INTRODUCTION

• Previous studies on the regional heterogeneity of microglia found that their concentration varies in different brain regions, making some areas more susceptible to neuroinflammation.

• Neuroinflammation due to a peripheral immune challenge has been associated with reduced cognition in female breast cancer patients; however, little is understood about the effects on male breast cancer patients.

• We will observe microglial morphology and cell counts in the substantia nigra and hippocampus of male MMTV-PyMT carrier mice. We aim to understand if cognitive decline may be due to evidence of hyper-vigilant hippocampal microglia.

We predict higher immunoreactivity to an acute peripheral immune challenge in microglia in the hippocampus compared to the substantia nigra of male MMTV-PyMT carrier mice.

EXPERIMENTAL DESIGN

ANIMALS

Male MMTV-PyMT carrier mice.

TREATMENT

Received 0.5 mg/kg LPS or saline injection. Mice were sacrificed 75-90 minutes after injection.

TISSUE COLLECTION

Brains were mounted and 40 um brain slices were obtained using a cryostat.

IMMUNOHISTOCHEMISTRY

Brain sections were treated with Iba-1 primary antibody followed by a streptavidin secondary antibody. Tissues were mounted and stained with DAPI.

MICROSCOPY

Images of microglia in brain regions of interest were taken with fluorescent microscopy (20x) and confocal microscopy (63x, 1-micron Z-stack).

SECTIONING

Male MMTV-PyMT carrier mice.

EXPERIMENTAL DESIGN

INCREASED MICROGLIAL ACTIVATION IN THE HIPPOCAMPUS DUE TO LPS CHALLENGE

Figure 1. Effects of LPS on microglial cell counts in the hippocampus of male MMTV-PyMT carrier mice (a) Cell counts of control (n = 2) and LPS-treated (n = 3) mice. Asterisk indicates statistical significance with a p-value < 0.05. (unpaired t-test = 3.917, df = 3). (b-c) 20x fluorescent microscopy images of the hippocampus. (b) Control with a mean cell count = 62.00. (c) LPS-treated with a mean cell count = 105.3.

LPS CHALLENGE MAY HAVE AN INSIGNIFICANT EFFECT ON SUBSTANTIA NIGRA MICROGLIAL ACTIVATION

Figure 2. Effects of LPS on microglial cell counts in the substantia nigra of male MMTV-PyMT carrier mice. p-value > 0.05 (unpaired t-test = 0.8453, df = 3). (a) Cell counts of control (n = 2) and LPS-treated (n = 3) mice. (b-c) 20x fluorescent microscopy images of the substantia nigra. (b) Control with a mean cell count = 46.50. (c) LPS-treated with a mean cell count = 123.7.

NO SIGNIFICANT LPS-INDUCED CHANGES IN MICROGLIAL MORPHOLOGY IN THE HIPPOCAMPUS

Figure 3. Effects of LPS on microglia morphology in the hippocampus (a) Microglial cell processes lengths in the hippocampus of control (n = 5) and LPS-treated (n = 7) mice. Units are in pixels. p-value > 0.05 (unpaired t-test = 0.1648, df = 10). (b-c) 63x confocal microscopy images of the hippocampus stained with anti-IBA-1. (b) Control with mean process length = 16.62. (c) LPS-treated with mean process length = 15.61.

CONCLUSIONS

• LPS challenge resulted in a significant increase in cell counts in the hippocampus compared to the control which could indicate an increase in microglial activation. While the substantia nigra also demonstrated an increase in cell counts, the changes were insignificant.

• Measurements of proportional area, microglial soma area, and microglial cell processes length showed only insignificant changes in response to LPS-challenge. We are only able to weakly suggest higher immunoreactivity of the hippocampus due to changes in cell count.

• This experiment is limited by the small sample size and an exclusively morphological approach. Further immunohistochemical analysis of microglial cell density and morphology in these brain regions as well as pro-inflammatory cytokine IL-1B will be necessary. Revisions to the experimental design to reduce background noise should also improve the accuracy of the analysis.

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 Lindsley 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 Dr. Monica Gaudier-Diaz, Shveta Parekh, and Olivia Tarpley for their assistance in this research.

References

