Project Title

Live imaging of microglial contributions to neurodevelopmental circuit refinement in health and disease

Investigator

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Statement of Purpose

In vivo microscopy of neuronal structure and function to assess the contributions of microglia in normal circuit refinement during development and following immune system activation, a risk factor for ASD.

Project Summary

Maternal infection during pregnancy has emerged as one of the strongest non-genetic risk factors for autism spectrum disorder. Activation of the immune system during infection drives the production of cytokines that enter the developing infant's brain and alter neurogenesis, connectivity, and function. Our recent work using the zebrafish model for in vivo imaging (Solek, Farooqi et al., J Neurosci, 2021) has shown that even brief exposure of larval fish to bacterial lipopolysaccharide (LPS) drives a transient cytokine response that activates microglia and alters the growth program of retinal ganglion cell (RGC) axons. Furthermore, we have found that it degrades visual acuity in the central nervous system (CNS), as measured by calcium imaging and behavioural assays.

Microglia have been the subject of numerous recent studies of neuron-glia interactions during development, including intriguing data from us and others that they are capable of trogocytosing ("nibbling") synaptic elements in the developing CNS and that this may contribute to the pruning of inappropriate synaptic contacts formed during early circuit development. We developed a novel assay for quantifying trogocytosis by microglia in the developing brain (Lim & Ruthazer, eLife, 2021) which involves optically measuring the changes in microglial levels of an axonally expressed fluorescent protein along with morphological reconstructions of the labeled axons. However, this assay has not yet been applied to the immune activation model.

In the current project, we will exploit in vivo 2-photon microscopy to perform live imaging of neurons and microglia as they interact in the developing retinotectal circuit, the visual system of the zebrafish. We will quantify the dynamic remodeling of RGC axonal arbors as they interact with microglia in the brain, examine how LPS-induced immune activation alters this interaction and cellular dynamics, and then use a transgenic line lacking microglia to better understand the importance of microglia in the remodeling process under healthy and inflammatory conditions.

Using state-of-the-art GCaMP transgenic animals expressing calcium indicators in neurons we will simultaneously measure the responses of thousands of tectal neurons

to visual stimuli designed to measure visual acuity and selectivity, and determine how the depletion of microglia may alter these properties under conditions of normal rearing versus brief immune activation, which we have previously shown can alter visual responsiveness.

Finally, we will assess the specific contributions of microglial trogocytosis of synapses, which we have previously demonstrated takes place in the developing retinotectal system, to axonal arbor pruning in the healthy and LPS-treated zebrafish larvae. Using the assays we recently published, we will measure the amount of trogocytosis per fish under a range of conditions and in animals where the Complement signaling pathway has been disrupted. In parallel we will examine axonal remodeling and visually evoked calcium signal to understand the consequences of trogocytosis by microglia on circuit structure and function.

As maternal immune activation is a key a risk factor for ASD, it is essential to gain greater understanding of precisely how it alters developing circuits and the role of the innate immune system in this process.