Establishing TEM markers of motor neuron death and atrophy in a novel dysphagia rodent model

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Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease that results in dysphagia due to weakness of the tongue and other cranial muscles. We propose that swallowing function may be preserved in ALS by enhancing neuroplasticity, particularly in the hypoglossal (XII) motor nucleus that innervates the tongue. One spontaneous mechanism that we hypothesize enhances plasticity in ALS is motor neuron death. However, it is difficult to study XII plasticity in ALS models because the amount and rate at which motor neuron death occurs cannot be controlled, and degeneration is not limited to the XII. Thus, we have developed a novel and inducible model of XII motor neuron death that mimics the behavioral phenotype of dysphagia observed in ALS models. Specifically, we injected cholera toxin B conjugated to saporin (CTB-SAP) into the genioglossus muscle of the tongue; CTB-SAP is then retrogradely transported via the XII nerve to the XII motor nucleus in the brainstem medulla, where it produces targeted death of XII motor neurons. This inducible model appears to meet our needs in terms of targeting the XII motor neurons and recapitulating dysphagia as observed in ALS, but it remains unknown whether ultrastructural changes related to atrophy are occurring. In this study we investigated signs of ultrastructural changes in the tongue, XII nerve, and XII nucleus in CTB-SAP treated rats (N=6) vs. controls (N=5) using transmission electron microscopy (TEM). Our results demonstrate degenerative and ultrastructural changes in the XII nucleus (Fig. 1), XII nerve (Fig. 2), and tongue (Fig. 3). Specifically, our results suggest that there are decreased mitochondrion in the tongue, and a decreased number of axons in the XII nerve in CTB-SAP treated rats. We are currently in the process of quantifying morphological differences in the XII nerve (e.g., size of axons).

Figure 1: Representative TEM images of hypoglossal neurons from a control (left) and CTB-SAP treated rat (middle, right). The yellow dotted line denotes a healthy neuron in the control and the yellow arrows are pointing to the nucleolus.

Figure 2: Representative TEM images from the distal XII nerve from a control (left) and CTB-SAP treated rat (right). Controls have intact axons surrounded by compact myelin sheaths (yellow arrows), whereas the myelin sheaths of CTB-SAP treated are thickened, consistent with degeneration. The number of axons appear to be decreased with CTB-SAP treatment (right), suggesting LMN degeneration in this model.

Figure 3: Representative TEM images from the posterior genioglossus from a control (left) and CTB-SAP treated rat (right). Mitochondrial density (depicted by yellow arrows) appears to be decreased with CTB-SAP treatment (right).