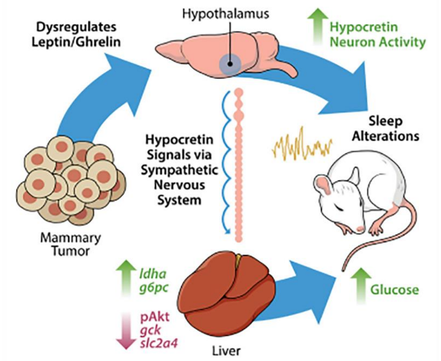
# Characterization of Microglia in the Hypothalamus and Amygdala of Male MMTV-PyMT Carriers

Dhatri Kakarla, Angelina Ross, Lauren Sidelinger, Sabian Martinez
Department of Psychology and Neuroscience, University of North Carolina at Chapel Hill

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

## Introduction

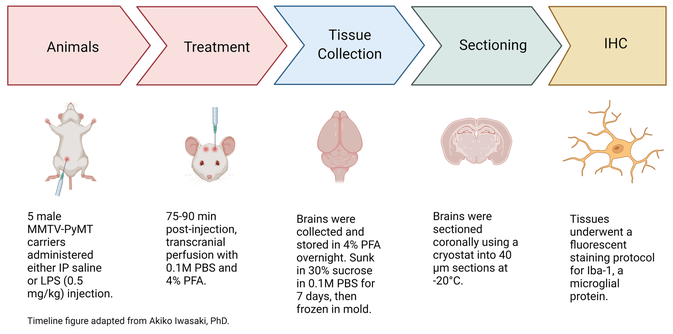
- Although characterized in female mice,[1] the microglial response has not been studied in male MMTV-PyMT carriers.



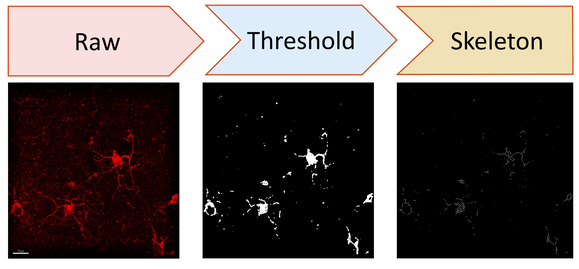

- Investigating the microglial response to an immune challenge[2] in male carriers may suggest mechanisms by which sickness behaviors manifest.
- We compared responses in the hypothalamus and amygdala; these are two brain regions that are implicated in an immune response to stress.[3]

**We hypothesized the hypothalamus and amygdala will have similar microglial activation after saline treatment; the amygdala will have greater activation after LPS.**

## Experimental Design




Timeline figure adapted from Akiko Iwasaki, PhD.




- Images were processed and analyzed using ImageJ. Comparisons were made using unpaired t-tests in GraphPad Prism. Experimental design figures were made with Biorender.com

## Results

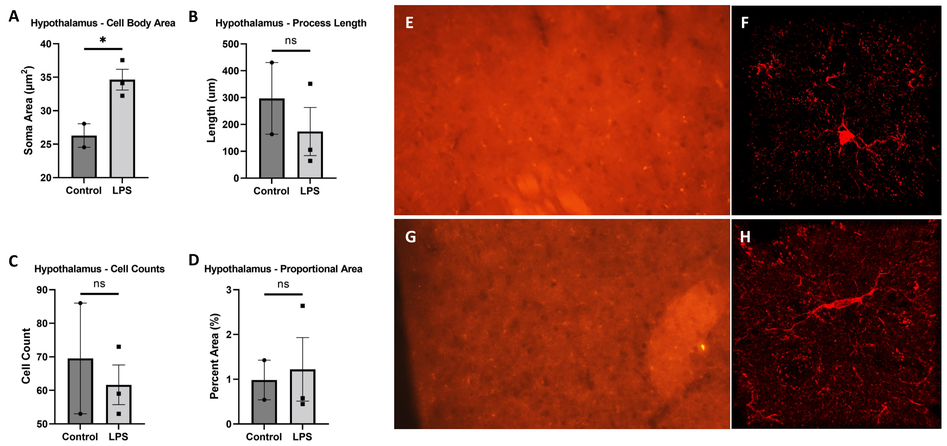



**Figure 1. Effects of LPS injection compared to saline injection in the hypothalamus.** (A) Cell body area, (B) process length, (C) cell counts, and (D) proportional area for microglia in the hypothalamus. *P < 0.05. (E) Representative wide-field (20x magnification) and (F) confocal (63x magnification) images of Iba-1 (AlexaFluor 568 Streptavidin, red) immunofluorescence in the hypothalamus of LPS animal. (G) Representative wide-field and (H) confocal images of Iba-1 immunofluorescence in the hypothalamus of control animal.

