Effects of LPS Immune Challenge on TNF-α Expression and Microglial Activation in the Hippocampus of Female Rats

Maya Arora[1], Mahlet Gebrekidan[1], Mia Keller[1], Hari K. Pinapaka[1], Shveta Parekh, PhD (Advisor)

Department of Psychology and Neuroscience, The University of North Carolina at Chapel Hill

UNC COLLEGE OF ARTS AND SCIENCES Psychology and Neuroscience

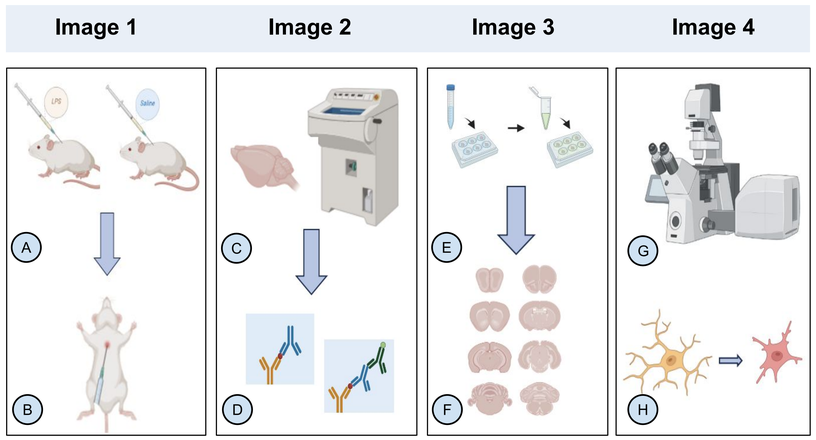
INTRODUCTION

- TNF-α has been implicated in learning and memory dysfunction. For example, Alzheimer's patients with difficulties in learning and memory have high TNF-α levels.[1]
- Following stress, hippocampal microglia release elevated levels of TNF-α in male rats which subsequently impairs working memory.[2]
- There is limited research on the impairment of working memory due to elevated TNF-α levels in female rats.
- The goal of this experiment is to understand how instituting an immune-challenge in a female rodent brain will affect expression of TNF-α in hippocampal microglia.
- Our results aim to begin establishing a link between TNF-α expression and neurodegenerative disorders in female rats.

HYPOTHESIS

TNF-α expression in CA1 hippocampal microglia of female rats will increase following an LPS-induced inflammatory response, as well as their soma size and process length, compared to saline-treated rats.

EXPERIMENTAL DESIGN



RESULTS

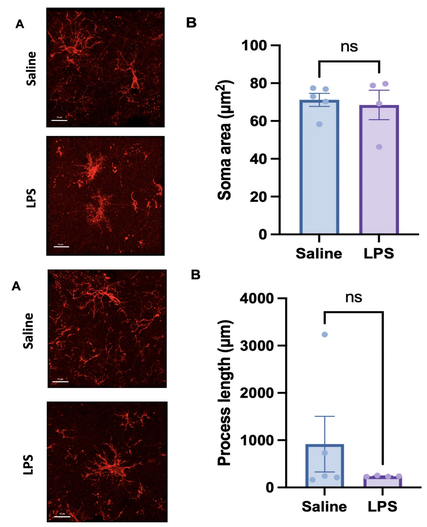


Figure 1. Microglial soma size in the CA1 is not significantly affected 24 hours after LPS immune challenge (n = 4) compared to saline control (n = 5). A) Representative image of 63X confocal microscopy of saline and LPS condition microglia in the CA1. **B)** Area of the microglial soma (µm2) in the LPS versus the saline condition (unpaired two-tailed t(7) = 0.3392, p = 0.744, SEM = 3.5, 7.8 [saline, LPS])) with no significance (ns). Cohen's d = 0.21. Error bars represent SEM. α = 0.05.

Figure 2. Microglial process length in the CA1 is not significantly affected 24 hours after LPS immune-challenge (n = 4) compared to saline control (n = 5). A) Representative image of 63X confocal microscopy of saline and LPS condition microglia in the CA1. **B)** Microglial process length (µm) in the LPS vs saline condition (unpaired two-tailed t(7) = 1.017, p = 0.3432, SEM = 589, 6.2 [saline, LPS])) with no significance (ns). Cohen's d = 0.80.

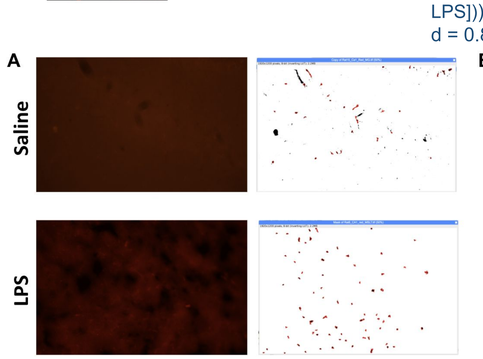


Figure 3. Iba-1 cell count in the CA1 is significantly affected 24 hours after LPS immune-challenge (n = 8) compared to saline control (n = 6). A) Widefield Iba-1 image and corresponding image generated by ImageJ software displaying Iba-1 cell count in CA1 region in saline and LPS condition. **B)** Iba-1 cell count in CA1 region in saline vs. LPS condition (unpaired, two-tailed t(12) = 3.832, p = 0.0024, SEM = 6.2, 13.5 [saline, LPS])) showing significance. Cohen's d = 4.98. Error bars represent SEM.

