Investigating microglia-mediated engulfment of astrocyte processes during brain development

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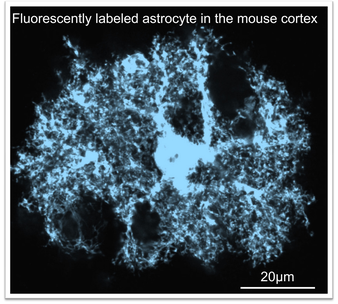
Introduction

Background and Research Overview

The brain contains neurons and non-neuronal glial cells. Astrocytes are one of the major glial cell types and have a variety of important functions within the central nervous system (CNS).



Astrocytes have a complex morphology which allows them to partake in cellular crosstalk meaning that they play an integral role in cell to cell signaling during disease development and progression. Some of these diseases include Huntington's, Alzheimer's, and Parkinson's diseases.

The central goal of the larger project is to determine whether microglia-mediated engulfment refines astrocyte processes in the developing brain. Preliminary evidence from the lab strongly supports a novel, microglia-mediated mechanism of astrocyte morphological refinement where microglia engulf astrocyte processes in a developmentally regulated manner.

My project examines microglia-astrocyte engulfment in the visual cortex at different developmental timepoints, with a specific focus on the adult brain.

Preliminary evidence for microglia-astrocyte engulfment

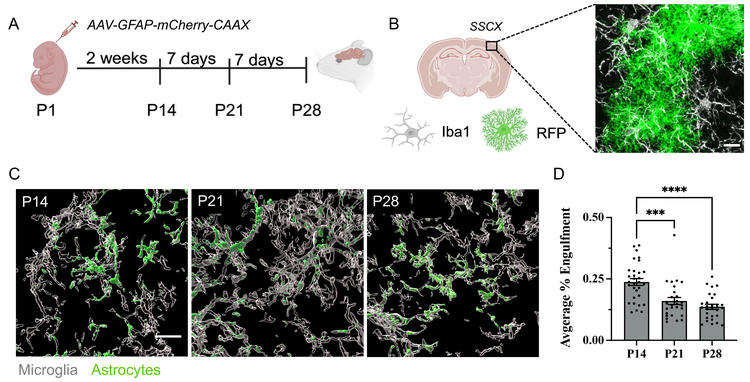


Figure 1. ***Preliminary evidence for microglia engulfment of astrocyte processes.*** (A) Injection process for labeling of cortical astrocytes. (B) Sample image of astrocyte labeling in the primary somatosensory cortex (SSCX; scale = 10µm). (C) Reconstruction of microglia and astrocytes done on Imaris (RFP; green; scale=10µm). (D) Average percent engulfment of astrocyte processes by microglia; individual microglia represented by each dot; n=3 mice per timepoint; ***p=0.0002, ****p<0.0001.

Hypothesis

In adult mice, microglia-mediated engulfment serves an important homeostatic function, enabling regulation of astrocyte morphology with important consequences for astrocyte function.

Results

Workflow for Aldh1l1-EGFP transgenic mouse brains

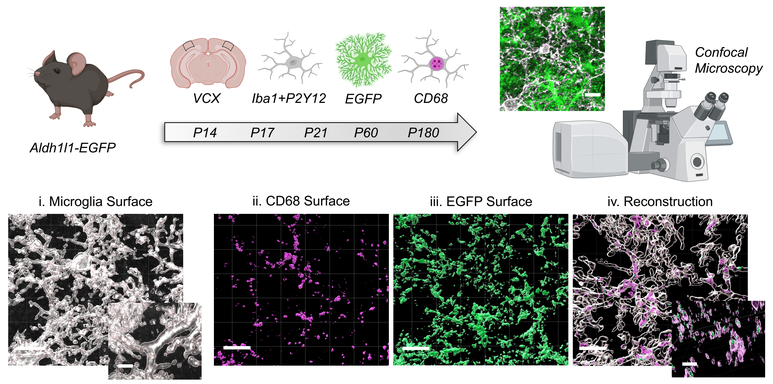


Figure 2. ***Schematic of workflow to analyze astrocyte morphology.*** Aldh1l1-EGFP transgenic strategy; sample collection timepoints; staining by immunohistochemistry; primary visual cortex (VCX) image acquisition amongst layers 2 and 5; microglia reconstruction and analysis using Imaris (scale=5µm).

