Motor neuron death and increased microglia (resident immune cells in the CNS) are observed in many neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS). Loss of motor neurons (e.g., phrenic and intercostal) innervating respiratory muscles (e.g., diaphragm and intercostal muscles) leads to impaired respiratory function and eventually death in ALS. In addition, increased microglial density correlates with motor neuron death, but whether this increase is beneficial or harmful remains unknown. Microglia can exist in multiple states including resting (ramified morphology) and activated (amoeboid morphology). Increased microglial density is also observed in the phrenic motor nucleus in a novel rat model of respiratory motor neuron death induced by intrapleural injections of cholera toxin B conjugated to saporin (CTB-SAP); however, less is known about microglia in the intercostal motor nucleus. In this study, microglial density and morphology in the intercostal motor nucleus as well as microglial morphology in the phrenic motor nucleus will be analyzed. Cervical and thoracic spinal cord sections containing the phrenic and intercostal motor nucleus from CTB-SAP treated rats will be stained for microglia using immunohistochemistry techniques, visualized using confocal microscopy, and analyzed using ImageJ and IMARIS software. We hypothesize that there will be an increase in microglial density in the intercostal motor nucleus, as well as amoeboid morphological changes in both the phrenic and intercostal motor nuclei. If the data support our hypotheses, this would indicate increased microglial activation in areas controlling respiration and suggest that microglia may play a role in respiratory function.

**Phrenic motor neuron survival**

<table>
<thead>
<tr>
<th>Variables</th>
<th>25 µg CTB-SAP, 7d</th>
<th>25 µg CTB-SAP, 28d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phrenic motor neuron survival</td>
<td>~40%</td>
<td>~40%</td>
</tr>
<tr>
<td>Phrenic motor output</td>
<td>Decreased &gt; 50%</td>
<td>Decreased &gt; 50%</td>
</tr>
<tr>
<td>Respiratory function</td>
<td>Decreased</td>
<td>Decreased</td>
</tr>
</tbody>
</table>

**Rationale**

1. Intrapleural cholera toxin B fragment conjugated to saporin (CTB-SAP) mimics aspects of neuromuscular disorders and neurodegenerative diseases.
2. Microglial density is increased in the phrenic motor nucleus in CTB-SAP treated rats.

**Hypothesis**

In CTB-SAP treated rats, there will be an increase in microglial density in the intercostal motor nucleus, as well as amoeboid morphology in both the phrenic and intercostal motor nuclei.

**Materials & Methods**

**Bilateral, intrapleural injection and tissue preparation:**

A. A 25 µg Cholera toxin B conjugated to saporin (CTB-SAP) was bilaterally, intrapleurally injected into adult male Sprague-Dawley rats (A.).

B. C and D. 7 and 28 day treated control and CTB-SAP treated rats were then perfused with 4% paraformaldehyde, the cervical and thoracic spinal cords containing the phrenic and intercostal nuclei, respectively, were isolated and sectioned at 40 µm using a freezing-sliding microtome, and then sections from C4 (C) and T2–T7 (D) were prepared for immunohistochemistry.

**Implications & Future Directions**

 Increased microglial density and amoeboid morphology would suggest increased microglial activation in the intercostal and phrenic motor nuclei, areas that control inspiration.

Increased microglial activation in these regions could suggest that microglia may play a role in respiratory function. Since respiratory function is impaired in patients that suffer from respiratory motor neuron death (e.g., ALS), knowing the role microglia play can contribute to knowledge of the disease process and potential avenues of therapy.

Future directions will be focused on understanding which factors are produced by microglia in the phrenic and intercostal motor nuclei in CTB-SAP treated rats, and whether these factors impact breathing.

**Expected Results**

- We expect to see increased microglial density in the intercostal motor nuclei of CTB-SAP treated rats vs. controls.
- Amoeboid microglial morphology (e.g., decreased number and length of branches) is expected to be seen in the intercostal motor nuclei and phrenic motor nuclei of CTB-SAP treated rats vs. controls.

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