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Cluster analysis profiling of behaviors in zebrafish larvae treated with antidepressants and pesticides

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Abstract

Antidepressants are used by a substantial number of women in their childbearing years. Treatment may continue during pregnancy, since untreated depression poses a risk to the mother and child. However, many antidepressants readily pass through the placental barrier to reach the fetus or may be ingested by the newborn via breastmilk. Little is known about the effects of antidepressants on brain development and subsequent behavior in young children. In the current study, we used zebrafish as a model system to examine the neurodevelopmental effects of three commonly prescribed antidepressants, sertraline, duloxetine and bupropion. Zebrafish were exposed to these antidepressants during development and were examined for changes in larval avoidance behavior, activity, social behaviors, and anxiety-related behaviors. The results show that antidepressants commonly affect larval swim speeds and resting, and differentially affect other behaviors depending upon the exposure period. Using cluster analysis profiling, we compared the obtained results to previous reports on behavioral defects induced by organophosphate pesticides. We found that the behavioral profiles induced by antidepressants and pesticides overlap, indicating a common mechanism of action. We conclude that developmental antidepressant exposures lead to specific behavioral changes in zebrafish larvae. At present, it is not known if antidepressants have similar effects in human development.

Keywords

antidepressants; cluster analysis; zebrafish; high-throughput assay; anxiety; avoidance; activity; development

1. Introduction

ADHD, autism spectrum disorders, and anxiety are becoming more prevalent in children and young adults (CDC, 2017a, b, c). There is still no clear cause for the development of these

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Conflicts of Interest

We declare that there are no conflicts of interest.

disorders. Pesticides are thought to be one factor contributing to the development of these disorders because they are readily absorbed into the body by inhalation, ingestion, and dermal contact. However, the medicines taken during pregnancy may also be a contributing factor in the development of these disorders.

Women that have been diagnosed with depression or anxiety disorders benefit from taking medications that alleviate or decrease depression and anxiety. Some women are advised by their doctors to continue on the medications while pregnant because the benefits of taking the medications during pregnancy outweigh the risks that could potentially be involved (Cogill et al., 1986; Philipps and O'Hara, 1991; Steer et al., 1992). The percentage of women in their childbearing years that take maintenance anxiolytics or anti-depressants is estimated to be 12–25% (CDC, 2011; Medco, 2011). These medications can readily cross through the placenta and breast milk to be taken up by the developing fetus and newborn (Boyce et al., 2011; Capello et al., 2011). There are several classes of anxiolytics and antidepressants classified dependent upon their mode of action. The most commonly used for long-term maintenance therapy are the selective serotonin reuptake inhibitors (SSRIs) and selective serotonin and norepinephrine reuptake inhibitors (SNRIs) (Alwan and Friedman, 2009; Alwan et al., 2011).

The three medications used in the present study are Sertraline (Zoloft), a commonly prescribed SSRI for anxiety and depression; duloxetine (Cymbalta), a commonly prescribed SNRI for anxiety, depression, and neuropathy and bupropion (Wellbutrin), an atypical antidepressant that is also prescribed for smoking cessation (GlaxoSmithKline, 2017; Lilly, 2009; Pfizer, 2002). The chosen drugs are categorized in pregnancy class C, indicating that there aren't enough adequate studies in humans to determine if there is a high enough risk during pregnancy to discontinue their use or if their benefits outweigh the risks.

Animal model systems are available to examine developmental effects of various chemicals, including anxiolytics, antidepressants and environmental toxicants. However, a detailed understanding of the risks and underlying mechanisms is hampered by the large number of chemicals that needs to be examined, the broad range of signaling molecules that may be affected and the complexity of neural targets in the developing brain.

Zebrafish is an excellent model system to address these challenges, because hundreds of embryos can be collected on a daily basis, the embryos can be easily exposed to pharmaceuticals in culture dishes during critical periods of early development, neural networks develop rapidly, and the automated analysis of larval behavior can reveal subtle defects in neural function that are easily missed by studying signaling pathways or brain morphology (Cachat et al., 2011; Champagne et al., 2010; Colwill and Creton, 2010; Colwill and Creton, 2011; Schmidt et al., 2013).

The current study assessed differences in larval behavior via cluster analysis using data previously published in our lab that utilized pesticides and new data from larval treatment with antidepressants. If the antidepressants and pesticides act on development via different mechanisms, one would expect one cluster with antidepressants and another cluster with pesticides. Instead, we found that the clustering profiles of some of the pesticide and

antidepressant concentrations overlap, pointing to a common mechanism, most likely neurotransmitter alterations during development.

2. Materials and Methods

2.1 Animal Care and Housing

Adult wild type zebrafish were obtained from Carolina Biological Supply and have been housed at Brown University over several generations in multiple 10 and 20 gallon tanks. For breeding purposes, the zebrafish were kept in mixed male and female populations on a 14 hour light/10 hour dark cycle. The fish were fed a combination of Gemma fish food, frozen brine shrimp, and frozen bloodworms. Embryos were collected from the tanks at the beginning of the light cycle (“dawn”) and were immediately transferred to deep Petri dishes (Fisher 08-752-11Z) containing ‘egg water’ (0.06 g of Instant Ocean and 0.25 mg of methylene blue per liter of deionized water). The embryos were then treated with various dilutions of bupropion hydrochloride (Sigma B102), sertraline (Sigma S6319), or duloxetine hydrochloride (Sigma SML0474) and grown in an incubator at 28.5 °C with a 14 hour light/10 hour dark cycle at a density of 60–70 embryos per culture dish.

2.2 Antidepressant and Pesticide Exposure

Larvae were exposed to egg water (EW), dimethyl sulfoxide (DMSO) and four concentrations of duloxetine, bupropion, or sertraline (from 0.01– 10 μ M) during 0–4 and 0–5 days post-fertilization (dpf) for behavioral assays. Pesticide exposure of zebrafish larvae to chlorpyrifos and malathion included both 1–4 and 1–5 dpf with concentrations between 0.001–1 μ M (Richendrfer and Creton, 2015). We selected a broad concentration range for the antidepressants, since it can be challenging to directly compare developmental exposures in humans and various animal model systems. Moreover, it is possible that embryonic development is affected by low doses of pharmaceuticals that are well below medically-relevant concentrations in fully grown organisms. We thus included concentrations that are similar to the doses used in animal model systems and doses prescribed for human use, as well as lower concentrations. For a comparison of concentrations in other species we refer to (Fraher et al., 2016; GlaxoSmithKline, 2017; Lilly, 2009; Lu et al., 2008; Lu et al., 2006; Pfizer, 2002; Vermoesen et al., 2011). On days larvae were not treated, all groups were housed in egg water. All behavioral analyses were performed at 5 dpf. Therefore, some experimental groups were in the antidepressant and pesticide treatment during behavioral testing depending upon the time point of exposure. All antidepressants and pesticides were dissolved in dimethyl sulfoxide (DMSO) as 1000 $\times$ stocks that were stored at –20 °C. The control groups were housed in egg water containing 0.1% DMSO. To obtain the desired final concentrations, 50 μ L of each 1000 $\times$ stock was dissolved into 50 ml of egg water in respective culture dishes. Dead embryos were removed daily from culture dishes and all solutions were replaced daily until 5 dpf, when behavioral analyses were conducted.

