Microglia Immunoreactivity: The Regional Heterogeneity of Microglia Cell Responses to a Peripheral Immune Challenge

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INTRODUCTION

- One possible approach to understanding the possible causes and sequence of neuroinflammation is the activation of microglial cells, which act as the innate immune cells of the CNS.
- Activated microglia possess a more amoeboid morphology, with shorter processes and a larger soma body area, as well as produce both pro- and anti-inflammatory markers
- For our experiment, we assessed microglial activation in male MMTV-PyMT carrier mice by presenting an immune challenge in the form of a lipopolysaccharide (LPS) injection, alongside a saline control.
- We predict that if the amygdala and substantia nigra are injected with a single dosage of peripheral LPS, there would be greater microglial activation in the substantia nigra (SN) compared to the basolateral amygdala (BLA) due the differences in microglia density between the two brain regions.

METHODS

Male MMTV-PyMT carrier mice were administered either a 0.5 mg/kg LPS or saline injection

Mice were trans-cardially perfused with 0.1 M PBS and 4% paraformaldehyde (PFA). After ~7 days in sucrose, brains were frozen.

Brains were submersed in tissue-freezing medium and sectioned on a coronal plane using a cryostat machine.

Slices were washed with PBS and blocked with 5% normal goat serum in phosphate buffer solution (PBS). Slices were incubated in antibodies and fluorescently stained for 1ba-1 protein.

Slices were imaged using confocal and fluorescent microscopy. Images were opened in ImageJ/Fiji for morphological and quantitative analysis.

- Iba-1 is a protein on the surface of M1 activated microglia cells. Fluorescent Staining for Iba-1 characterized microglia activation in response to LPS challenge.
- 20X images were taken using a Nikon ECLIPSE Ts2 Microscope. The Zeiss LSM 800 Confocal Microscope was used to create Z-stacks at 63X magnification using oil immersion. Imaris was used to subtract background using a 3D-blind deconvolution.
- By imaging, microglial immunoreactivity was quantified by cell counts, soma body area, proportional area, and average process lengths of microglia cells in the SN and BLA.
- Statistical analysis was conducted with unpaired t-tests.

