The Effects of LPS Exposure on the Dynamic Function of Microglia in a Developing Zebrafish

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Abstract

Our lab utilizes Danio rerio (zebrafish) as a versatile and robust model organism for studying microglia-neuron interactions in the CNS. Microglia, the resident immune cells of the brain, have a distinct spectrum of morphologies that correlate to their main functions. Alterations in immune-neuron interactions, such as the overactivity of microglia, during development and throughout life are suggested to play a role in many neurodevelopmental and neurodegenerative disorders. We utilize transgenic lines with microglia expressing RFP and neurons expressing GFP to live confocal image fry following exposure to lipopolysaccharide (LPS), a known microglia activator. Our initial findings uncovered a dose-dependent activation of microglia morphology following early embryo treatment with LPS.

Here, we expanded our studies to evaluate for dynamic changes in a key function of microglia, the surveillance of the neural network by processes. We captured a time-lapse Z-stack of individual RFP expressing microglia cells in contact with GFP expressing neurons by live *in vivo* imaging of LPS exposure zebrafish fry. We will quantify the amount of processes movement, hoping to prove statistical significance in increased movement signifying heightened dynamic motility. These studies will further establish our zebrafish model and techniques as a rapid-throughput system to improve the current toxicological standard assessment of lethality, sub-lethality, and physical abnormalities to include evaluation of cellular modifications, to more strictly define toxic levels following chemical exposures.

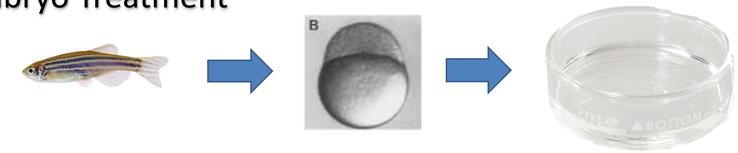
Background

In the early stages of development, microglia have a plastic morphology, transitioning between a ramified, surveillance state and an active, "ameboid" phagocytotic state (Lull and Block 2010). With thin, finger-like projections that extend into their immediate surveillance environment, microglia assist in shaping CNS development by dynamically tracking, pruning, and engulfing unwanted or unhealthy neurons, and cellular death and debris (Svhan, Graeber et al., 2013).

Current model systems are limiting for CNS studies. Mouse models have a long developmental timeline and are expensive, and cell culture fails to reproduce the intricate cell-to-cell interactions. Thus, zebrafish have become a choice model in CNS research. We seek to confirm that the zebrafish model can efficiently and effectively be used to study microglia-neuron interactions.

Methods and Materials

Embryo Treatment



Male and Female zebrafish of GL23tg and islet strains were crossbred and their embryos were collected and treated at approximately 3 hours post-fertilization (hpf). After embryos were collected, they were cleaned and separated into four different small petri dishes, one of each treatment, and were treated on 4 dpf. Treatments were lipopolysaccharide (LPS) diluted in egg water at concentrations of 0 µg/mL, 10 µg/mL, 25 µg/mL, and 50 µg/mL. Egg water with no LPS composition was used as a control. Treated embryos were then imaged via confocal microscopy at 5dpf.

Confocal Microscopy

At 5 dpf, the fry are live-imaged. The fry are anesthetized with tricaine mesylate and plated in 0.8% agarose to stabilize, then dorsally oriented for imaging. Fry are imaged using an Olympus FV1000MPE 2-photon confocal microscope (Olympus XLPan N 25x objective NA 1.05). Z-stack images of the zebrafish brain within a time-lapse video are condensed for analysis.

For data analysis, we will use ImageJ, and Z-stack images will be compressed at each time point for a set of images. Next, the time-lapse stack will also be compressed, creating a complete compressed set of all the time points on top of each other. The amount of microglia processes movement will be determined by subtracting the total area of surveillance from the first image acquired from the total area surveyed in the complete compressed set. We will quantify following completing triplicate experiments each dosage.

Preliminary Results

Live *In Vivo* imaging

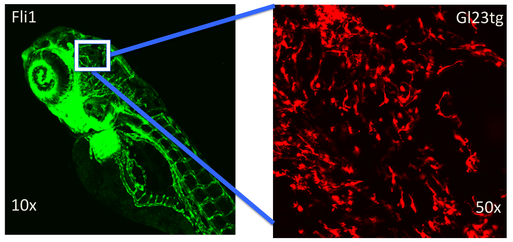


Figure 1. Live *in vivo* imaging.
Left: Representative confocal image of a live 5 dpf Fli1 fry where all blood vessels and vasculature express enhanced green fluorescent protein (EGFP).
Right: representative confocal image of a live 5 dpf Gl23tg fry where mCherry is expressed in microglia/macrophages.

Microglia morphology *in vivo* following LPS treatment

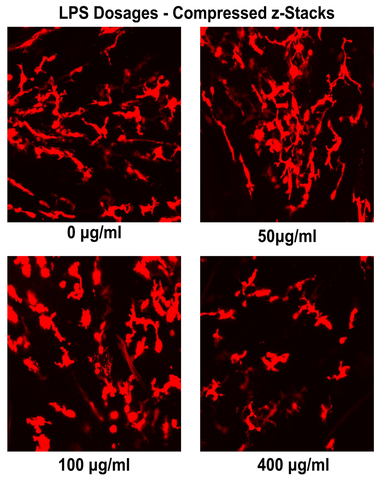


Figure 2. Visualization of Microglia morphology following LPS treatment.
Following 24 hour exposure to varying dosages of LPS, Z-stack images of 5pdf fry brains were obtained. Individual microglia will be 3D rendered to quantify changes in overall microglia morphology.

Microglia dynamic motility *in vivo* following LPS treatment

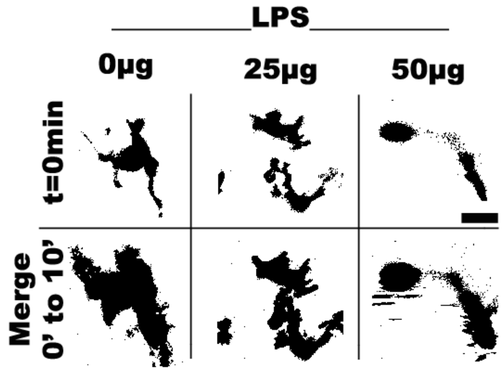


Figure 3. Analyses of microglia processes dynamic motility *in vivo*.
Following 24 hour exposure to varying dosages of LPS, time-lapse Z-stack images of microglia within 5pdf fry were obtained over 10 minutes. Each Z-stack was compressed and made binary, and the entire stack merged to create one complete image. We will be quantifying the percent area coverage to reveal the total area surveyed. Scale bar = 15µm

Preliminary Conclusions

- LPS treatment results in altered microglia morphology in a dose dependent manner, with decreased ramified processes at higher dosages of LPS. This indicated LPS to be capable of activating microglia in a zebrafish model.
- LPS treatment seems to limit microglial function dynamic response, with visually reduced area surveillance over a short time span.
- Microglia within a zebrafish model are have a spectrum of morphologies and function responses, making them an impactful rapid-throughput model to study the CNS.

Future Direction

- Complete 3D renderings of the microglia and neuronal interactions of the mCherry strain and begin morphological measurements on the 3D renderings such as Sholl Analysis and branch complexity
- Utilize this technique for environmental toxins our lab has determined the morphological effects of, including Chlorpyrifos, Deltamethrin, Bifenthrin/Pyrethrin, and PFOS.
- Begin RNA-Sequencing and Proteomics in order to assess mechanisms involved in environmental toxin exposure.

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