2.3 Behavioral Analysis

The behavioral assays were carried out in 5 lane plates, which were made by placing a specialized mold into liquid agarose and then letting the mold cool. Five larvae were placed in each lane in 5 lane plates; the solution that the larvae were housed in was used to fill up

the agarose lanes. The plates were positioned on top of a laptop screen; four plates fit onto one laptop screen. Lane assignment was not randomized, but consistency of imaging conditions was confirmed before each experiment by checking tilt, screen brightness and zoom. The assay utilized a PowerPoint presentation shown to the larvae for 30 minutes (Richendrfer and Creton, 2013). The results of the behavioral assays were used to analyze thigmotaxis, avoidance behavior, rest, and swim speed.

For the first half of the behavioral assay there were no visual stimuli. In the second half of the assay, a red moving bar was shown to the larvae which is a visually aversive stimulus and causes the larvae to swim away to the “horizontal” bottom half of the lane. Time lapse imaging was used to capture larval placement within the wells. Images were imported into Image J and a macro developed in our laboratory was used to analyze x,y coordinates of the larvae within the lanes (Richendrfer and Creton, 2013). Results were then imported into Excel to calculate the placement of larvae within the lane in addition to swim speed and rest. Based on previous reports in our lab, avoidance behavior can be measured by determining “percent down” in the lane (percentage of fish down in the lane) and thigmotaxis behavior can be measured by determining “percent on the side, end, and edge” of the lane (percentage of fish on the side, end and edge of the lane) (Richendrfer, H. et al., 2012; Richendrfer, H.A. et al., 2012). It should be noted that the aversive red bar stimulus evokes avoidance behavior in the larvae which is determined by comparing larval locations with and without visual stimuli (Pelkowski et al., 2011; Richendrfer, H. et al., 2012). In a 15 minute period, the imaging system collects 750 ‘larval measurements’ per lane (150 images $\times$ 5 larvae per lane). The values of these larval measurements are first averaged per lane and the per-lane values are then averaged over multiple lanes. Behavioral assays for each exposure group were run in triplicates using 10 lanes per experiment ($n = 30$ lanes). Thus, for each experimental group, the measurements were based on a total of 150 larvae (30 lanes $\times$ 5 larvae per lane) and a total of 22,500 larval measurements (30 lanes $\times$ 750 larval measurements per lane). Larvae that are together in a lane can influence each other’s behavior. To maintain independence of the measurements, all values were compared on a per-lane basis ($n = 30$ lanes in the statistical analysis). For a detailed methodology for the behavioral assay setup see our previous report (Richendrfer and Creton, 2013).

2.4 Statistical Analysis

Differences in the effects between duration of treatment, pesticide and concentrations were analyzed using the general linear multivariate analysis in SPSS 22 with Tukey post-hoc analysis. Subsequently, differences in behavior before and during the visual stimuli and between groups were tested for significance for each behavioral variable and pesticide concentration using a one way ANOVA in SPSS 22 to determine the effects of treatment during each specific exposure period. If the treatments showed significance ($p < 0.05$), Tukey’s multiple comparison test was used to determine any significant differences between and within treatment groups compared to the DMSO control groups (before visual stimulus and during visual stimulus). The asterisks in the graphs indicate a significant difference between the pesticide exposures and the DMSO controls from the Tukey posthoc analysis; $* = < 0.05$ and $** = < 0.001$. With limited prior knowledge on the effective concentrations, we examined four concentrations per treatment group over a 1000-fold range. The current

studies are too broad for detailed dose-response analyses. However, such analyses would be feasible in future studies, after an effective dose has been identified.

2.5 Cluster analysis

All behaviors from the five treatments were assigned a number between 0–2 based upon their statistical p values. Any behaviors that were statistically lower than the DMSO control were given a 0 or 0.5 (0 for $p<0.01$ or 0.5 for $p<0.05$). Any behaviors that were statistically higher than the DMSO control were given a 1.5 or 2 (1.5 for $p<0.05$ or 2 for $p<0.01$). Behaviors were assigned the number one if they were not statistically different from the DMSO control.

2.6 Cluster Tree View

The numbers assigned to the varying behaviors were imported into the cluster Tree View analysis program to determine relationship between the array of pesticide and antidepressant concentrations and their impact on behaviors. Colors were assigned to the numbers in order to illustrate clustering of behaviors. Green clusters are numbers from 0 to <1 , black is 1, and red is >1 to 2. Data from malathion and chlorpyrifos treated larvae (Richendrfer and Creton, 2015) were compared to behaviors in larvae treated with duloxetine, sertraline, and bupropion.

3. Results

3.1 Antidepressant behavioral results – treatment from 0–5 dpf

There were no increased larval deaths or morphological abnormalities in fish treated with antidepressants for any concentration used.

3.1.1 Sertraline behavioral results – without stimulus—Sertraline at a concentration of 1 μM significantly increased the larval preference for the end of the lane compared to the DMSO control (Figure 1A) ($p<0.05$). Swim speeds were significantly decreased in larvae treated with both 1 and 10 μM sertraline compared to the DMSO control ($p<0.001$ for both) (Figure 1B). Similarly, rest was increased in larvae treated with both 1 and 10 μM sertraline compared to the DMSO control ($p<0.001$) (Figure 1C). All other behavioral parameters were not significantly different compared to the DMSO control group.

3.1.2 Sertraline behavioral results – with stimulus—The percentage of larvae down in the lane were significantly decreased with 1 and 10 μM Sertraline ($p=0.017$ and $p<0.001$) (Figure 1D). Swim speeds were significantly decreased in larvae treated with both 1 and 10 μM Sertraline compared to the DMSO control ($p<0.001$ for both) (Figure 1B). Similarly, rest was increased in larvae treated with both 1 and 10 μM Sertraline compared to the DMSO control ($p<0.001$) (Figure 1C). All other behavioral parameters were not significantly different compared to the DMSO control group.