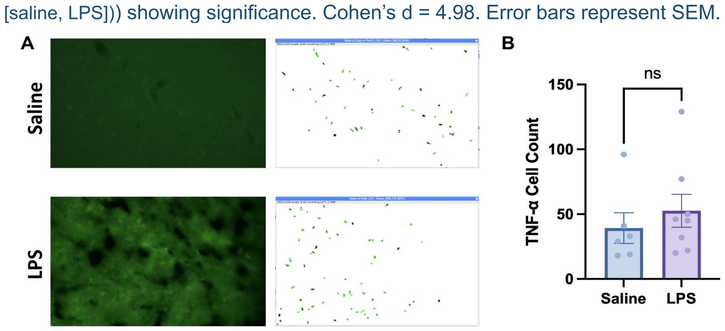


Figure 4. TNF-α cell count in the CA1 is not significantly affected 24 hours after LPS immune-challenge (n = 8) compared to saline control (n = 6). A) Widefield TNF-α image and corresponding image generated by ImageJ software displaying TNF-α cell count in CA1 region in saline and LPS condition. **B)** TNF-α cell count in CA1 region in saline vs. LPS condition (unpaired, two-tailed t(12) = 0.7423, p = 0.4722, SEM = 11.9, 12.7 [saline, LPS])) with no significance. Cohen's d = 0.40.

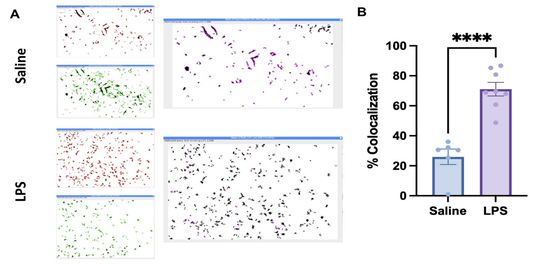


Figure 5. The percentage of colocalization in the CA1 is significantly affected 24 hours after LPS immune-challenge (n = 8) compared to saline control (n = 6). A) Image generated by ImageJ software displaying percent colocalization in CA1 region in saline and LPS condition. **B)** Percent colocalization in CA1 in saline versus LPS condition (unpaired, two-tailed t(12) = 6.532, p < 0.0001, SEM = 4.6, 5.2 [saline, LPS]) with statistical significance between the two groups. Cohen's d = 3.53. Error bars represent SEM of colocalization.

DISCUSSION

- There was no significant difference in the process length and soma area between the LPS and saline groups.
- Colocalization of IBA-1 and TNF-α in the CA1 region was significantly greater in LPS-treated rats.
  - Since there was no significant change in TNF-α cell counts but increased IBA-1 cell count, colocalization indicates that CA1 microglia are producing elevated TNF-α levels.
  - The neuroinflammation associated with increased TNF-α expression aids in the progression of neurodegenerative disorders.
- Understanding the damaging effects of increased TNF-α following immune can provide insight into new therapies via TNF-α inhibition.
- Further studies might:
  - Explore sex differences in TNF-α expression following immune challenge using both male and female rats.
  - Perform behavioral analysis on LPS-treated rats to examine behavioral consequences of TNF-α upregulation.

REFERENCES

1. Takahashi, S., Fukushima, H., Yu, Z., Tomita, H., & Kida, S. (2021). Tumor necrosis factor α negatively regulates the retrieval and reconsolidation of hippocampus-dependent memory. Brain, Behavior, and Immunity, 94, 79–88. https://doi.org/10.1016/j.bbi.2021.02.033
2. Ohgidani, M., Kato, T. A., Sagata, N., Hayakawa, K., Shimokawa, N., Sato-Kasai, M., & Kanba, S. (2016). TNF-α from hippocampal microglia induces working memory deficits by acute stress in mice. Brain, Behavior, and Immunity, 55, 17–24. https://doi.org/10.1016/j.bbi.2015.08.022
3. Images 1-4 were created with BioRender.com

ACKNOWLEDGEMENTS

We would like to express our sincere gratitude to Dr. Shveta Parekh and Elena Vidrascu for their guidance and mentorship throughout this project. Additionally, we extend our appreciation to the Department of Psychology and Neuroscience for their financial support of the NSCI laboratories. Finally, we would like to thank the UNC College of Arts and Sciences for the opportunity to pursue this project.