
2) Larval Mortality- Yes
3) Locomotion- Yes
4) Stress responses – Yes

Future Directions

Does early life exposure to anti-depressants affect:

1) Cortisol levels in response to stressors
2) Locomotor behavior as adults
3) Physiology of adults (mass, lifespan, etc)
4) Stress responses of adults
5) Long-term survival

Acknowledgements

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