3.1.3 Duloxetine behavioral results – without stimulus—The percentage of larvae on the end of the lane were significantly increased with 1 and 10 μM Duloxetine compared

to the DMSO control ($p<0.001$ and $p=0.011$) (Figure 2A). The percentage of larvae on the side of the lane were significantly increased with 1.0 and 10 μM Duloxetine compared to the DMSO control ($p=0.005$ and $p<0.001$) (Figure 2B). The percentage of larvae on the edge of the lane was significantly higher with 1 and 10 μM Duloxetine ($p<0.01$ for both) (Figure 2C). Swim speed was significantly decreased with 0.1, 1, 10 μM Duloxetine compared to DMSO control ($p<0.001$ for all) (Figure 2D). Percent rest was significantly increased in larvae treated with 0.1, 1, and 10 μM Duloxetine ($p<0.001$ for all) (Figure 2E). The percentage of larvae that remained together during the experiment was significantly increased with 1 μM duloxetine ($p = 0.018$) (Figure 2F). All other behavioral parameters were not significantly different compared to the DMSO control group.

3.1.4 Duloxetine behavioral results – with stimulus—The percentage of larvae down in the lane was significantly lower with 1 and 10 μM Duloxetine ($p=0.015$ and 0.011) (Figure 2G). The percentage of larvae on the end of the lane was significantly higher with treatment of 1 μM duloxetine compared to the DMSO control ($p=0.003$) (Figure 2A). The percentage of larvae on the edge of the lane was higher with 1 μM duloxetine compared to DMSO ($p<0.01$) (Figure 2C). Swim speed was significantly decreased with 0.1, 1, 10 μM Duloxetine compared to DMSO control ($p=0.025$ for 0.1 μM , $p<0.001$ for 1 and 10 μM) (Figure 2D). Percent rest was significantly increased in larvae treated with 0.1, 1, and 10 μM Duloxetine ($p=0.013$ for 0.1 μM , $p<0.001$ for 1 and 10 μM) (Figure 2E). The percentage of larvae that remained together during the experiment was significantly increased with 1 μM duloxetine ($p < 0.001$) (Figure 2F). All other behavioral parameters were not significantly different compared to the DMSO control group.

3.1.5 Bupropion behavioral results – without stimulus—Percentage of larvae on the edge of the lane was significantly decreased with treatment of 1 μM bupropion compared to DMSO control ($p=0.008$) (Figure 3A). Swim speed was significantly increased in larvae treated with 0.1 μM bupropion ($p<0.001$) (Figure 3B). Rest was significantly decreased in larvae treated with 0.1 and 1 μM bupropion ($p<0.001$ and $p=0.043$) (Figure 3C). All other behavioral parameters were not significantly different compared to the DMSO control group.

3.1.6 Bupropion behavioral results – with stimulus—Swim speed was significantly increased in larvae treated with 0.1 μM bupropion ($p=0.047$) (Figure 3B). All other behavioral parameters were not significantly different compared to the DMSO control group.

3.2 Antidepressant behavioral results – treatment from 0–4 dpf

There were no increased larval deaths or morphological abnormalities in fish treated with antidepressants for any concentration used.

3.2.1 Sertraline behavioral results – without stimulus—Swim speed was significantly decreased in larvae treated with 1 and 10 μM sertraline compared to DMSO controls ($p<0.001$) (Figure 4A). Percent rest was significantly increased in larvae treated with 1 and 10 μM sertraline compared to DMSO controls ($p=0.03$ and $p<0.001$) (Figure 4B).

All other behavioral parameters were not significantly different compared to the DMSO control group.

3.2.2 Sertraline behavioral results – with stimulus—Percentage of larvae on the side of the lane was significantly increased in larvae treated with 1 μ M sertraline compared to DMSO controls ($p=0.042$) (Figure 4C). Swim speed of larvae was significantly decreased in larvae treated with 1 and 10 μ M sertraline compared to DMSO controls ($p=0.01$ and $p<0.001$) (Figure 4A). Percent rest was significantly increased in larvae treated with 1 and 10 μ M sertraline compared to DMSO controls ($p=0.03$ and $p<0.001$) (Figure 4B). All other behavioral parameters were not significantly different compared to the DMSO control group.

3.2.3 Duloxetine behavioral results – without stimulus—Percentage of larvae on the end of the lane was significantly decreased when treated with 1 μ M duloxetine when compared to DMSO controls ($p=0.022$) (Figure 5A). Swim speed was significantly increased in larvae treated with 0.1 μ M duloxetine ($p=0.036$) but was significantly decreased in larvae treated with 10 μ M duloxetine compared to DMSO controls ($p<0.001$) (Figure 5B). Rest was significantly increased in larvae treated with 1 and 10 μ M duloxetine ($p=0.002$ and $p<0.001$) when compared to DMSO controls (Figure 5C). Percent of fish together was increased when larvae were treated with 10 μ M duloxetine ($p<0.001$) (Figure 5D). All other behavioral parameters were not significantly different compared to the DMSO control group.

3.2.4 Duloxetine behavioral results – with stimulus—Percent of larvae down in the lane was significantly less in larvae treated with 10 μ M duloxetine when compared to DMSO controls ($p<0.001$) (Figure 5B). Swim speed was significantly decreased in larvae treated with 10 μ M duloxetine compared to DMSO controls ($p<0.001$) (Figure 5C). Rest was significantly increased in larvae treated with 1 and 10 μ M duloxetine ($p=0.014$ and $p<0.001$) when compared to DMSO controls (Figure 5E). All other behavioral parameters were not significantly different compared to the DMSO control group.

3.2.5 Bupropion behavioral results – without stimulus—All behavioral parameters were not significantly different compared to the DMSO control group (Figure 6).

3.2.6 Bupropion behavioral results – with stimulus—Percentage of larvae on the edge of the lane was significantly increased in larvae treated with 0.01 μ M when compared to DMSO controls ($p=0.016$) (Figure 6A). Percentage of larvae on the side of the lane was significantly increased in larvae treated with 0.01 μ M when compared to DMSO controls ($p=0.028$) (Figure 6B). All other behavioral parameters were not significantly different compared to the DMSO control group.