RESULTS

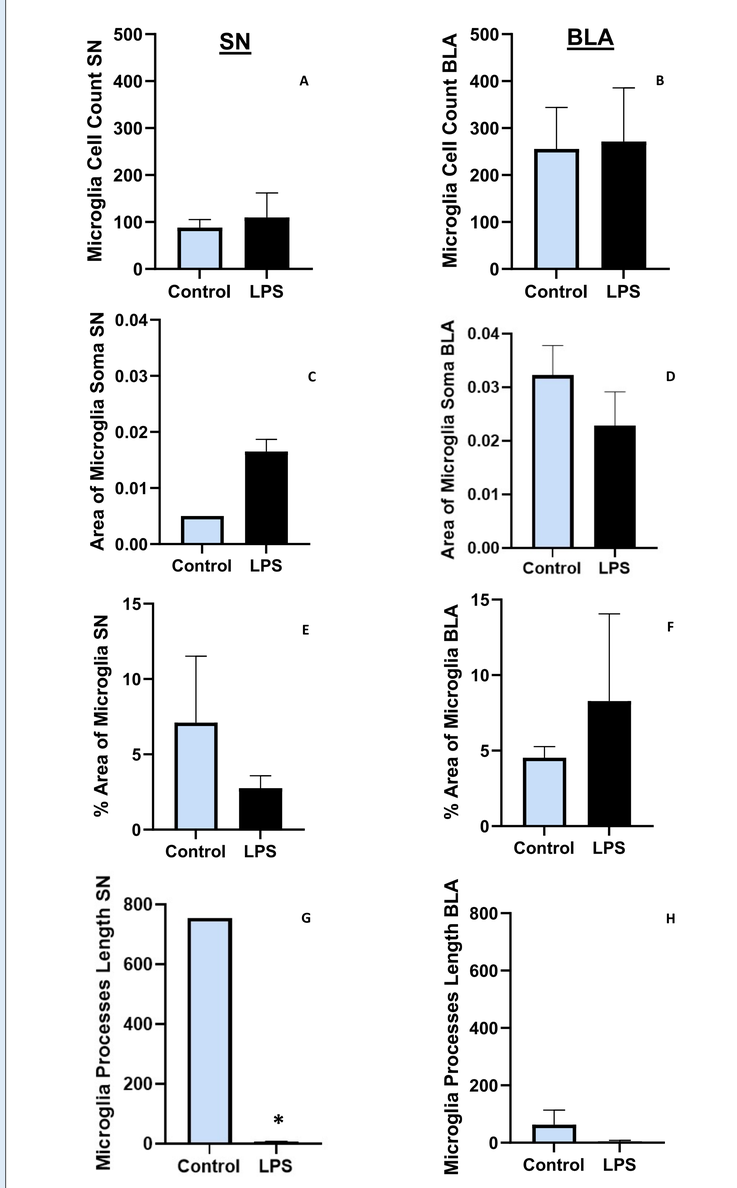

Figure 1. Images were analyzed using Image J software. **A**) Graph of the microglial cell count in the substantia nigra **B**) Graph of the microglia cell count in the basolateral amygdala **C**) Graph of microglia soma body area for substantia nigra between LPS injected mice and control **D**) Graph of microglia soma body area for basolateral amygdala between LPS-injected mice and control. **E**) Graph of microglial percent area in substantia nigra between LPS-injected mice and control **F**) Graph of microglial percent area in basolateral amygdala between LPS-injected mice and control **G**) Graph of microglial process length in substantia nigra between LPS-injected mice and control **H**) Graph of microglial process length in basolateral amygdala between LPS-injected mice and control.

RESULTS CONT.

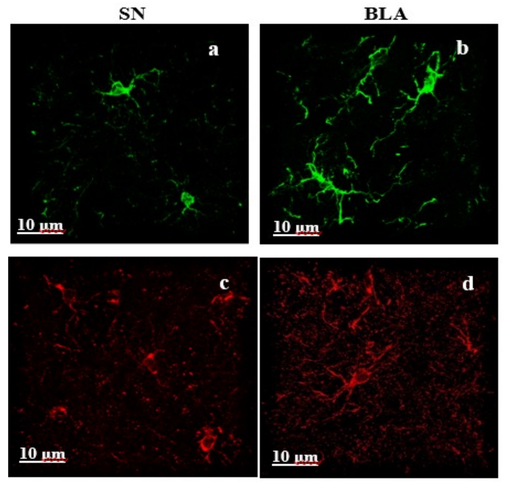

Figure 2. Effects of LPS challenge on microglia cell body area and process lengths. (**a**) 63X z-stack fluorescent image of microglia in the substantia nigra (SN) of LPS subject. (**b**) image of microglia in the basolateral amygdala (BLA) of LPS subject. (**c**) image of microglia in the SN of control subject. (**d**) image of microglia in the BLA of control subject.

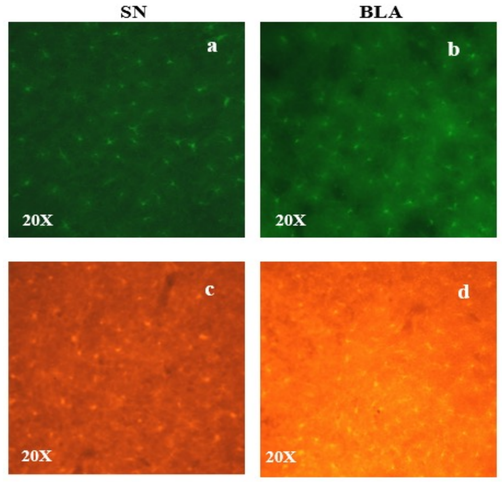

Figure 3. Effects of LPS challenge on microglia cell counts and proportional area of Iba-1 staining. (**a**) Iba-1-stained microglia in the substantia nigra (SN) of LPS subject. (**b**) Iba-1-stained microglia in the basolateral amygdala (BLA) of LPS subject. (**c**) Iba-1-stained microglia in the SN of control subject. (**d**) Iba-1-stained microglia in the BLA of control subject.

CONCLUSIONS

- There is not a significant difference between LPS-injected mice and saline-injected mice in several key morphometric factors, such as cell count, soma body area, percent area of microglia, and average process length. These results suggest that LPS does not induce significant activation in the microglia of both the substantia nigra and basolateral amygdala
- These results can give us insight as to various factors that are involved in the activation of microglia, which is known to lead to neuroinflammation. Potential new treatments for neurodegenerative disorders such as Alzheimer's disease can focus not only on preventing neuronal degeneration but include ways of moderating the microglial activation pathway to prevent or diminish symptoms of neuroinflammation.

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