Behavioral and Transcriptomic Effects of Paroxetine on the Fathead Minnow (*Pimephales promales*)

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INTRODUCTION

The fathead minnow (FHM) is a native freshwater species, widely distributed throughout North America. FHM are an ideal model for toxicological studies of chemical exposures in the environment. Pharmaceuticals are commonly found in freshwater ecosystems due to improper disposal of prescriptions and through human excretion.[1] Selective serotonin reuptake inhibitors (SSRIs) are some of the most prescribed pharmaceuticals and treat disorders including depression and anxiety.[2] SSRIs are highly water soluble, making it difficult for wastewater treatment plants to filter them out, allowing them to enter freshwater environments, impacting aquatic fauna.[1,3] SSRIs, such as paroxetine (PXT), have been shown to have effects on the behavior and transcriptomic ('omics) expression in organisms.[4,5] We aimed to use behavior and transcriptomics as two endpoints to provide further insight on the effects PXT has on the early life-stage FHM.

MATERIALS & METHODS

1. Egg Collections: FHM eggs were collected in 4 one-hour window to ensure larvae were at similar developmental stages at time of exposure. Three egg collection went towards each of three behavior exposure replicates. The fourth collection went towards the –omics exposure.
2. Behavior Exposure Layout: Seven 5-day post-fertilization (dpf) FHM exposed in beakers with 70mL media each. One set had 4 beakers (Control + 3 Doses, Table 1). Three sets of beakers represented three replicates. After a 24-hour exposure, FHM were transferred to a 24-well plate (Figure 1), and movement data was collected using the ViewPoint ZebraBox[6] behavior assay protocol outlined in Table 2, with photoperiodicity as a stimulus.
3. 'Omics Exposure Layout: Eight 5-dpf FHM larvae exposed in beakers with 80mL media each for 24-hours. Exposure concentrations are outlined in Table 1, showing the Control and 10 PXT treatments.
4. Sample Processing: RNA was isolated from samples using the MagMAX-96 Total RNA Isolation Kit.[7] Libraries were prepped using the CORALL mRNA-Seq Library Prep V1 kit with Poly-A Selection.[8] Samples were sequenced by RTSF Genomics Core at Michigan State.
5. Behavior Analysis: Movement data was plotted in 30-second intervals over the experimental period (excluding acclimation period). Movement was then aggregated for all light photoperiods and all dark photoperiods. An ANOVA and Dunnett's Test were performed to compare activity between doses and photoperiods.
6. 'omics Analysis: Number of differentially expressed genes (DEGs) will be calculated based on sequencing data. A QIAGEN Ingenuity Pathway Analysis (IPA)[9] will be performed to determine the pathways affected by PXT exposure.

Workflow	Concentration (mg/L)
Omics + Behavior	1.5
Omics	0.5
Omics + Behavior	0.15
Omics	0.05
Omics + Behavior	0.015
Omics	0.005
Omics	0.0015
Omics	0.0005
Omics	0.00015
Omics	0.00005
Omics + Behavior*	0

Table 1. Table showing the ten PXT concentrations used in the –omics workflow. Three concentrations, and a control, also were used for the behavioral assay. * denotes the control. All treatments used DMSO as a solvent at 1% v/v.

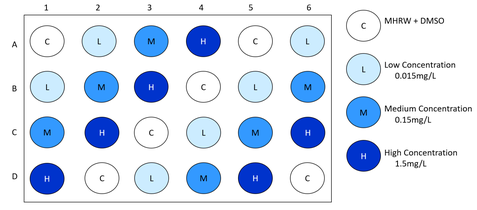

Figure 1. Plate layout for the behavioral assay, showing a diagonal format of the controls (C), low (L), medium (M), and high (H) concentrations of PXT. Each treatment per plate has 6 replicates. There are 3 identical plates from three sets of beakers. Each well had 2mL exposure media and one larval FHM. A diagonal layout was used to avoid positional bias.[10]

Photoperiod	Duration (sec)	Duration (min)	% Light
Acclimation	0-600	00:00-10:00	0%
Light 1	600-900	10:00-15:00	100%
Dark 1	900-1200	15:00-20:00	0%
Light 2	1200-1500	20:00-25:00	100%
Dark 2	1500-1800	25:00-30:00	0%
Light 3	1800-2100	30:00-35:00	100%
Dark 3	2100-2400	35:00-40:00	0%

Table 2. Table showing each phase of the behavioral assay, using light intensity as a stimulus. The acclimation period lasted ten minutes, followed by six 5-minute photoperiods, alternating between complete light (100% light) and complete darkness (0% light). Data was sampled every 30-seconds. The acclimation period data was excluded from analysis.

RESULTS

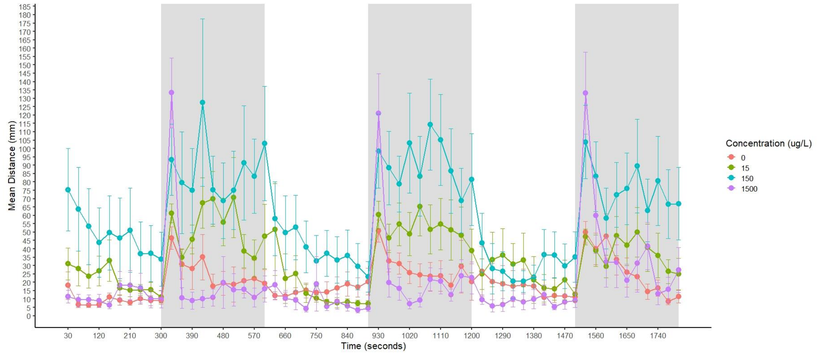

Figure 2. Plot showing mean distance over time for each treatment during six alternating photoperiods. Each data point is the mean cumulative distance for each 30-second interval. Each treatment has n=18, aggregated across 3 experimental plates. Error bars are standard error. Shaded regions are time periods where the light intensity was 0%. White regions are time periods where light was at 100%. Distances are only from activity >3mm/s.