3.3 Cluster analysis comparison results

3.3.1 Pesticide behavioral results—Detailed behavioral results from pesticide treatments can be found in our previous publication (Richendrfer and Creton, 2015). Briefly, the results of our previous study indicated that chlorpyrifos and malathion cause opposite

effects on certain behaviors in zebrafish larvae such as swim speed (hypoactivity vs. hyperactivity) and rest. Additionally, the pesticides affected only certain behaviors, such as thigmotaxis, during specific time points in development that were found to be unrelated to changes in acetylcholinesterase (AChE) activity. Larvae that were treated with malathion but not chlorpyrifos also had significantly smaller forebrain and hindbrain regions compared to controls by 5 days post-fertilization.

3.3.2 Behavioral profiling from larvae treated 0–5 dpf—Cluster analysis was used in order to compare the behavioral profiles of both pesticide and antidepressant treatment in larvae. In larvae treated with these compounds from 0–5 dpf, there were several groupings of similar behavioral outcomes (Figure 7). The results indicate that there are two main clusters with the first cluster including duloxetine 0.1, 1 and 10 μ M, sertraline 1 and 10 μ M, and 0.1 μ M chlorpyrifos. The second main cluster included bupropion 0.1, 1, and 10 μ M, malathion 0.001, 0.01, 0.1, and 1 μ M, chlorpyrifos 0.001 and 0.01 μ M, sertraline 0.01 and 0.1 μ M, and bupropion 0.01 and 10 μ M. Both main clusters had nested pairings of various treatments. Duloxetine 1 and 10 μ M had high similarity between each other in their behavioral profiles, which were similar in comparison to two other nested pairings, sertraline 1 and 10 μ M and duloxetine 0.1 and chlorpyrifos 0.1 μ M. There were also nested pairings within the second main cluster. Bupropion 0.1 and malathion 0.1 and 1 μ M showed high similarity in their behavioral profiles. The other treatments listed on the Tree View had mostly no change in behaviors in comparison to DMSO control groups.

3.3.3 Behavioral profiling from larvae treated 0–4 dpf—In larvae treated with pesticides or antidepressants from 0–4 dpf, there were also several clusters of behavioral profiles that were found (Figure 8). The groups with the most differences in comparison to control groups that were similar with each other included duloxetine 10 μ M, chlorpyrifos 0.1 μ M, and sertraline 1 and 10 μ M. Sertraline 0.1 μ M and bupropion 0.01 μ M were also grouped similarly. All other groups either were not changed significantly compared to DMSO control groups or were not similar in behavior profiles compared to other groups.

4. Discussion

The behavioral measures of zebrafish recorded in this study can be used to assess avoidance, activity, and anxiety-related behaviors (Richendrfer and Creton, 2013; Richendrfer, H. et al., 2012). Our cluster profiling results indicate that early developmental exposure to environmental compounds including pesticides and drugs including antidepressants had significant impacts on behavior. It must be noted that while many of the treatment groups in the current study had decreases in swim speeds, the larvae were physically identical to the control groups which allows us to postulate that the increase in rest and decrease in swim speeds were from changes in brain function and not from physical abnormalities. Additionally, cluster analysis results showed that both pesticide and antidepressant exposure lead to similar behavioral profiles indicating that they may have similar modes of action. Moreover, exposure period was also a significant factor that affected behavioral results.

4.1 Sertraline behavioral assay

In larvae treated 0–5 dpf with sertraline, results show that there was a slight preference for the end of the lane without visual stimulus in the 1 μ M treatment group, which can be indicative of increased anxiety in zebrafish (Richendrfer and Creton, 2013; Richendrfer, H. et al., 2012; Richendrfer, H.A. et al., 2012). As it was not elicited by the visual stimulus, the result indicates a generalized anxiety in the zebrafish. Sertraline also significantly decreased swim speed and increased resting behavior regardless of presence or absence of a visual stimulus in the two largest concentrations, indicating that early treatment of sertraline affects activity in zebrafish larvae, similar to reports in rats with chronic use of sertraline (Mukherjee et al., 2015). Moreover, avoidance behavior, as indicated by percent of larvae down in the lane, was decreased in the two highest concentrations when the visual stimulus was present, similar to results in human subjects with social anxiety disorder that had decreased avoidance behavior after taking sertraline (Connor et al., 2006). Similar results were found in larvae treated with sertraline from 0–4 dpf but were less pronounced. Swim speed and rest were affected like in the 0–5 dpf treatment group. However, the only other behavior that was affected was increased larval preference for the side of the lane with a visual stimulus in the 1 μ M treatment group, indicating that anxiety related behavior in the zebrafish was only influenced by a stimulus during this shorter treatment period. Differences between the 0–4 and 0–5 dpf exposure groups may be explained by the total length of the exposure, specific developmental processes affected during the fifth day of development, or acute effects of the antidepressants during the behavioral assay. While we cannot exclude any of these options at this time, we can conclude that the exposure period (0–4 or 0–5 dpf) differentially affects behavior and that behavioral changes observed in both exposure groups are likely caused by developmental defects induced during the first 4 days of development. Overall, the sertraline experiments indicate that the exposure period differentially affects specific behaviors in zebrafish larvae in addition to the observed differences in behavior between treatment groups and DMSO control.

4.2 Duloxetine behavioral assay

Duloxetine treated larvae had a large variety of behavioral differences in comparison to the control group in which the number of differences were increased with a longer exposure time. In larvae treated from 0–5 dpf, there were increased preferences for larvae to be on the edge, side, and end of the lane with higher concentrations (edge and end preference were both affected with and without visual stimulus), all indicating increased anxiety related behavior in zebrafish from early duloxetine treatment. Similar to sertraline, swim speeds were significantly decreased and rest was significantly increased in the three highest treatment groups both with and without the visual stimulus showing that early duloxetine treatment also affects zebrafish activity levels. The percent of larvae close together increased with the 1 μ M treatment group, which could indicate a social preference or it could be related to the fact that larvae in the same treatment group had the lowest swim speeds and did not move around as much as the other groups. The percentage of larvae down in the dish decreased in the highest two treatment groups with a visual stimulus, indicating that the larvae had a decrease in avoidance behavior. However, this result may also be a residual behavior due to the fact that larvae in the two highest concentration groups had lower swim speeds and thus moved around less.

