Effects of Lipopolysaccharide Immune Challenge on Microglial Activation and CD3+ T-cells in the Substantia Nigra of Female Rats

Mary Linares, Zhuo Yun Song, Sean Ahaotu-Simelane, Phoebe Pak, Samanyu Kunchanapalli, Shveta Parekh Ph.D

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

Introduction

- Parkinson’s disease (PD) is a debilitating motor disorder caused by dopaminergic neuron death in the substantia nigra that affects up to one million Americans.
- Previous research suggests that microglial activation and T-cell infiltration may be associated with PD.
- Few studies have looked at the combined role of CD3+ and microglia in PD pathology.
- The use of female animals in neuroscience research has traditionally remained low compared to the use of males.
- By examining the interaction between microglia and CD3+ T-cells in the substantia nigra of female rats, there is an opportunity to develop and examine new therapies for PD.

Hypothesis

**CD3+ T-cells will be highly colocalized with the activated microglia in the substantia nigra of LPS treated rats compared to saline treated rats.**

Experimental Design

**Rat Brain Treatment**

- LPS
- Saline
- 24 hrs
- Rats sacrificed by transcardial perfusion
- Brains extracted, post-fixed, and stored in 30% sucrose

**Immunohistochemistry**

- Brains sliced to 40 μm
- Immunostained slices with CO3 and Iba-1 antibodies with secondary green and red antibodies to visualize T-cells and microglia
- Image analysis with imageJ

### Results

**MICROGLIAL ACTIVATION BASED ON MORPHOLOGY**

**A** Microglia Process Length

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>LPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Process Length (μm)</td>
<td>250</td>
<td>150</td>
</tr>
</tbody>
</table>

**B** Microglia Soma Size

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>LPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area of Soma (μm²)</td>
<td>75</td>
<td>50</td>
</tr>
</tbody>
</table>

**COLOCALIZATION OF MICROGLIA AND T-CELLS**

**A** IBA-1 Cell Count

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>LPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Cells</td>
<td>150</td>
<td>200</td>
</tr>
</tbody>
</table>

**B** CD3+ Cell Count

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>LPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Cells</td>
<td>150</td>
<td>200</td>
</tr>
</tbody>
</table>

**C** Colocalization of IBA-1 and CD3+

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>LPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Colocalization</td>
<td>0</td>
<td>20</td>
</tr>
</tbody>
</table>

**Discussion**

- The results for colocalization were not significant, but the data shows a pattern of increased colocalization in LPS challenged rats. Therefore, the relationship between microglia and T-cells should continue to be explored in PD pathology and therapies.
- The results for IBA-1 & CD3+ cell count and microglial process length do support our hypothesis. Shorter process lengths indicate microglial activation, and the morphology of microglia in LPS challenged rats changed in this characteristic manner.
- Future directions for research:
  - Analysis of differentiated T-cell presence in the substantia nigra (SN)
  - Examination of cytokine presence to further investigate microglial activation in the SN

References

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