Using a zebrafish model for evaluating alterations of microglia morphology following lipopolysaccharides exposure

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Abstract

Microglia cells are macrophages located in the central nervous system that are known to mediate an immune response that clears cellular death and debris. The main function of microglia cells is important for normal brain development and regeneration. Current research is lacking on the effects of toxicant exposure during fetal development, including from mother's diet or proximity to farms that spray pesticides.

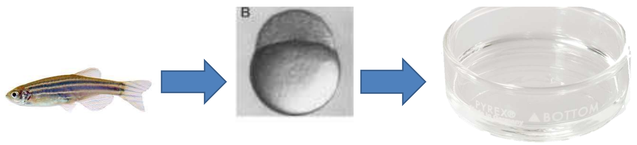
To this end, we are first reconstructing whole microglia cells from five day post fertilized (5 dpf) zebrafish to assess for alteration in cellular morphology following varying dosage of the known immune activator lipopolysaccharides (LPS). These reconstructions will allow us to establish if microglia from the zebrafish model do have a spectrum of morphology, allowing us to next evaluate the effects on pesticide exposed zebrafish.

Background

Microglia morphology can be associated with neurodevelopmental disorders. 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). Microglia is crucial to the central nervous system development by tracking unhealthy and unwanted neurons, and cellular death and debris (Svhan, Graeber, et al, 2013).

Exposure to pesticides during fetal development has been linked to neurological disorders, infertility and birth defects. Pesticide exposure may increase microglia activation. We propose that early pesticide exposure disrupts microglia-neuron interaction leading to disrupt neurocircuitry formation.

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.

Methods and Materials

Physical Morphometrics

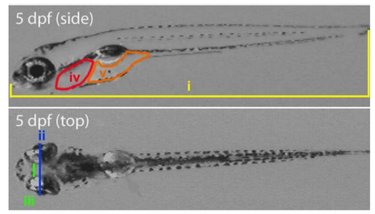
Following fixation and Alcian Blue staining, images of fry were obtained for body and organ measurements.

i. standard length – measure from mouth to the end of the spine.

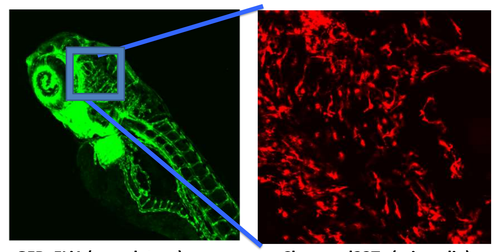
ii-iii. Occular distance – measure between eyes (average between interocular (ii) and intraoccular (iii).

iv. pericardial sac area

v. yolk sac area – measure of yolk sac



Live *in vivo* Confocal Microscopy Imaging



GFP: FLi1 (vasculature) mCherry: gl23Tg (microglia)

Fry are live-imaged at 5 days post-fertilization (5dpf), first anesthetized with tricaine mesylate and plated in 0.8% agarose to stabilize and 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 and collapsed at each time point for analysis.

Preliminary Results

Morphometrics Post-Treatment

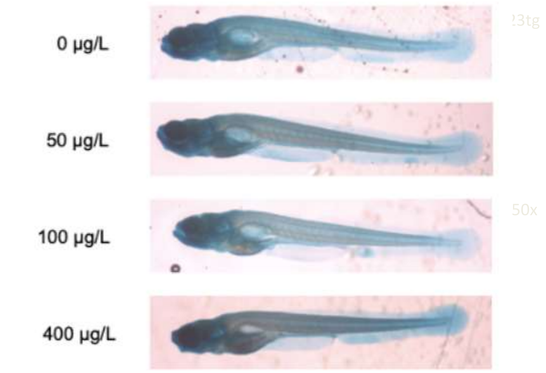


Figure 1. Fry morphology following treatments of LPS at 4dpf and processed at 5dpf

Following 24 hour exposure to varying dosages of LPS, fry were buffered formalin fixed and bone and cartilage stained. Following imaging, measurements of length, ocular distance, pericardial and yolk sac area will be quantified.

Microglia morphology *in vivo* following LPS treatment

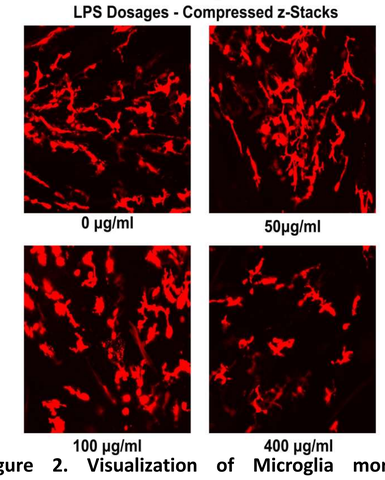
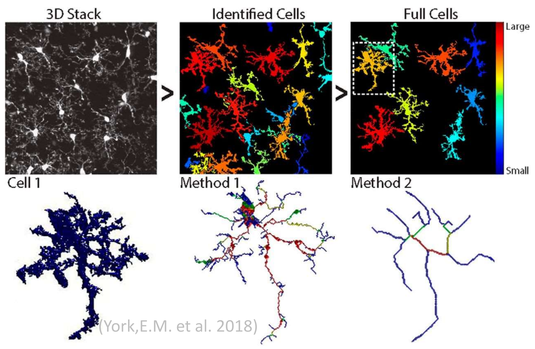


Figure 2. Visualization of Microglia morphology following LPS treatment.

Following 24 hour exposure to varying dosages of LPS, Z-stack images of 5pdf fry brains were obtained. Visually, we observe an increase of amoeboid/rounded cell bodies, indicative of activation when compared controls displaying branshed/ramified processes.



3D reconstruction software

Figure 3. Reconstruction and quantification of Microglia morphology following LPS treatment.

Following 24 hour exposure to varying dosages of LPS, Z-stack images of 5pdf fry brains were obtained.

A specialized free-open source program through MatLab will be used to 3D render individual microglia cells. Quantifications of microglia morphological structure will be performed including, branch length, nodes, endings, and processes order (primary, secondary, etc.).

Preliminary Conclusions

- Visually, we do not observe any major alterations in fish morphometric following any LPS treatment at 4dpf and processed via bone/cartilage staining at 5dpf.
- Measurements and quantifications are currently verifying.
- Visually, LPS treatment seems to result in altered microglia morphology with increasing LPS dosage. The decrease of ramified process with increasing dosages suggests that LPS is capable of activating microglia in a spectral manner.
- Once confirmed that microglia in a zebrafish model do display a spectrum of morphology following insult/injury, our model system will be a powerful high- and rapid-throughput system to evaluate environmental toxicants.

Future Direction

- Matlab will be used to reconstruct whole 3D microglia cells from zebrafish following varying dosages of LPS.
- Utilize these techniques to test known pesticides for neurological activity including Chlorpyrifos, Deltamethrin, Bifenthrein, and PFOS.
- Additional environmental toxicants can be evaluated.

Acknowledgements

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