NRSC 510A – project proposal
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Due: Oct 4 2024 at 11:59 pm

Title: Exploring Microglial Heterogeneity: Transcriptomic Insights into Maternal Stress Responses

OSF Page: https://osf.io/475ug/?view_only=ca9801b037a340898f36e7279c05e689

Background, research question and significance

Maternal stress is a significant issue that disrupts offspring neurodevelopment, leading to long-term behavioral and cognitive challenges.[4] It activates microglia, the brain's immune cells, which play a crucial role in shaping neural progenitor development.[1] Studies have shown that maternal stress leads to altered microglial activity in the hypothalamus, a key brain region for regulating the stress response, contributing to disruptions in neurodevelopmental processes, such as neuronal differentiation and synaptic pruning.[5]

Although this link, between stress-induced microglial changes and hypothalamic dysfunction, highlights a pathway, by which maternal stress influences offspring development, the mechanism, by which fetal hypothalamic microglia interact with neural cells to change their development, is understudied.[6] Using a toolbox of techniques, including scRNA-seq, Rosin et al. (2021) found that there are four distinct microglial clusters of hypothalamic microglia, one of which is in direct contact with nearby NSCs and responded to maternal cold stress. While Rosin et al. (2021) provide valuable insights into the role of stress-responsive microglia, the heterogeneity within microglial subpopulations remains underexplored. I will try to answer the question: how do transcriptomic differences, within a specific subtype of hypothalamic microglia identified in Rosin et al. (2021) reflect the heterogeneity in microglial responses to maternal stress?

By exploring the heterogeneity within cluster 3, this project aims to improve our understanding of how microglial diversity impacts neurodevelopmental programs in response to maternal stress. These findings could help uncover underlying mechanisms involved in NDDs, enabling identification of vulnerable pathways for interventions to reduce these insults on the developing brain.

Detailed work plan

I will use samples from Rosin et al. (2021), "GSM3901919 E15.5 microglia from CD1 embryos" and "GSM3901920 E15.5 microglia from stressed CD1 embryos," publicly available on GEO (no access permission required). Each has supplementary files containing datasets: the barcode file (identifiers for 2,623 hypothalamic microglia cells), the feature file (a list of gene identifiers expressed in the microglia), and the matrix file (normalized expression levels of these genes in each cell). Mean reads per cell are 96,753.

Data analysis will be conducted with R, a language well-suited for analyzing large and complex datasets like scRNA-seq due to its powerful packages, such as Seurat.[2]

Project timeline:

- October 3 – November 14: loading files, removing low-quality cells, normalizing data, ensuring integrity for subsequent analyses. Then, subsetting cluster 3, identifying and extracting the specific microglial population for analysis, and visualizing with UMAP. Conducting differential expression analysis to identify genes (Seurat).[7]
- November 15 – 18: conducting pathway enrichment analysis to uncover pathways potentially affected by maternal stress (clusterProfiler).[9]
- November 19 – 22: performing Gene Set Enrichment Analysis to determine whether predefined gene sets (control and stress) show significant differences, providing insight into functional implications of transcriptomic changes observed (fgsea).[3]
- November 23 – 30: carrying out pseudotime analysis to investigate potential developmental trajectories of cells, offering insights into how stress may alter microglial development (monocle3).[8]

The key milestone is understanding the theory underlying the Seurat analysis code, necessary for implementation of subsequent steps in R. Margo Kapustina (TA), a PhD student in the Cembrowski lab, will support me.

Learning objectives

I am new to programming and have no experience with R. Throughout the term, I want to learn basic concepts and operations in R used in data processing. I need to learn how to analyze scRNA-seq data using the Seurat package and others, necessary to create code that generates graphical representations of cell subpopulations and characterizes their transcriptomic profiles. I aim to achieve working knowledge of reclustering data, differential expression, pathway enrichment analysis, GSEA and Pseudotime. In the long term, my goal is to achieve fluency in R and write code tailored to analyze my own experimental data.

References

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