Treatment of larvae with duloxetine from 0–4 dpf had several interesting results that were different from larvae treated from 0–5 dpf. Larvae treated with 1 μ M duloxetine actually showed a decreased preference for the end of the lane, opposite of what was found with 0–5 dpf treatment, indicating that 0–4 dpf exposures to the drug can lower anxiety related behaviors whereas 0–5 dpf exposures increase anxiety related behaviors. Also interesting is the fact that zebrafish had higher swim speeds with 0.1 μ M treatment but significantly lower swim speeds in 10 μ M treatment with the 1 μ M treatment not different from control. These results indicate that 0–4 dpf treatments allow for opposite levels of activity in comparison to 0–5dpf exposures depending upon the concentration of the drug. Duloxetine has been shown to increase the activity of motor neurons in the spinal cord, which could explain the higher swim speeds with certain concentrations (Redaelli et al., 2015; Schuessler, 2006). The two highest concentrations of duloxetine affected rest, as they significantly increased it compared to controls, similar to the results found in the 0–5dpf group. Similar to the results found here with increased rest and decreased swim speeds, a decrease of hyperactivity in encounter-induced mice has been found with treatment of duloxetine, which is thought to be modulated from 5-HT₄ and α ₂ receptors, indicating a potential mechanism of activity induced changes after duloxetine exposure in zebrafish (Hasebe et al., 2015). The percentage of fish together was significantly increased in the highest concentration group, differing from the results found with longer duloxetine exposure which increased it in the 1 μ M group. Also, like stated above, this could be the result of an increased social preference, increased preference to be on the edges/ends of the lane, or due to a lack of activity in those specific treatment groups. In humans, duloxetine treatment did not have an effect on the social adjustment scale in comparison to the placebo (Hellerstein et al., 2012). Lastly, the percentage of fish down in the lane was significantly decreased in the highest treatment group with the visual stimulus only, indicating that a stimulus is needed to cause a decrease in avoidance behavior in that treatment group. Because percent of fish down in the lane without a visual stimulus was the same across treatment groups, these results signify that the measurement for differences in avoidance behavior was in fact caused by the visual stimulus.

4.3 Bupropion behavioral assay

Treatment of larvae with bupropion elicited fewer changes in behavioral parameters than sertraline and duloxetine did when compared to DMSO controls. Treatment of larvae with 1 μ M bupropion from 0–5 dpf decreased the percentage of larvae on the edge of the lane, signifying a possible decrease in anxiety related behavior, similar to what was found in mice subjected to the elevated plus maze (Carrasco et al., 2004). Additionally, bupropion increased swim speeds with 0.1 μ M and decreased rest with 0.1 and 1 μ M concentrations. These results indicate that treatment with bupropion has overall opposite effects compared to the other two antidepressants used in this study indicating that development exposure to bupropion increases activity and decreases anxiety. Bupropion efficacy has been examined in children with ADHD and has been found to alleviate the symptoms of ADHD differentially depending upon treatment and evaluation, indicating a potential cause for altered activity of zebrafish found in the current study (Ng, 2016). In mice, bupropion was found to increase locomotion in animals pre-exposed to the tail suspension test and to mice subjected to the elevated plus maze, also in line with the results of the current study (Carrasco et al., 2004; Kale et al., 2013).

Treatment of larvae from 0–4 dpf with bupropion did not affect activity but did affect anxiety related behaviors by increasing the larval preference for the edge and side of the lane in the lowest concentration treatment group. However, these results only occurred with the presence of a visual stimulus and indicate overall anxiety behaviors were not affected unless a stimulus was given. In mice treated with bupropion, increased anxiety was found using the hole-board test, suggesting that depending on exposure period and concentration, anxiety can be differentially affected by this drug (Redolat et al., 2005).

4.4 Cluster analysis comparison of pesticide vs. antidepressant behavioral responses

There were several similarities between behaviors in larvae treated with pesticides and antidepressants, especially between sertraline, duloxetine and chlorpyrifos. We previously reported that several behavioral abnormalities caused by pesticide exposure are not likely linked to changes in AChE levels (Richendrfer and Creton, 2015). Because of the similarity of behavioral profiles between larvae treated with antidepressants and those treated with pesticides are highly similar, it is probable that similar mechanisms were affected in both cases. Some studies suggest a link between maternal antidepressant use and autism spectrum disorders in the subject's children (Croen et al., 2011; Harrington et al., 2013, 2014; Torjesen, 2014); others suggest a link to attention deficit hyperactivity disorder (Clements et al., 2014) all of which have underlying correlation to abnormalities in neurotransmitters such as serotonin, noradrenaline and dopamine, the same neurotransmitters that are affected by the antidepressants used in the current study (Harrington et al., 2013; Muhle et al., 2004; Oades et al., 2008; Sharp et al., 2009; Smit-Rigter et al., 2010). Zebrafish develop dopaminergic, noradrenergic and serotonergic neurons in addition to BDNF very early in development (by 1–2 dpf) (Brustein et al., 2003; Cacialli et al., 2016; De Felice et al., 2014; McLean and Fetcho, 2004). In minnows exposed to either sertraline or chlorpyrifos, serotonin transporter binding was reduced by 50%, indicating a common mechanisms between these two agents (Gould et al., 2007). In adult rats, chlorpyrifos exposure decreased dopamine levels in the brain (Savy et al., 2015). Additionally, norepinephrine levels were differentially affected by chlorpyrifos depending upon time of developmental exposure in rats (Seidler and Slotkin, 2011). Together, the molecular targets of antidepressants and the behavioral defects observed in the current study suggest that neurotransmitter levels during development are important for neural development and subsequent behavior. At this point, the location of targeted neurons is unknown. For example, changes in visually-guided behaviors may be caused by neural defects at various locations in the visual system, including the retinal ganglion cells or optic tectum in the brain, or may be caused by areas in the brain that regulate anxiety and stress responses. Neurons in zebrafish embryos are accessible to various imaging techniques and it may be possible to identify the exact neural targets in future research.

There is also the potential that neurotrophins were altered in the zebrafish embryos after exposure to antidepressants and pesticides. BDNF is known to have a regulatory role in the presentation of major depressive disorder. In rats, chronic exposure to chlorpyrifos caused differential gene expression (of neuropeptides and neurotrophins including BDNF) in the hippocampus, indicating another common mechanism of action between pesticides and antidepressants. (Lee et al., 2016). In line with this argument, sertraline has been found in

increase concentrations of peripheral BDNF in humans (Zhou et al., 2017) and duloxetine has been found to increase concentrations of BDNF in the medial prefrontal cortex (Xu et al., 2016). As seen in our results, the effects of bupropion were not similar to those found with high concentrations of chlorpyrifos treatment. Along these lines, it was also found that bupropion does not have an effect on BDNF levels in mice with chronic treatment (Mlyniec and Nowak, 2015). Also along these lines, malathion decreased BDNF levels in the rat hippocampus (Dorri et al., 2015). All of these studies point to a role of BDNF in the differential behavioral parameters seen in early zebrafish exposure.

