Effects of Lipopolysaccharide Immune Challenge on Microglial Activation and TNF-α Colocalization in the Nucleus Accumbens of Female Rats

Harshita Gudipudi, Vidya Gadikota, Marissa Jeganathan, Sarah Smith, Dr. Shveta Parekh

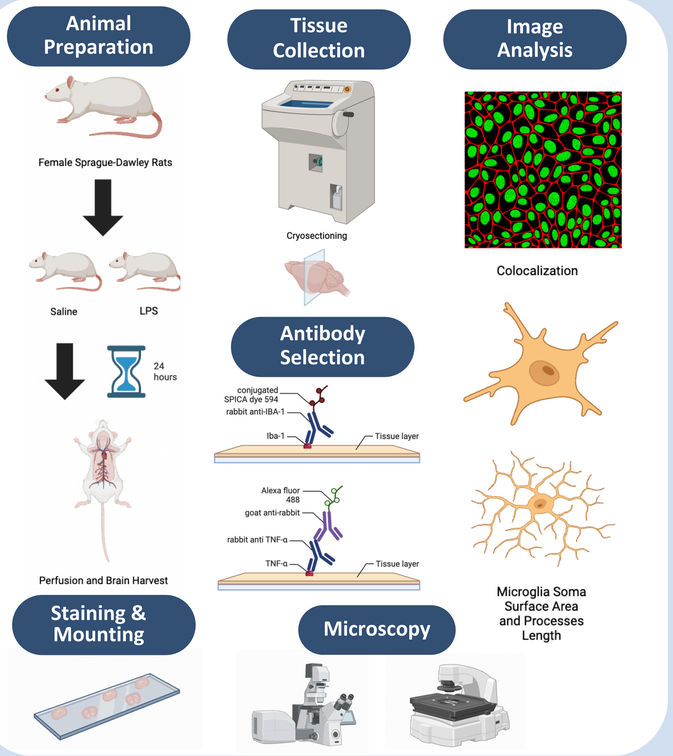
UNC PSYCHOLOGY & NEUROSCIENCE

Introduction

- Microglia are the resident immune cells of the brain, serving a vital role in nervous system regulation.
- TNF-α is a proinflammatory cytokine emitted from microglia, involved in both stress response and addiction[7].
- The nucleus accumbens is a crucial component of the mesolimbic reward pathway, by which it is associated with TNF-α regarding substance use and stress[13].
- Thorough research exists on the effects of stress on glial biology, however, the effects of the lipopolysaccharide challenge on females has yet to be elucidated.
- The purpose of this study is to investigate the interaction between stress, microglia, and TNF-α through measuring microglial morphology, microglial cell count, TNF-α cell count, and TNF-α colocalization with microglia in the nucleus accumbens post-LPS challenge in females.

As a result of the LPS challenge, microglial deramification and increased TNF-α colocalization occurs in the nucleus accumbens of female rats. For the deramified state, the processes length decreases and the soma size increases.

