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Research Article

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Effects of Fluoxetine on Anxiety-Like Behaviours of Zebrafish (*Danio Rerio*)

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Abstract

The zebrafish (Danio rerio) has emerged as a powerful model organism for studying anxiety. In this regard, a host of anxiolytic agents are available for testing, including fluoxetine - a selective serotonin reuptake inhibitor (SSRI). Adult wild-type zebrafish were used in this study which was approved by the Institutional Animal Use and Care Committee of St. Mary's College of Maryland. Fluoxetine-treated and control groups underwent a startle cue test which consisted of three-time intervals: pre-stimulus, stimulus and post-stimulus. Average proportion of time spent in the top half of the tank, and the average number of erratic movements were assessed. The difference between the average proportion of time spent in the top half of the tank was significantly different between the fluoxetine and control groups ($t(22) = 2.074$, $p < 0.05$). In the fluoxetine group, there was a statistical difference between the proportion of time spent in the top half of the tank during the pre-stimulus, stimulus, and post stimulus intervals for both fluoxetine [$F(2,11) = 3.98$, $p = 0.004$] and control groups [$F(2,11) = 3.98$, $p = 0.004$]. Analysis of the average number of erratic movements revealed no statistical difference between the means of the fluoxetine and control groups. However, differences were observed in the average number of erratic movements between the three intervals of the startle cue test for both the fluoxetine and control groups ($p = 0.005$, $p = 0.001$). Fluoxetine was therefore shown to exert anxiolytic effects on a stereotypic anxiety-like behaviour of the zebrafish. This provides proof-of-principle for the behavioural assay involving a looming cue to evoke anxiety-like behaviour in this model animal.

Key words: Zebrafish, Anxiety, Fluoxetine

1. Introduction

Globally, there is a high prevalence of anxiety disorders to the extent that one study reported that 31% of US adults experience one or more forms of anxiety disorder, indicating that these conditions can last temporarily or life-long [1]. There are significant socioeconomic consequences of anxiety disorders, as an individual's ability to work, perform recreational activities, socialize, and carry out day- to-day tasks is compromised [2]. Over the last several decades, the zebrafish (*Danio rerio*) has emerged as a powerful model organism in biomedical research. This species offers the advantages of being inexpensive, low- maintenance, and shows high fecundity [3]. Indeed, zebrafish are excellent test subjects because they have the same vertebrate neurotransmitters as humans and they exhibit measurable behaviour to stress [4]. For example, the tank diving test assesses the anxiety-like reactions of zebrafish when exposed to stressors. More frequent irregular movements, taking longer to reach and spending less time in the top half of the tank are thought to be correlated with increased anxiousness [4,5]. The primary objective of this study was to provide proof-of-principle for using anxiety- like behaviours of the zebrafish for the study of anxiolytic agents [6]. Selective serotonin reuptake inhibitors (SSRIs), like fluoxetine, are extensively used for the treatment of anxiety disorders and depression [7]. It was hypothesized that fluoxetine-treated fish would demonstrate reduced anxiety-like responses to a looming cue.

2. Methods

This investigation was approved by the Institutional Animal Care and Use Committee (IACUC) of St. Mary's College of Maryland. The average amount of time spent in the top half of the tank, and the average number of erratic movements in response to a computer-generated looming cue were analyzed to determine the potential effects of fluoxetine in these behavioural assays. A total of 16 young adult AB wild-type zebrafish (*Danio Rerio*) were used (sourced from ZIRC, Oregon, USA). These fish were divided into two groups, one of which was exposed to fluoxetine and the other was untreated (control). For the treatment group, fish were immersed in 400 µg/ml of fluoxetine solution for a 12-minute period daily, over 14 days.

Observation of fish behaviours was conducted over a 12-minute period in response to an anxiety-inducing stimulus - the looming cue. A camera on the tripod was directed at the test tank and remained in a constant position for the entire duration of the experiment. The 12- minute period was initiated with a pre-stimulus interval, then a stimulus interval and terminated with a post-stimulus interval. Each of these intervals was 4 minutes in duration. The stimulus interval was characterized by a 3-second presentation of the looming cue followed by the absence of the cue for five seconds then the reappearance of the cue. This cycle was repeated for the 4-minute duration of the stimulus interval.

The behaviours assessed included time spent in the top half of the tank and the number of erratic movements defined by a sudden increase in velocity while swimming rapidly back and forth in the tank. Anxiety-like behaviour in this experiment was defined by increased erratic movement, increased freezing (motionless state) and less time spent in the top half of the tank. All data collected were analyzed using Microsoft Excel 2016. To determine any statistical differences in the behaviours of treated and control groups, one-way independent t- testing and ANOVA single factor followed by Tukey post hoc analyses were performed.

