Microglial Immunity: Differential Activation between Motor Cortex and Dentate Gyrus

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INTRODUCTION

•Microglia are the major innate immune cells of the central nervous system.

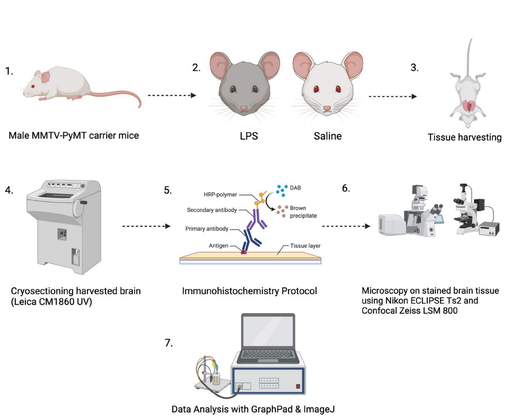
•During an immune challenge, microglial activation within specific brain regions have been shown to elicit aspects of the sickness response such as decreased locomotion and spatial learning deficits.

•This study focuses on the differential expression and activation patterns of microglia in the motor cortex (M1) and the dentate gyrus (DG) in control and immune-challenged male MMTV-PyMT carrier mice.

•Our study works to establish a model to discover which brain regions are more vulnerable to microglial activation and how that translates to the extent of sickness behavior in hopes to provide valuable information in prevention and treatment plans for infections and neurodegenerative diseases.

Is there variation in the microglia activation response in the DG and M1 following LPS injection in immune-challenged mice?

METHODS & MATERIALS



Materials

Solution	Brand
Normal Goat Serum	Vector Labs – S-1000
Streptavidin 568	Thermos – S11226
Streptavidin/Biotin Blocking Kit	Vector Labs – SP-2002
Iba-1 biotinylated	Wako – 016-26461
DAPI Mounting Medium	Vectashield Mounting Medium

Table 1. Materials used during the experiment and their respective manufacturers.

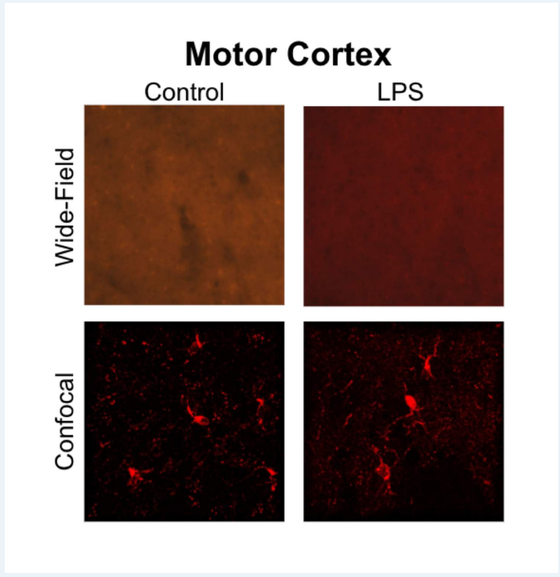
Solution Recipes

Solution	Ratio
0.1 M Phosphate Buffer (~1.3L)	12g sodium phosphate monobasic anhydrous, 500 ml dH_2O. 28.4 g of sodium phosphate dibasic anhydrous, 1L of dH_2O. 500 mL 0.2 M Phosphate buffer, 500 ml dH_2O
Blocking Solution (3ml)	5% Normal Goat Serum, 0.1% Tritonx100
IBA-1 Solution (3ml)	5% Normal Goat Serum, 0.1% Tritonx100, 1:500 rabbit anti-Iba-1-Biotinylated Wako
Streptavidin Solution (3ml)	5% Normal Goat Serum, 0.1 % TritonX100, 1:1000 streptavidin-568 Thermo S11226

Table 2. Ratios of made reagents, including the primary antibody and secondary antibody.

A timeline of the procedure, the materials used, and the dilutions for the created solutions.