Materials and Methods



Figures

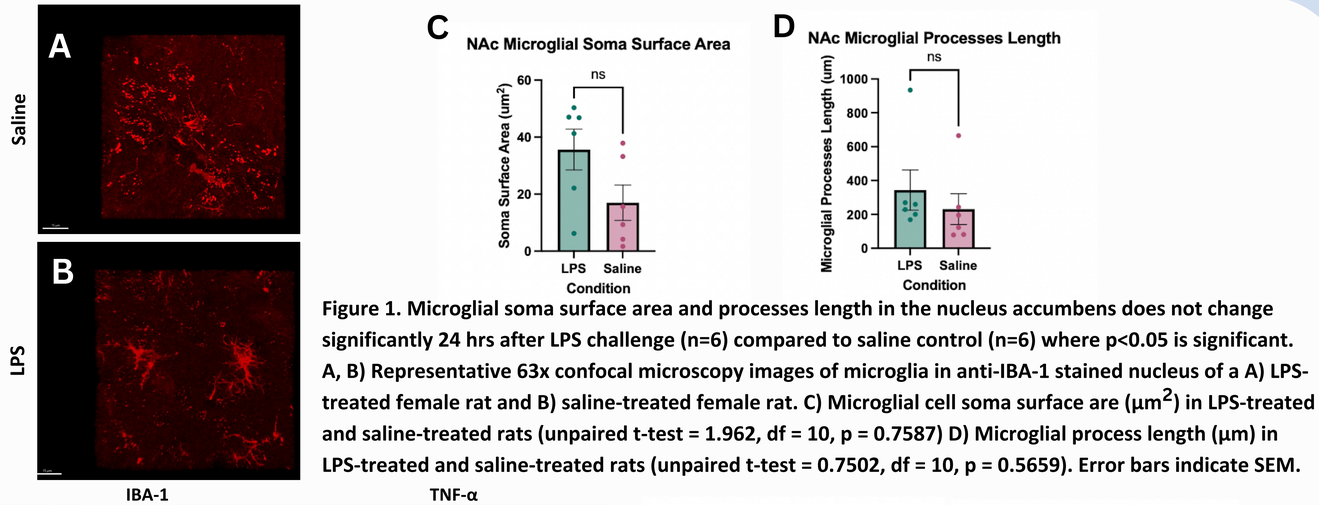


Figure 1. Microglial soma surface area and processes length in the nucleus accumbens does not change significantly 24 hrs after LPS challenge (n=6) compared to saline control (n=6) where $p<0.05$ is significant. A, B) Representative 63x confocal microscopy images of microglia in anti-IBA-1 stained nucleus of a A) LPS-treated female rat and B) saline-treated female rat. C) Microglial cell soma surface are (μm^2) in LPS-treated and saline-treated rats (unpaired t-test = 1.962, df = 10, p = 0.7587) D) Microglial process length (µm) in LPS-treated and saline-treated rats (unpaired t-test = 0.7502, df = 10, p = 0.5659). Error bars indicate SEM.