3. Results

The average proportion of time spent in the top half of the tank by each zebrafish in the fluoxetine and control over the 12-minute duration of the startle cue test displayed notable variation (Figure 1). The overall averages for the entire test are 0.320 and 0.648 for the control and fluoxetine groups respectively. Independent t-testing highlighted statistical differences between these two groups, $t(22) = 2.074$, ($p < 0.05$). On the other hand, the average number of erratic movements was not significantly different between the fluoxetine and control groups [Figure 2; $t(22) = 2.074$, ($p>0.05$)].

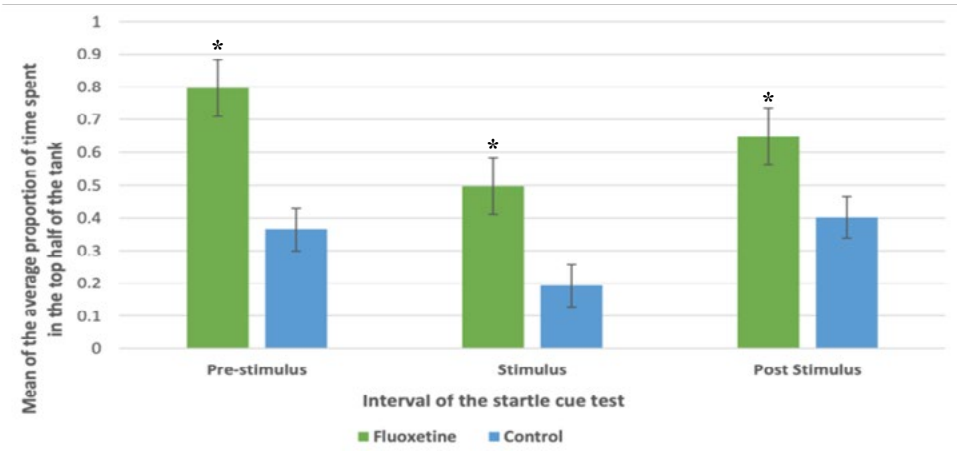

Figure 1: Average time spent in the upper half of test tank during the pre-stimulus, stimulus and post-stimulus periods, for fluoxetine-treated and control fish. Standard error bars are shown. Statistically significant differences between control and fluoxetine-treated means are indicated by "*".

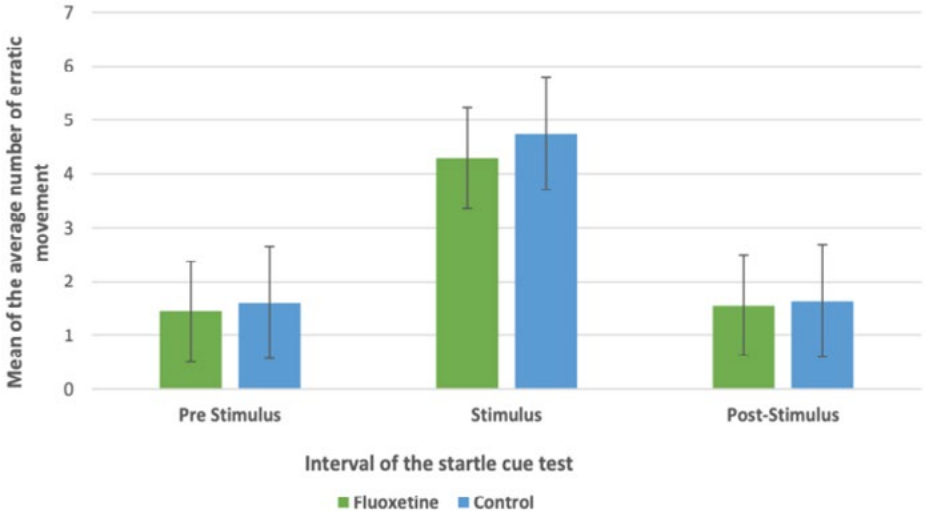

Figure 2: Average number of erratic movement observed during the pre-stimulus, stimulus and post-stimulus periods, for fluoxetine-treated and control fish. Standard error bars are shown.

4. Discussion

The anxiety-reducing effects of fluoxetine demonstrated in this study are consistent with those in the scientific literature and strongly support the use of the zebrafish model in anxiety-related investigations [8]. Observed differences in the average proportion of time spent in the top half of the tank between the fluoxetine and control groups were statistically different. Also, a difference in the pre-stimulus and post-stimulus intervals seen in the fluoxetine-exposed zebrafish suggest a period of acclimatization after being exposed to a fear-inducing stimulus. However, in contrast to previous research that illustrate measurable differences between frequency of erratic movements in zebrafish in high- and low-anxiety states, the average number of erratic movements between the control and fluoxetine-exposed groups showed no statistical difference, suggesting that fluoxetine did not significantly erratic movements in the wake of the looming anxiety-inducing cue [9]. We suggest that the anxiety-inducing stimulus more significantly affected position in the tank, as fish may have been attempting to escape the predator-like cue. Our results illustrate the effectiveness of visual stimuli in the form of the looming cue in producing measurable behavioural responses in zebrafish. Furthermore, time spent in the top of the tank proved to be a powerful assay for fluoxetine-induced anxiolytic effects.

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