RESULTS



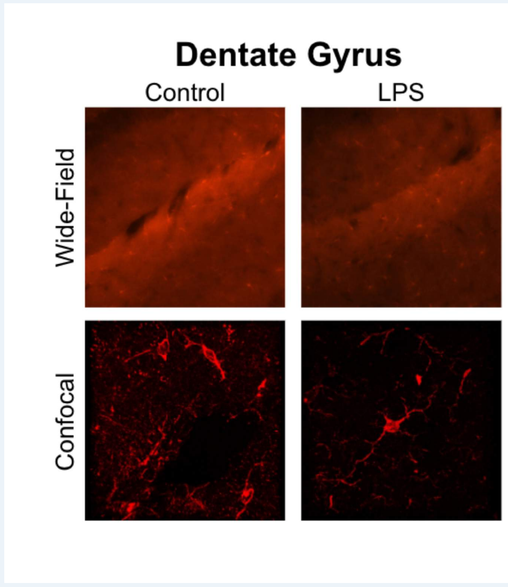


Figure 1. Sample images from wide-field and confocal microscopy. Wide-field images were taken at 568nm excitation at 20x magnification with a Nikon ECLIPSE Ts2 Microscope. Confocal images were taken at 568nm exciatation at 63x magnification with a Zeiss LSM 800 Confocal Microscope.

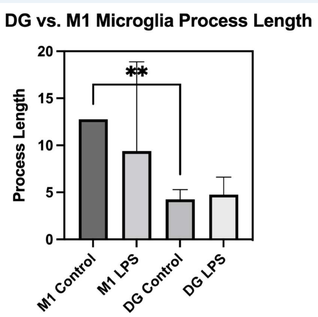
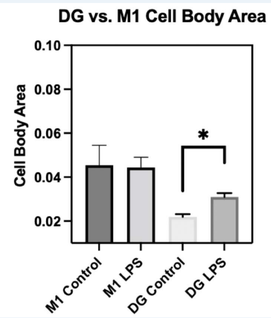


Figure 2. Significant difference in microglia process length between M1 and DG within control group (left). Paired two-tailed t-test between control groups of M1 and DG (p = 0.0049). No significance among the other groups.

Figure 3. Significant increase in cell body area following LPS injection in DG; no significant difference between regions (right). Unpaired t-test between Control and LPS conditions for M1 (p=0.927) and DG (p=0.005). Paired t-test for control (p=0.07) and LPS mice (p=0.052).



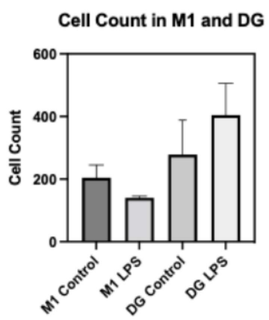
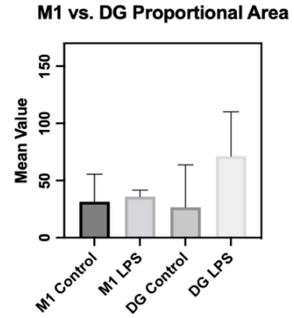


Figure 4. No significant difference in cell count between regions (left). Unpaired, two-tailed t-tests between Control and LPS conditions for M1 (p = 0.1263) and DG (p = 0.2894).

Figure 5. No significant difference in microglial proportional area between or regions (right). Unpaired two-tailed t-test between Control and LPS conditions for M1 (p = 0.8252) and DG (p = 0.2848).



CONCLUSION

The results gathered did not provide many significant relationships, and the ones that manifested did not relate to supporting or refuting our hypothesis.

We were able to draw conclusions, however, such as cell body area increasing in the DG following LPS injection. (Figure 3) Process lengths of microglia were also greater in the motor cortex than the DG. (Figure 2)

Resulting significant differences
Microglia in the M1 region had longer process lengths than microglia in the dentate gyrus region prior to LPS injection
Microglia in the dentate gyrus region experienced an increase in cell body area following LPS injection

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References:

Hines, Dustin J., et al. "Prevention of LPS-Induced Microglia Activation, Cytokine Production and Sickness Behavior with TLR4 Receptor Interfering Peptides." PLoS ONE, vol. 8, no. 3, 2013, doi:10.1371/journal.pone.0060388.

Keller, D., Erö, C., & Markram, H. (2018). Cell Densities in the Mouse Brain: A Systematic Review. Frontiers in Neuroanatomy, 12, 83.

Norden, D. M., Trojanowski, P. J., Villanueva, E., Navarro, E., & Godbout, J. P. (2016). Sequential activation of microglia and astrocyte cytokine expression precedes increased Iba-1 or GFAP immunoreactivity following systemic immune challenge. Glia, 64(2), 300–316.

Tan, Y.-L., Yuan, Y., & Tian, L. (2020). Microglial regional heterogeneity and its role in the brain. Molecular Psychiatry, 25(2), 351–367.

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