Microglia Density and Morphology at P10 in the Early Postnatal Male and Female C57BL/6J Mouse Hippocampus

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PROJECT OVERVIEW

QUESTIONS

- How does sex impact early development of microglia in the hippocampus?
- Is there a difference in density of microglia between male and female mice and across hippocampal subregions?

KEY TERMS

- Morphology = shape and structure of a cell, which for microglia is indicative of function
- P10 = postnatal day 10
- Microglia = neuronal cell that controls nervous system immune defense
- Hippocampus = portion of brain's limbic system that impacts memory

POTENTIAL IMPLICATIONS

- Database of annotated morphology for future experiments
- Can help us identify potential sex differences in neuronal development
- Differences in understanding of pediatric hippocampus across sex

ABSTRACT

Microglia, the brain's resident macrophages, play critical roles in the maintenance of the healthy brain and the brain's response to disease and injury. Microglia also contribute to learning and memory through synaptic pruning. The hippocampus is an area of focus for microglial study due to the hippocampus's role in learning and memory. Interestingly, microglia have innate sex differences. There is conflicting literature regarding the developmental time course of male and female microglia in the hippocampus during early development. Additionally, the P10 time point has never been measure with respect to sex differences in hippocampal microglia. My experiment fills the knowledge gaps by quantifying male and female microglial densities in the mouse model within the four most microglial dense hippocampal subregions at the P10 timepoint. Subregions were imaged using an Olympus light microscope. Then we analyzed these images for Iba1+ cell density and morphology. Our analysis reveals no sex-based differences in Iba1+ cell density across subregions in the anterior hippocampus. However, female mice did show more microglia with "Thick" morphology in the DG Mol and DG Hilus relative to males, which showed more "Thin" cells.

OBJECTIVES/METHODS

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- Determine methodology for quantification and morphological classification
- Determine if there is a baseline difference in microglial densities or microglia morphology across male and female P10 sham mice

METHODS

- With a 400x magnification on the Olympus microscope I imaged anterior hippocampal subregions for the 6 male and 7 female mice quantified.
- I virtually isolated our tissue of interest, also known as region of interest (ROI), using the free-handed cropping tool in FIJI software
- Using offline ROIs, I quantified Iba1+ cells. Throughout the quantification process, I simultaneously subclassified the morphology of each microglia into one of four categories outlined by the protocol I designed.
- 4 categories of morphology: Round or ameboid (round-ish soma, no processes), Stout (round-ish soma, short process), Thick (irregular soma with few, thick processes), or Thin (irregular soma with multiple thin processes).

METHODS CONTINUED

PROTOCOLS DESIGNED

- Imaging: Outlined guidelines to ensure subregion imaging was consistent across mice.
- ROI: I designed a protocol that details how to isolate the tissue from the region of interest (ROI) for each of the hippocampal subregions that are being imaged (dentate gyrus molecular layer, dentate gyrus hilus, CA1, CA3) (figure 1).
- Quantification and Morphology: Relying on sample images and previous literature, I outlined a set of criteria that determined what constituted a microglial cell and how to subclassify these microglia into one of four different morphology categories.

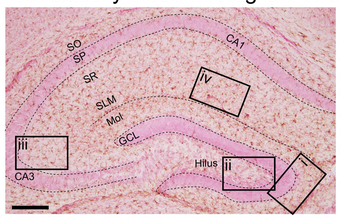

Figure 1. Hippocampal subregions analyzed. This photomicrograph displays a P10 mouse hippocampal coronal brain section (-2.05mm from bregma) immunostained with an anti-Iba1 antibody and counterstained with FastRed. Image was taken at 400x (objective: 40x, 0.9 NA). Principal cell layers of the dentate gyrus (DG; granule cell layer, GCL) and Ammon's horn (pyramidal cell layer or stratum pyramidal [SP] in CA1 and CA3) are darker pink and outlined with dashed lines. The hippocampal fissure that separates the DG molecular layer (Mol) from the CA1 stratum lacunosum moleculare (SLM) is shown as a single dashed line. The four outlined rectangles show the precise placement used during data collection: (i) DG Mol, (ii) DG Hilus, (iii) CA3, and (iv) CA1 SR and SLM. SO, stratum oriens. Scale bar=200um.

