Effects of Lipopolysaccharide Immune Challenge on Microglial Activation and CD3+ T-cells in the Substantia Nigra of Female Rats

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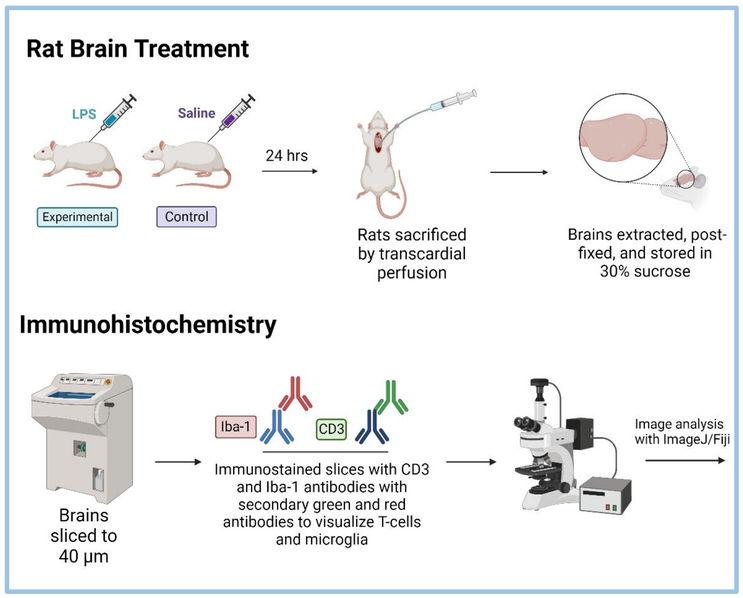
Introduction

- **Parkinson's disease (PD)** is a debilitating motor disorder caused by dopaminergic neuron death in the substantia nigra that affects up to one million Americans[1]
- Previous research suggests that **microglial activation and T-cell infiltration** may be associated with PD[2]
- Few studies have looked at the **combined role** of CD3+ and microglia in PD pathology
- The use of **female animals** in neuroscience research has traditionally remained **low** compared to the use of males[3]
- By examining the **interaction** between microglia and CD3+ T-cells in the substantia nigra of **female** rats, there is an opportunity to develop and examine new therapies for PD

Hypothesis

CD3+ T-cells will be highly colocalized with the activated microglia in the substantia nigra of LPS treated rats compared to saline treated rats

Experimental Design



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Results

MICROGLIAL ACTIVATION BASED ON MORPHOLOGY

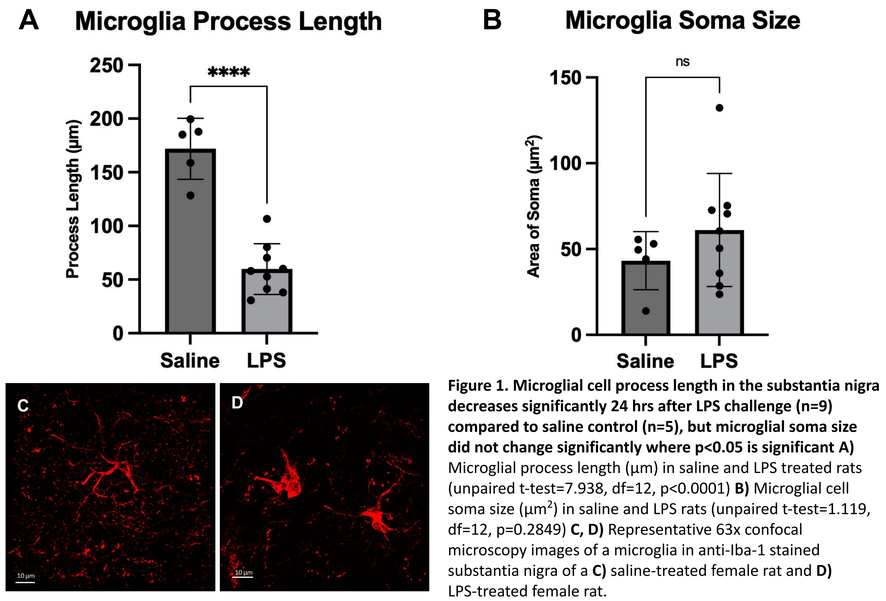
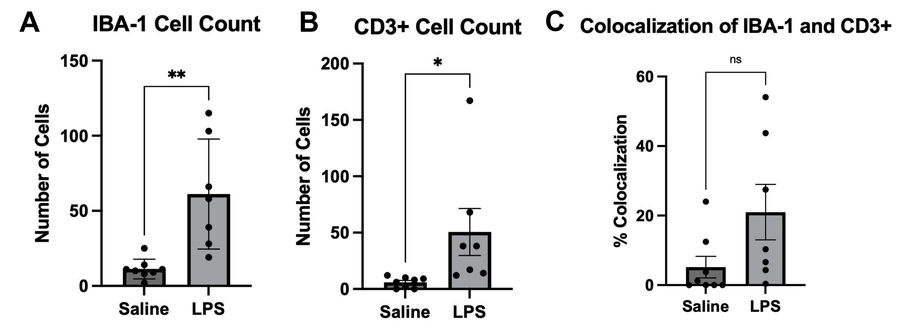


Figure 1. Microglial cell process length in the substantia nigra decreases significantly 24 hrs after LPS challenge (n=9) compared to saline control (n=5), but microglial soma size did not change significantly where p<0.05 is significant A) Microglial process length (µm) in saline and LPS treated rats (unpaired t-test=7.938, df=12, p<0.0001) **B)** Microglial cell soma size (µm^2) in saline and LPS rats (unpaired t-test=1.119, df=12, p=0.2849) **C, D)** Representative 63x confocal microscopy images of a microglia in anti-Iba-1 stained substantia nigra of a **C)** saline-treated female rat and **D)** LPS-treated female rat.