Taken together, it seems as though the antidepressants used in the current study and the pesticides used in our previous studies exert their effects through BDNF, serotonin, dopamine and noradrenaline alterations. We propose that these alterations are what caused the behavioral differences found during early zebrafish embryonic treatment.

5. Conclusions

The current study shows that developmental exposures to antidepressants lead to changes in zebrafish larval behavior. It is not known if antidepressants induce similar effects in human development.

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References

- Alwan S, Friedman J. Safety of selective serotonin reuptake inhibitors in pregnancy. *CNS Drugs*. 2009; 23(6):493–509. [PubMed: 19480468]
- Alwan S, Reefhuis J, Rasmussen S, Friedman J. Patterns of antidepressant medication use among pregnant women in a United States population. *J Clin Pharmacol*. 2011; 51(2):264–270. [PubMed: 20663997]
- Boyce PM, Hackett LP, Ilett KF. Duloxetine transfer across the placenta during pregnancy and into milk during lactation. *Arch Womens Ment Health*. 2011; 14(2):169–172. [PubMed: 21359876]
- Brustein E, Chong M, Holmqvist B, Drapeau P. Serotonin patterns locomotor network activity in the developing zebrafish by modulating quiescent periods. *J Neurobiol*. 2003; 57(3):303–322. [PubMed: 14608665]
- Cachat J, Stewart A, Utterback E, Hart P, Gaikwad S, Wong K, Kyzar E, Wu N, Kalueff AV. Three-Dimensional Neurophenotyping of Adult Zebrafish Behavior. *PLoS ONE*. 2011; 6(3):e17597. [PubMed: 21408171]
- Cacialli P, Gueguen MM, Coumailleau P, D'Angelo L, Kah O, Lucini C, Pellegrini E. BDNF Expression in Larval and Adult Zebrafish Brain: Distribution and Cell Identification. *PLoS One*. 2016; 11(6):e0158057. [PubMed: 27336917]
- Capello C, Bourke C, Ritchie J, Stowe Z, Newport D, Nemeroff A, Owens M. Serotonin transporter occupancy in rats exposed to serotonin reuptake inhibitors in utero or via breast milk. *J Pharmacol Exp Ther*. 2011; 339(1):275–285. [PubMed: 21775476]
- Carrasco MC, Vicens P, Vidal J, Redolat R. Effects of acute administration of bupropion on behavior in the elevated plus-maze test by NMRI mice. *Prog Neuropsychopharmacol Biol Psychiatry*. 2004; 28(7):1135–1141. [PubMed: 15610926]
- CDC. Antidepressant Use in Persons Aged 12 and Over: United States, 2005–2008. 2011. <http://www.cdc.gov/nchs/data/databriefs/db76.htm>

- CDC. Attention-Deficit / Hyperactivity Disorder (ADHD). 2017a. <https://www.cdc.gov/ncbddd/adhd/data.html>
- CDC. Autism Spectrum Disorder (ASD). 2017b
- CDC. Mental Health. 2017c. https://www.cdc.gov/mentalhealth/data_stats/surveillance.htm
- Champagne DL, Hoefnagels CCM, de Kloet RE, Richardson MK. Translating rodent behavioral repertoire to zebrafish (*Danio rerio*): Relevance for stress research. *Behav Brain Res*. 2010; 214(2): 332–342. [PubMed: 20540966]
- Clements C, Castro V, Blumenthal S, Rosenfield H, Murphy S, Fava M, Erb J, Churchill S, Kaimal A, Doyle A, Robinson E, Smoller J, Kohane I, Perlis R. Prenatal antidepressant exposure is associated with risk for attention-deficit hyperactivity disorder but not autism spectrum disorder in a large health system. *Mol Psychiatry*. 2014 Epub ahead of print.
- Cogill S, Caplan H, Alexandra H, Robson K, Kumar R. Impact of maternal postnatal depression on cognitive development of young children. *Br Med J (Clin Res Ed)*. 1986; 292(6529):1165–1167.
- Colwill RM, Creton R. Automated Imaging of Avoidance Behavior in Larval Zebrafish. In: Kalueff AV, Cachat JM, editors *Neuromethods*. Springer Science+Media, LLC 2011; New York: 2010.
- Colwill RM, Creton R. Locomotor behaviors in zebrafish (*Danio rerio*) larvae. *Behav Processes*. 2011; 86(2):222–229. (NIHMS264983). [PubMed: 21147203]
- Connor KM, Davidson JRT, Chung H, Yang R, Clary CM. Multidimensional effects of sertraline in social anxiety disorder. *Depression and Anxiety*. 2006; 23(1):6–10. [PubMed: 16216019]
- Croen L, Grether J, Yoshida C, Odouli R, Hendrick V. Antidepressant use during pregnancy and childhood autism spectrum disorders. *Arch Gen Psychiatry*. 2011; 68(11):1104–1112. [PubMed: 21727247]
- De Felice E, Porreca I, Alleva E, De Girolamo P, Ambrosino C, Ciriaco E, Germana A, Sordino P. Localization of BDNF expression in the developing brain of zebrafish. *J Anat*. 2014; 224(5):564–574. [PubMed: 24588510]
- Dorri SA, Hosseinzadeh H, Abnous K, Hasani FV, Robati RY, Razavi BM. Involvement of brain-derived neurotrophic factor (BDNF) on malathion induced depressive-like behavior in subacute exposure and protective effects of crocin. *Iran J Basic Med Sci*. 2015; 18(10):958–966. [PubMed: 26730329]
- Fraher D, Hodge JM, Collier FM, McMillan JS, Kennedy RL, Ellis M, Nicholson GC, Walder K, Dodd S, Berk M, Pasco JA, Williams LJ, Gibert Y. Citalopram and sertraline exposure compromises embryonic bone development. *Mol Psychiatry*. 2016; 21(5):656–664. [PubMed: 26347317]
- GlaxoSmithKline. WELLBUTRIN® (bupropion hydrochloride) Tablets. 2017. https://www.gsksource.com/pharma/content/dam/GlaxoSmithKline/US/en/Prescribing_Information/Wellbutrin_Tablets/pdf/WELLBUTRIN-TABLETS-PI-MG.PDF
- Gould GG, Brooks BW, Frazer A. [(3)H] citalopram binding to serotonin transporter sites in minnow brains. *Basic Clin Pharmacol Toxicol*. 2007; 101(3):203–210. [PubMed: 17697042]
- Harrington R, Lee L, Crum R, Zimmerman A, Hertz-Picciotto I. Serotonin hypothesis of autism: implications for selective serotonin reuptake inhibitor use during pregnancy. *Autism Res*. 2013; 6(3):149–168. [PubMed: 23495208]
- Harrington R, Lee L, Crum R, Zimmerman A, Hertz-Picciotto I. Prenatal SSRI use and offspring with autism spectrum disorder or developmental delay. *Pediatrics*. 2014 epub ahead of print.
- Hasebe S, Ago Y, Nishiyama S, Oka S, Hashimoto H, Takuma K, Matsuda T. Pharmacological profile of encounter-induced hyperactivity in isolation-reared mice. *Behav Pharmacol*. 2015; 26(7 Spec No):681–690. [PubMed: 25932719]
- Hellerstein DJ, Stewart JW, McGrath PJ, Deliyannides DA, Batchelder ST, Black SR, Withers A, O'Shea D, Chen Y. A randomized controlled trial of duloxetine versus placebo in the treatment of nonmajor chronic depression. *J Clin Psychiatry*. 2012; 73(7):984–991. [PubMed: 22901348]
- Kale PP, Addepalli V, Ghadawale SR. Impact of pre-exposure of tail suspension on behavioural parameters like locomotion, exploration, and anxiety in mice. *Indian J Exp Biol*. 2013; 51(9):732–738. [PubMed: 24377133]