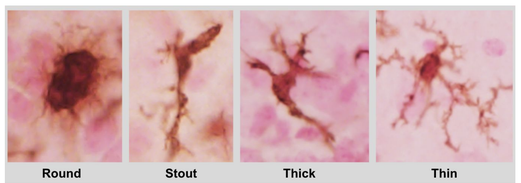

Figure 2. Morphological Classification. The figure above contains example images of microglia from each of the four morphological stages I used during subclassification. The microglia and their processes appear dark brown due to immunohistochemistry for Iba1+ markers.

RESULTS

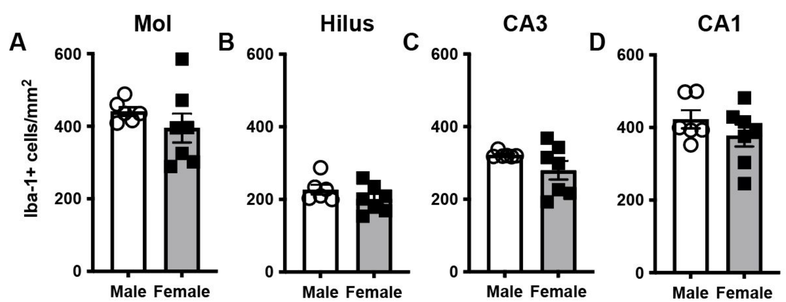

Figure 3. Iba1+ cell densities in four hippocampal subregions are not different between male and female C57BL/6J mice at postnatal day 10 (P10). Subregions analyzed are shown in Fig. 1. Subregions analyzed: **(A)** dentate gyrus molecular layer (Mol), **(B)** dentate gyrus hilus, **(C)** CA3 stratum radiatum and stratum lucidum (CA3), **(D)** CA1 stratum radiatum and stratum lacunosum moleculare (CA1). Y axis in **A** applies to **B-D**. Male n = 6, Female n = 7. Unpaired t-tests, all p's>0.05.

RESULTS CONTINUED

- The analyzed results reveal that there is no significant difference between microglia levels of male and female mice at P10.
- Although we do not see sex differences in P10 mice, it remains possible a difference will be observable at other time points.
- There are sex-dependent difference in Iba1+ cell morphology classification for the DG Mol and DG Hilus. Females showed more Thick Iba1+ cells and males showed more Thin Iba1+ cells.
- This sex difference in morphology signified that Iba1+ microglia in female mice are "younger" or "more active" than those in male mice. Additionally, the difference in morphology signifies functional differences in P10 male and female microglia. Thick microglia are more motile and contribute to removal of debris. Thin microglia are stationary and involved with sense and maintain homeostasis.

FUTURE DIRECTIONS

- This project establishes a baseline of normal hippocampal development in the mouse model that we will later use as a baseline when considering an injury model. The injury we will be studying is HI (hypoxia ischemia). This future experiment aims to reveal if there is a quantitative difference in hippocampal microglial density between male and female mice after HI.
- Additionally, our research will benefit from the addition of other surrounding time points including P3, P7, and P13.

ACKNOWLEDGMENTS

This research was supported by The Ruth Marcus Kanter College Alumni Society Undergraduate Research Grant and Pincus-Magaziner Family Undergraduate Research and Travel Fund. This work was supported by the Children's Hospital of Philadelphia (CHOP) Development Fund and Bridge Funding and an NINDS Institutional Postdoctoral T32 and by Supplemental Support for Clinicians to Pursue Research Training in Neuroscience from the CHOP Research Institute.

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