COLOCALIZATION OF MICROGLIA AND T-CELLS



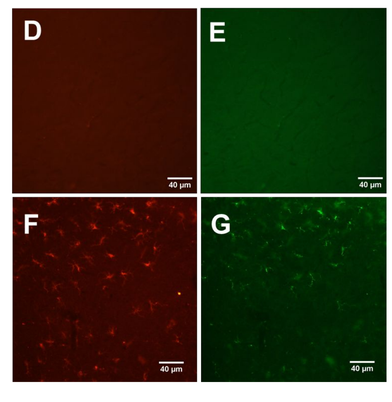


Figure 2. T-cell and microglia cell count is increased significantly in the substantia nigra of female rats 24 hrs after LPS injection (n=7) compared to saline injection (n=8), but colocalization of T-cells and microglia does not increase significantly with LPS injection where p<0.05 is significant A) Microglia cell count in the substantia nigra in saline-and LPS-treated female rats (unpaired, two-tailed t-test=3.804, df=13, p=0.0022). **B)** T-cell count in the substantia nigra of saline- and LPS-treated female rats (unpaired, two-tailed t-test=2.303, df=13, p=0.0384) **C)** Colocalization of T-cells and microglia in the substantia nigra of saline- and LPS-treated rats (unpaired, two-tailed t-test=1.944, df=13, p=0.0739) **D, E, F, G)** Representative 20x widefield fluorescent microscopy images of the substantia nigra of female rat brains stained with anti-Iba-1 and anti-CD3 antibodies. **D)** Red anti-Iba-1 AlexaFluor 568 fluorescent stain and **E)** green anti-CD3 AlexaFluor 488 fluorescent stain of cells in the substantia nigra of saline-treated female rat brain. **F)** Red anti-Iba-1 AlexaFluor 568 fluorescent stain and **G)** green anti-CD3 AlexaFluor 488 fluorescent stain of cells in the substantia nigra of LPS-treated female rat brain.

COLOCALIZATION (CONT.)

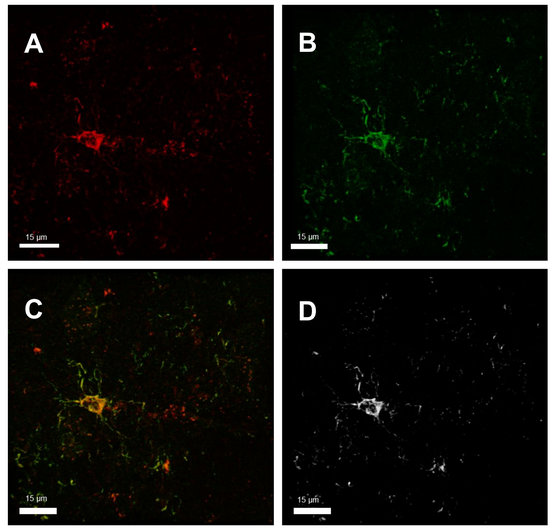


Figure 3. CD3 is colocalized with Iba-1 in the substantia nigra of female rats 24 hours after LPS injection. A, B, C, D) Representative 63x confocal microscopy images of cells in the substantia nigra of an LPS treated female rat brain stained with anti-Iba-1 and anti-CD3 antibodies. **A)** Red anti-Iba-1 AlexaFluor 568 fluorescent stain and **B)** green anti-CD3 AlexaFluor 488 fluorescent stain of cells in the substantia nigra of LPS-treated female rat brain. **C)** Combined overlay of red and green fluorescent stains with colocalization shown in yellow and **D)** white representation of Iba-1 and CD3 colocalization demonstrate that T-cells and microglia are colocalized in the substantia nigra after LPS immune challenge in female rats.

Discussion

- The results for colocalization were not significant, but the data shows a pattern of increased colocalization in LPS challenged rats. **Therefore, the relationship between microglia and T-cells should continue to be explored in PD pathology and therapies**
- **The results for IBA-1 & CD3+ cell count and microglial process length do support our hypothesis.** Shorter process lengths indicate microglial activation, and the morphology of microglia in LPS challenged rats changed in this characteristic manner
- **Future directions** for research:
  - Analysis of differentiated T-cell presence in the substantia nigra (SN)
  - Examination of cytokine presence to further investigate microglial activation in the SN

References

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2. Chen, Z., Chen, S., & Liu, J. (2018). The role of T cells in the pathogenesis of Parkinson's disease. *Progress in Neurobiology*, *169*, 1–23. https://doi.org/10.1016/j.pneurobio.2018.08.002
3. Mamlouk, G. M., Dorris, D. M., Barrett, L. R., & Meitzen, J. (2020). Sex bias and omission in neuroscience research is influenced by research model and journal, but not reported NIH funding. *Frontiers in Neuroendocrinology*, *57*, 100835. https://doi.org/10.1016/j.yfrne.2020.100835

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