- Lee YS, Lewis JA, Ippolito DL, Hussainzada N, Lein PJ, Jackson DA, Stallings JD. Repeated exposure to neurotoxic levels of chlorpyrifos alters hippocampal expression of neurotrophins and neuropeptides. *Toxicology*. 2016; 340:53–62. [PubMed: 26775027]
- Lilly E. Cymbalta (Duloxetine hydrochloride). 2009. http://www.accessdata.fda.gov/drugsatfda_docs/label/2014/021427Orig1s0451bl.pdf
- Lu C, Barr DB, Pearson MA, Waller LA. Dietary intake and its contribution to longitudinal organophosphorus pesticide exposure in urban/suburban children. *Environ Health Perspect*. 2008; 116(4):537–542. [PubMed: 18414640]
- Lu C, Toepel K, Irish R, Fenske RA, Barr DB, Bravo R. Organic Diets Significantly Lower Children's Dietary Exposure to Organophosphorus Pesticides. *Environ Health Perspect*. 2006; 114(2)
- McLean DL, Fetcho JR. Ontogeny and innervation patterns of dopaminergic, noradrenergic, and serotonergic neurons in larval zebrafish. *J Comp Neurol*. 2004; 480(1):38–56. [PubMed: 15515022]
- Medco. America's State of Mind. 2011. <http://apps.who.int/medicinedocs/documents/s19032en/s19032en.pdf>
- Mlyniec K, Nowak G. Up-regulation of the GPR39 Zn²⁺-sensing receptor and CREB/BDNF/TrkB pathway after chronic but not acute antidepressant treatment in the frontal cortex of zinc-deficient mice. *Pharmacol Rep*. 2015; 67(6):1135–1140. [PubMed: 26481532]
- Muhle R, Trentacoste SV, Rapin I. The genetics of autism. *Pediatrics*. 2004; 113(5):e472–486. [PubMed: 15121991]
- Mukherjee S, Sen S, Biswas A, Barman TK, Tripathi SK. Impact on behavioral changes due to chronic use of sertraline in Wistar albino rats. *Indian Journal of Pharmacology*. 2015; 47(6):657–662. [PubMed: 26729959]
- Ng QX. A Systematic Review of the Use of Bupropion for Attention-Deficit/Hyperactivity Disorder in Children and Adolescents. *J Child Adolesc Psychopharmacol*. 2016
- Oades RD, Lasky-Su J, Christiansen H, Faraone SV, Sonuga-Barke EJ, Banaschewski T, Chen W, Anney RJ, Buitelaar JK, Ebstein RP, Franke B, Gill M, Miranda A, Roeyers H, Rothenberger A, Sergeant JA, Steinhausen HC, Taylor EA, Thompson M, Asherson P. The influence of serotonin- and other genes on impulsive behavioral aggression and cognitive impulsivity in children with attention-deficit/hyperactivity disorder (ADHD): Findings from a family-based association test (FBAT) analysis. *Behav Brain Funct*. 2008; 4:48. [PubMed: 18937842]
- Pelkowski S, Kapoor M, Richendrfer H, Wang X, Colwill RM, Creton R. A novel high-throughput imaging system for automated analyses of avoidance behavior in zebrafish larvae. *Behav Brain Res*. 2011; 223(1):135–144. [PubMed: 21549762]
- Pfizer. ZOLOFT® (sertraline hydrochloride) Tablets and Oral Concentrate. 2002. http://www.fda.gov/ohrms/dockets/ac/04/briefing/4006b1_06_zoloft-label.pdf
- Philipps L, O'Hara M. Prospective study of postpartum depression: 4 1/2-year follow-up of women and children. *J Abnorm Psychol*. 1991; 100(2):151–155. [PubMed: 2040765]
- Redaelli M, Ricatti MJ, Simonetto M, Claus M, Ballabio M, Caretta A, Mucignat-Caretta C. Serotonin and noradrenaline reuptake inhibitors improve micturition control in mice. *PLoS One*. 2015; 10(3):e0121883. [PubMed: 25812116]
- Redolat R, Gomez MC, Vicens P, Carrasco MC. Bupropion effects on aggressiveness and anxiety in OF1 male mice. *Psychopharmacology (Berl)*. 2005; 177(4):418–427. [PubMed: 15289998]
- Richendrfer H, Creton R. Automated High-Throughput Behavioral Analyses in Zebrafish Larvae. *J. Vis. Exp*. 2013:e50622. [PubMed: 23851916]
- Richendrfer H, Creton R. Chlorpyrifos and Malathion have opposite effects on behaviors and brain size that are not correlated to changes in AChE activity. *Neurotoxicology*. 2015
- Richendrfer H, Pelkowski S, Colwill RM, Creton R. On the edge: pharmacological evidence for anxiety-related behavior in zebrafish larvae. *Behav Brain Res*. 2012; 228(1):99–106. [PubMed: 22155488]
- Richendrfer HA, Pelkowski S, Colwill R, Creton R. Developmental sub-chronic exposure to chlorpyrifos reduces anxiety-related behavior in zebrafish larvae. *Neurotoxicol Teratol*. 2012; 34:458–465. [PubMed: 22579535]