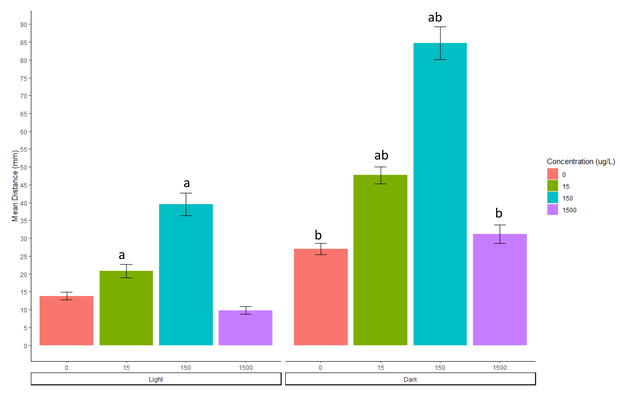

Figure 3. Bar graph showing mean distance (mm) aggregated by treatment and photoperiod. (a) indicates PXT doses differ significantly ($p<0.0001$) from the control within the same photoperiod. (b) indicates PXT doses and controls differed significantly ($p<0.0001$) from same treatment in opposing photoperiod. Error bars are standard error. Distances are from activity >3mm/s.

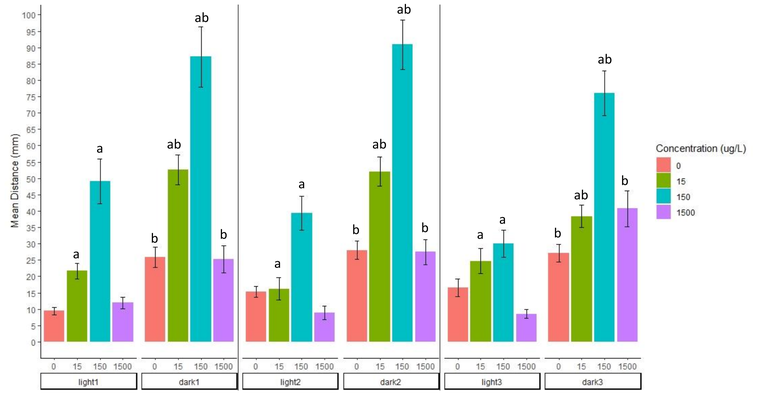

Figure 4. Bar graph showing mean distance (mm) aggregated by treatment but separated out into the six alternating photoperiods. (a) indicates PXT doses differ significantly ($p<0.0001$) from the control within the same photoperiod. (b) indicates PXT doses and controls differed significantly ($p<0.0001$) from same PXT treatment in opposing photoperiod within a set of light and dark (light1 vs dark1, etc.). Error bars are standard error. Distances are from activity >3mm/s.

IN PROGRESS

DEG counts are being calculated to determine which concentrations had greater effects on FHM larvae. In addition, an IPA analysis is being done to determine which biological systems are mostly impacted by PXT exposure.

DISCUSSION

1. Each PXT dose and the control showed a significant change in movement between the light and dark photoperiods ($p<0.0001$), which indicates that larvae are reacting to the light stimulus. PXT does not appear to affect the general behavioral response to a stimulus. Magnitude of response may still vary and is a point of consideration for further analysis.
2. The low (15 ug/L) and medium (150 ug/L) doses differ significantly in mean distance moved from the control (0 ug/L) in both the light and dark photoperiods. The high dose (1500 ug/L) does not show any differences from the control in either photoperiod. PXT is an anxiolytic pharmaceutical, however side effects include anxiety. Increased movement, which could possibly be associated with anxiogenic effects are seen in the low, and particularly the medium doses. This effect is not seen in the high dose and despite having a high concentration of PXT, there is no noticeable effect on behavior.
3. Based on Figure 2, the high dose (1500 ug/L) shows the largest response in magnitude when a stimulus is introduced. The mean movement is shown to be the lowest in the light photoperiods, and then mean movement shows the highest peak in the first sampling of the dark photoperiod. The response and sudden increase in movement will be a focus point for further analysis.
4. Based on Figure 4, the same patterns of behavior repeat in each of the three time points of stimulus introduction over the experimental period, indicating a consistent response to a stimulus. The medium (150ug/L) dose consistently shows the highest movement after the stimulus throughout the experimental protocol.

FUTURE WORK

1. Focus the behavioral analysis on the immediate stimulus response during the transition from light to dark, rather than total movement during extended periods of time.
2. Multiple weeks of behavioral data were previously collected with identical protocols. Future analyses will compare between weekly trials and determine if paroxetine has a consistent effect on FHM movement.
3. Upon completion of the –omics data analysis, we can compare behavioral and –omics results to see if there is a correlation between the two endpoints. We can also compare PXT results with results from other SSRIs to look for consistent effects within that chemical class.

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