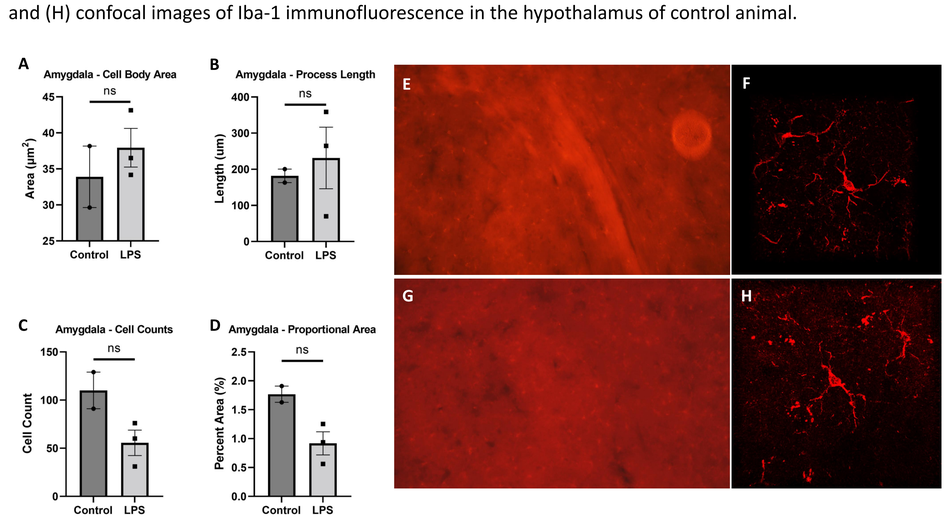



**Figure 2. Effects of LPS injection compared to saline injection in the basolateral amygdala.** (A) Cell body area, (B) process length, (C) cell counts, and (D) proportional area for microglia in the amygdala. $^{ns}P > 0.05$. (E) Representative wide-field (20x magnification) and (F) confocal (63x magnification) images of Iba-1 (AlexaFluor 568 Streptavidin, red) immunofluorescence in the amygdala of LPS animal. (G) Representative wide-field and (H) confocal images of Iba-1 immunofluorescence in the amygdala of control animal.

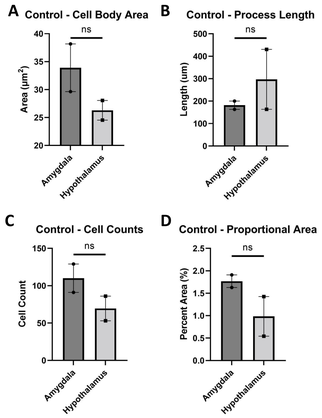



**Figure 3. Comparison of microglia morphology, number, and proportional area between the amygdala and hypothalamus of saline-injected mice.** Difference in (A) cell body area, (B) process length, (C) cell count, and (D) proportional area between the ROIs of saline-injected mice. $^{ns}P \geq 0.05$

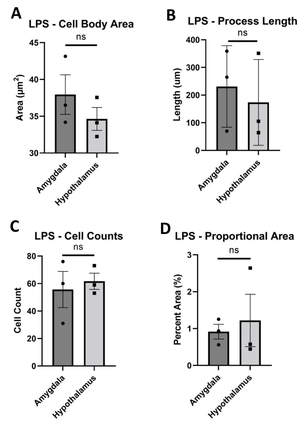



**Figure 4. Comparison of microglia morphology, number, and proportional area between the amygdala and hypothalamus of LPS-injected mice.** Difference in (A) cell body area, (B) process length, (C) cell count, and (D) proportional area between the ROIs of LPS-injected mice. $^{ns}P \geq 0.05$

## Conclusion

- The hypothalamus of LPS mice had significantly greater microglia soma areas than those injected with saline.
- The data suggests the LPS group shows higher microglial activation compared to control in both regions, however the difference is insignificant.
- No significant differences in microglial activation were observed between the amygdala and hypothalamus.

**Limitations**

- A small sample size (n=5) lowered the statistical power of this study.

**Future Directions**

- The activation state of microglia can be further defined as classical/alternative by examining cytokine expression via dual-staining.

## Acknowledgements

- We would like to thank Dr. Jeremy Borniger at Cold Spring Harbor Laboratory for donating the mice tissue.
- We would also like to thank the Graduate Research Consultant Program funded by OUR, Research and Discovery Course Development Grant funded by OUR, and the Psychology and Neuroscience Undergraduate Research Grant funded by Lindquist Undergraduate Research Award for funding.
- Additionally, we would like to thank the Center for Faculty of Excellence, the College of Arts and Sciences & the Department of Psychology and Neuroscience for additional funding and support.
- Finally, we would like to thank our classmates, Dr. Monica M. Gaudier-Diaz, Shveta Parekh, and Olivia Tarpley for their guidance and support.

## References

1. Borniger JC, Walker Ii WH, Surbhi, Emmer KM, Zhang N, Zalenski AA, Muscarella SL, Fitzgerald JA, Smith AN, Braam CJ, TinKai T, Magalang UJ, Lustberg MB, Nelson RJ, DeVries AC. A Role for Hypocretin/Orexin in Metabolic and Sleep Abnormalities in a Mouse Model of Non-metastatic Breast Cancer. Cell Metab. 2018 Jul 3;28(1):118-129.e5.
2. Dong H, Wang Y, Zhang X, Zhang X, Qian Y, Ding H, Zhang S. Stabilization of Brain Mast Cells Alleviates LPS-Induced Neuroinflammation by Inhibiting Microglia Activation. Front Cell Neurosci. 2019 May 3;13:191. doi: 10.3389/fncel.2019.00191. PMID: 31130850; PMCID: PMC6509474.
3. Troubat, R., Barone, P., Leman, S., Desmidt, T., Cressant, A., Atanasova, B., Brizard, B., El Hage, W., Surget, A., Belzung, C., & Camus, V. (2020). Neuroinflammation and depression: A Review. *European Journal of Neuroscience*, *53*(1), 151–171. https://doi.org/10.1111/ejn.14720