- Savy CY, Fitchett AE, McQuade R, Gartside SE, Morris CM, Blain PG, Judge SJ. Low-level repeated exposure to diazinon and chlorpyrifos decrease anxiety-like behaviour in adult male rats as assessed by marble burying behaviour. *Neurotoxicology*. 2015; 50:149–156. [PubMed: 26297601]
- Schmidt R, Strähle U, Scholpp S. Neurogenesis in zebrafish – from embryo to adult. *Neural Development*. 2013; 8(1):3. [PubMed: 23433260]
- Schuessler B. What do we know about duloxetine's mode of action? Evidence from animals to humans. *Bjog*. 2006; 113(Suppl 1):5–9.
- Seidler FJ, Slotkin TA. Developmental neurotoxicity targeting hepatic and cardiac sympathetic innervation: effects of organophosphates are distinct from those of glucocorticoids. *Brain Res Bull*. 2011; 85(3–4):225–230. [PubMed: 21453761]
- Sharp SI, McQuillin A, Gurling HM. Genetics of attention-deficit hyperactivity disorder (ADHD). *Neuropharmacology*. 2009; 57(7–8):590–600. [PubMed: 19715710]
- Smit-Rigter LA, Wadman WJ, van Hooft JA. Impaired Social Behavior in 5-HT(3A) Receptor Knockout Mice. *Front Behav Neurosci*. 2010; 4:169. [PubMed: 21103015]
- Steer R, Scholl T, Hediger M, Fischer R. Self-reported depression and negative pregnancy outcomes. *J Clin Epidemiol*. 1992; 45(10):1093–1099. [PubMed: 1474405]
- Torjesen I. Depression and SSRI use in pregnancy associated with traits of autism in children. *BMJ*. 2014; 349:g4835. [PubMed: 25084785]
- Vermoesen K, Serruys AS, Loyens E, Afrikanova T, Massie A, Schallier A, Michotte Y, Crawford AD, Esguerra CV, de Witte PA, Smolders I, Clinckers R. Assessment of the convulsant liability of antidepressants using zebrafish and mouse seizure models. *Epilepsy Behav*. 2011; 22(3):450–460. [PubMed: 21962757]
- Xu H, Zhang Y, Zhang F, Yuan SN, Shao F, Wang W. Effects of Duloxetine Treatment on Cognitive Flexibility and BDNF Expression in the mPFC of Adult Male Mice Exposed to Social Stress during Adolescence. *Front Mol Neurosci*. 2016; 9:95. [PubMed: 27757074]
- Zhou C, Zhong J, Zou B, Fang L, Chen J, Deng X, Zhang L, Zhao X, Qu Z, Lei Y, Lei T. Meta-analyses of comparative efficacy of antidepressant medications on peripheral BDNF concentration in patients with depression. *PLoS One*. 2017; 12(2):e0172270. [PubMed: 28241064]

Highlights

- The behavioral profiles stimulated by antidepressant and pesticide treatments overlap, indicating a common mechanism of action
- Zebrafish larvae are useful for behavioral assays due to their rapid development
- Exposure period differentially affected behavioral outcomes in zebrafish treated with antidepressants

Figure 1.

Embryonic exposure to sertraline from 0–5 dpf. Concentration is in μM . White bars indicate zebrafish behaviors without any visual stimulus. Red bars indicate zebrafish behaviors with a visual stimulus. Percent of larvae on the end of the lane (A), Swim speed of larvae (mm/min) (B), percent of larvae at rest (C), and percent of larvae down in the lane (D). Error bars in graph represent +SEM. Significance of $*=p<0.05$; $**=p<0.01$ for post hoc Tukey analysis.

Figure 2.

Embryonic exposure to duloxetine from 0–5dpf. Concentration is in μ M. White bars indicate zebrafish behaviors without any visual stimulus. Red bars indicate zebrafish behaviors with a visual stimulus. Percent of larvae on the end of the lane (A), side of the lane (B), edge of the lane (C), Swim speed of larvae (mm/min) (D), percent of larvae at rest (E), percent of larvae together (F) and percent of larvae down in the lane (G). Error bars in graph represent +SEM. Significance of *= p <0.05; **= p <0.01 for post hoc Tukey analysis.

Figure 3.

Embryonic exposure to bupropion from 0–5dpf. Concentration is in μ M. White bars indicate zebrafish behaviors without any visual stimulus. Red bars indicate zebrafish behaviors with a visual stimulus. Percent of larvae on the edge of the lane (A), swim speed of larvae (mm/min) (B), percent of larvae at rest (C). Error bars in graph represent +SEM. Significance of *= $p < 0.05$; **= $p < 0.01$ for post hoc Tukey analysis.

Figure 4.

Embryonic exposure to sertraline from 0–4dpf. Concentration is in μ M. White bars indicate zebrafish behaviors without any visual stimulus. Red bars indicate zebrafish behaviors with a visual stimulus. Swim speed of larvae (mm/min) (A), percent of larvae at rest (B), and percent of larvae on the side of the lane (C). Error bars in graph represent +SEM. Significance of *= $p < 0.05$; **= $p < 0.01$ for post hoc Tukey analysis.

Figure 5.

Embryonic exposure to duloxetine from 0–4dpf. Concentration is in μ M. White bars indicate zebrafish behaviors without any visual stimulus. Red bars indicate zebrafish behaviors with a visual stimulus. Percent of larvae on the end of the lane (A), Swim speed of larvae (mm/min) (B), percent of larvae at rest (C), and percent of larvae together (D), and percent of larvae down in the lane (E). Error bars in graph represent +SEM. Significance of *= p <0.05; **= p <0.01 for post hoc Tukey analysis.

Figure 6.

Embryonic exposure to bupropion from 0–4dpf. Concentration is in μ M. White bars indicate zebrafish behaviors without any visual stimulus. Red bars indicate zebrafish behaviors with a visual stimulus. Percent of larvae on the edge of the lane (A) and percent of larvae on the side of the lane (B). Error bars in graph represent +SEM. Significance of *= $p < 0.05$; **= $p < 0.01$ for post hoc Tukey analysis.

Figure 7.

Cluster analysis of behaviors from pesticide and antidepressant treated larvae from 0–5dpf. Green bars indicate values of 0–1, red bars indicate values between 1–2 and black indicates value of one. Brackets indicate embryonic treatments with similar behavioral outcomes.

Figure 8.

Cluster analysis of behaviors from pesticide and antidepressant treated larvae from 0–4dpf.

Green bars indicate values of 0–1, red bars indicate values between 1–2 and black indicates value of one. Brackets indicate embryonic treatments with similar behavioral outcomes.