Establishing TEM Markers of Laryngeal Nerve Injury in a Translational Mouse Model

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Dysphagia (swallowing dysfunction), dysphonia (voice dysfunction), and dyspnea (respiratory dysfunction) are common complications after surgical procedures targeting the anterior neck, such as cervical spinal surgery and thyroidectomy. During these procedures, the recurrent laryngeal nerve (RLN), a branch of the vagus (10th cranial nerve), may become inadvertently damaged. RLN injury causes ipsilateral vocal fold (VF) paralysis that contributes to dysphagia, dysphonia, and dyspnea. However, RLN injury is impossible to systematically investigate in human patients; therefore, we have created a translational mouse model to specifically address this major clinical problem. In this study, we used an aneurysm clip with a known closing force to provide a reliable crush injury in our model. Mice were randomly allocated to one of two study end-points to investigate critical phases of nerve regeneration: 3 days post-injury to capture degenerative changes, and 2 weeks post-injury to capture regenerative changes. After functional assessment of the VFs, mice were euthanized for transmission electron microscopy (TEM), using standard protocols, at each time point. Our results demonstrate consistent degenerative changes 3 days post-injury and regenerative changes 2 weeks post-injury in all mice (Figure 1). In addition, the mice regained near normal levels of VF mobility by 2 weeks. Thus, the injury was not severe enough to create long-term VF dysfunction which is required to investigate possible therapeutics for chronic nerve injury. We are currently developing a micro-manipulator controlled tool to deliver a higher compression force to the nerve in attempts at creating chronic dysfunction of the VFs. In addition, we are in the process of identifying metrics for automatic tracking to quantify the nerve cell structures after injury and during the recovery process.

Fig. 1: Representative TEM images of control and experimental RLN (1200x). Left control nerves showed thick myelination and tightly packed axons with associated Schwann cells (S). At 3 days post-crush, the right RLN showed extensive signs of degeneration, indicated by collapsed fibers and dense, compressed myelin debris (asterisks) within the Schwann cells. At 2 weeks post-crush, regeneration of thinly myelinated axons are evident (arrows) within an expanded endoneurium.