Environmental concentrations of fluoxetine disrupt total and serotoninergic enteric neurons in early *zebrafish* larvae *Danio rerio*

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INTRODUCTION: Fluoxetine (FLX) is the main representative of the selective serotonin reuptake inhibitor antidepressants. Part of the FLX that is not metabolized is eliminated into the environment. Due to concerns about the its effects on the environment, the aim of this study was to evaluate the potential effects of environmental concentrations of FLX on neurons (GFP) and serotoninergic neurons (SEN, 5-HT) from gut of *Danio rerio* larvae. MATERIAL AND METHODS: The exposure was carried out from 3 hours post fertilization (hpf) to 4 days post fertilization (4 dpf) *zebrafish* transgenic/mutant TgBAC(neurod:EGFP)nl1/Casper larvae in E3 medium. The control group was only with E3 medium, and the experimental groups were: 50; $5x10^2$; $5x10^3$; $5x10^4$ ng L^{-1} of FLX. At 4 dpf, the larvae were fixed in PFA 4% and then, proceeded to immunofluorescence routine. These mutant expresses fluorescence for pan-neural peptide GFP and the individuals were labeled with anti-serotonin. Then, the individuals were analyzed under LSM 700 confocal laser scanning microscope and made stacks of images to obtain maximum intensity projection images with ZEN 2009 software. The GFP and SEN densities were estimated by number of cells/μm^3 and then, calculated the % ratio of SEN in total GFP. The data were analyzed statically with $p<0.05$. RESULTS AND DISCUSSION: At 4 dpf, the enteric neurons densities were influenced by concentrations $5x10^2$ and $5x10^4$ ng L^{-1} of FLX. The $5x10^2$ ng L^{-1} of FLX presented the lowest 5-HT expression density, whereas the GFP expression density didn't differ, representing the lowest SEN percentage of enteric neurons. In this scenario, the SEN plasticity was disrupted in this concentration, affecting local metabolism, 5-HT signaling and co-factors related to development. The $5x10^4$ ng L^{-1} of FLX presented the lowest 5-HT expression density, whereas the GFP expression density didn't differ, as consequence, the SEN percentage of enteric neurons was significantly higher in relation to GFP enteric neurons. Therefore, in this concentration, the 5-HT misbalance in enteric neurons could alter the 5-HT signaling and stimulus-response and/or sensitization. So, the enteric neurons population and SEN showed to be sensible to environmental concentrations of FLX during the enteric neurogenesis in zebrafish larvae.

Keywords: enteric nervous system; developmental biology; morphological biomarker; antidepressant