Microglia-Astrocyte Engulfment Across Development

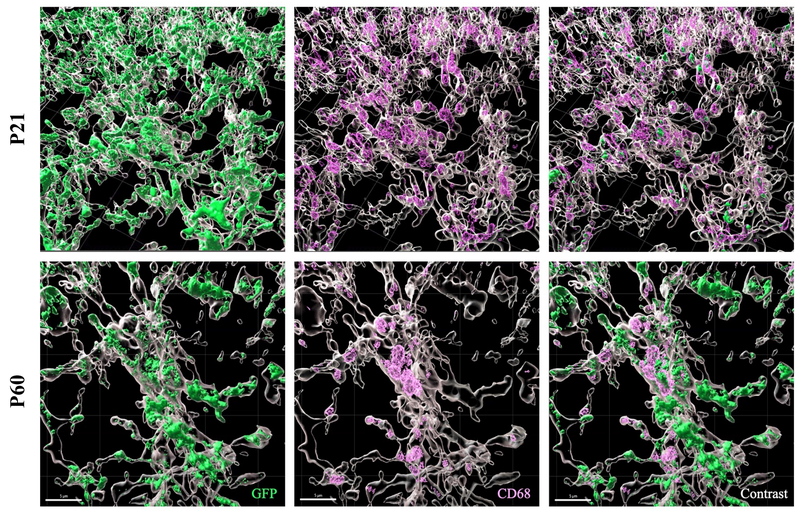


Figure 3. ***Sample image reconstruction of the visual cortex at two timepoints.*** Astrocyte inclusion in microglia is quantified using Imaris image analysis software. Microglia are seen in white. P21 and P60 timepoints are represented, both timepoints showcasing the reconstruction with GFP channel only, CD68 (lysosome) channel only, and a contrast image representing both GFP and CD68 inclusion in microglia.

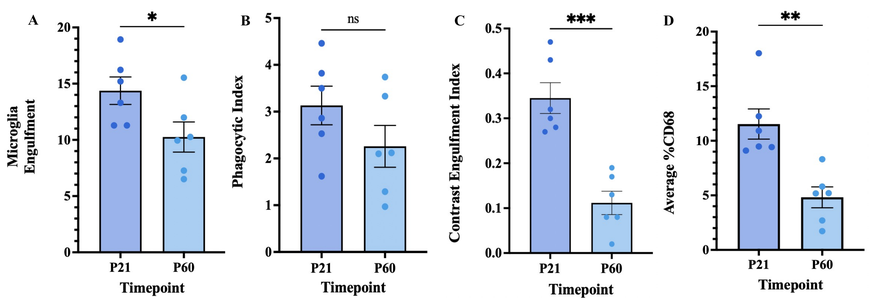


Figure 4. ***Unpaired t-tests for statistical categories of interest.*** (A) Average engulfment by microglia volume. (B) Average phagocytic index. (C) Average contrast engulfment index. (D) Average percent CD68 (lysosome content). Statistical significance between P21 and P60 timepoints for A, C, and D. ANOVA test and a subsequent unpaired t-test were ran; * indicates $p < 0.05$, ** indicates $p < 0.01$, and *** indicates $p < 0.001$.

Ongoing Work

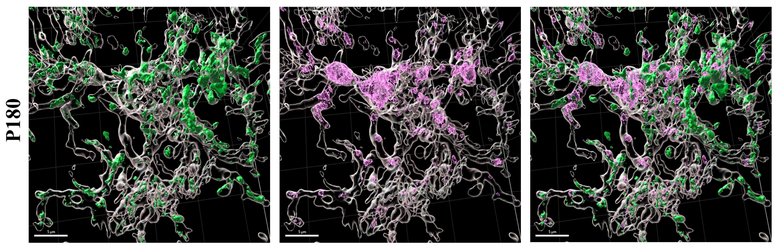


Figure 5. ***Sample image reconstruction of the visual cortex at the P180 timepoint.*** Astrocyte inclusion in microglia is quantified using Imaris image analysis software. Microglia are seen in white. P180, 6-month, timepoint is represented, showcasing the reconstruction with GFP channel only, CD68 (lysosome) channel only, and a contrast image representing both GFP and CD68 inclusion in microglia.

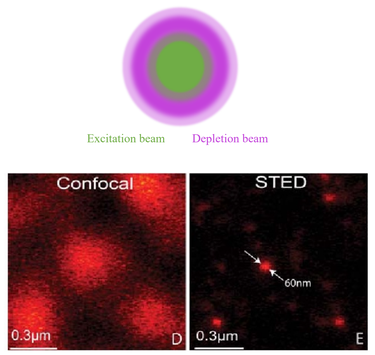
Conclusions and Future Directions

Conclusions

- Microglia-mediated mechanism of astrocyte morphological refinement is likely to continue into adulthood
- With lysosome content being significantly decreased in adulthood, these cells are less effective at removing waste and thus brain tissue may be getting damaged over time. This could have implications in the development of neurodegenerative disorders like Alzheimer's or Parkinson's disease.

Future Directions

- A larger sample size should be studied to make the research more comprehensive, impactful and representative of the population of adult timepoint mice studied; P180, 6-month, tissue will be analyzed next
- Super-resolution STED (stimulated emission depletion) microscopy; visualized to the right



References

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