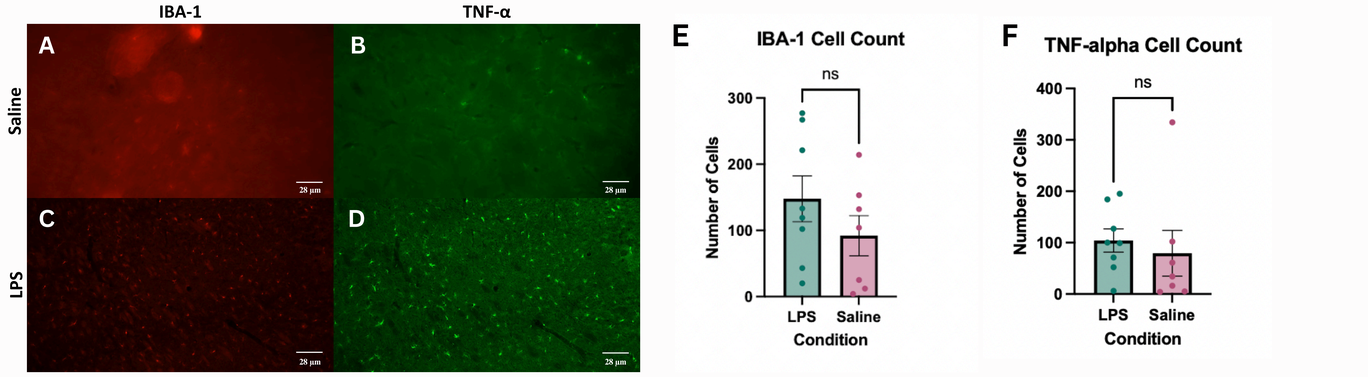


Figure 2. Microglial and TNF-alpha expressing cell count does not change significantly in the nucleus accumbens of female rats 24 hrs after LPS injection (n=8) compared to saline injections (n=7). A, B, C, D) Representative 20x widefield fluorescent microscopy images of the nucleus accumbens of female rat brains stained with anti-IBA-1 and anti-TNF-alpha antibodies. A) Red anti-IBA-1 SPICA Dye 594 fluorescent stain and B) green anti-TNF-alpha AlexaFluor 488 fluorescent stain of cells in the nucleus accumbens of saline-treated female rat brain. C) Red anti-IBA-1 SPICA Dye 594 fluorescent stain and D) green anti-TNF-alpha AlexaFluor 488 fluorescent stain of cells in the nucleus accumbens of LPS-treated female rat brain. E) Microglial cell count in the nucleus accumbens in saline- and LPS-treated female rats (unpaired, two-tailed t-test = 1.062, df = 11, p = 0.4648). F) Cell count of TNF-alpha expressing cells in the nucleus accumbens of saline- and LPS-treated female rats (unpaired, two-tailed t-test = 1.054, df = 11, p = 0.0622). Error bars indicate SEM.

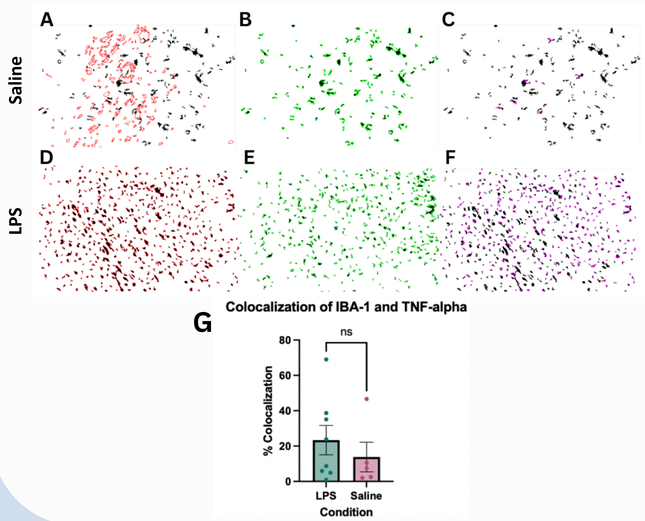


Figure 3. TNF-α colocalization with IBA-1 does not significantly change in the nucleus accumbens of female rats 24 hrs after LPS injections (n=8) compared to saline injections (n=5). A, B, C, D, E, F) Representative 20x widefield fluorescent microscopy images of cells in the nucleus accumbens of a saline-treated rat brain (A, B, C) and LPS-treated rat brain (D, E, F) stained with anti-IBA-1 SPICA Dye 594 and anti-TNF-alpha AlexaFluor 488 antibodies. A) Red anti-IBA-1 SPICA Dye 594 fluorescent stain of cells of the saline-treated female rat brain. B) Green anti-TNF-alpha AlexaFluor 488 fluorescent stain of cells of the saline-treated female rat brain. C) Combined ROI overlay of the GOI (green) and IBA-1 (red) fluorescent stains with colocalization shown in magenta of the saline-treated female rat brain. D) Red anti-IBA-1 SPICA Dye 594 fluorescent stain of cells of LPS-treated female rat brain. E) Green anti-TNF-alpha AlexaFluor 488 fluorescent stain of cells of the LPS-treated female rat brain. F) Combined ROI overlay of the GOI (green) and IBA-1 (red) fluorescent stains with colocalization shown in magenta of the LPS-treated female rat brain. G) % colocalization in the nucleus accumbens of saline-treated and LPS-treated rats (unpaired two-tailed t-test = 0.7713, df = 11, p = 0.6969). Error bars indicate SEM.

Results

Results of our five two-tailed independent samples t-tests indicated that there was NOT a statistically significant difference between our control group and our LPS group.

Implications

- Our results are most likely attributed to sex differences.
- Existing literature determines that it is inconclusive if female rats are more or less sensitive to lipopolysaccharide challenge compared to males[3,4,12].

Future Directions

Future research includes modeling our paradigm in the nucleus accumbens of male rats

Acknowledgements

We would like to thank Dr. Donald Lysle for donating the rodent brain tissue and the College of Arts and Sciences as well as the Department of Psychology & Neuroscience for funding and support of the undergraduate NSCI laboratories.

Additionally, special thanks to Dr. Parekh, Elena Vidrascu, Connie, and Druthi for their mentorship.

References: