Previously, electrical stimulation of a denervated canine laryngeal muscle was shown to promote reinnervation by original foreign motoneurons. A quantitative EMG technique was used to index the appropriateness of reinnervation of the vocal fold abductor (posterior cricoarytenoid, PCA) muscle by inspiratory versus foreign reflex glottic closure (RGC) motoneurons, following recurrent laryngeal nerve section and repair.

In the present study in six canines, a clinical model was used, where both nerves were sectioned and ventilation compromised due to loss of abduction. The PCA muscles were innervated bilaterally with electrodes leading to a pulse generator. Animals were randomly assigned to three groups to assess the effect of different stimulation paradigms on reinnervation quality and degree of functional recovery. 140 pps train, 2150 pps train 30% vs control period. One muscle was applied with 4 sec on 4 sec. off duty cycle during the 3-month post neurotomy regenerative period. In bimonthly sessions, spontaneous vocal fold movement was measured endoscopically during induced hypercapnia in the anaesthetized animal. A treadmill test was applied with a duty cycle of 4 seconds on 4 seconds off (Fig. 2).

Data acquisition in an awake animal: A treadmill exercise test was performed every 2 weeks both with and without stimulation. Blood hemoglobin oxygen saturation was measured with a sensor placed on a tail. The dogs ran for 12 minutes (gradually increasing from 4 mph to 3 miles per hour), or until oxygen saturation dropped below 90% (Fig 3).

In summary, low-frequency stimulation of a denervated muscle simulating activity inhibits motoneuron fibers that are reinnervated. 4 sec off duty cycle

Table 1. Animal Groupings and procedures. 1msec during delivery of a pulse train to a muscle simulating activity. This method was performed in the anesthetized animal. Exercise tolerance was measured on the treadmill exercise test. A 12-month study period showed.

Figure 2. Endoscopic session

Figure 3. Treadmill exercise test

Figure 4. Stimulation inhibited muscle reinnervation. The threshold responses to stimulation decreased over time with reinnervation of the muscles. Note that the 2 groups that received conditioned stimulation during the 3-month post neurotomy regenerative period showed a delayed drop in threshold, particularly muscles stimulated at higher frequency (40 pps), or continuously (left) versus intermittently (right).

Table 2. Evoked EMG recordings from the PCA muscles of the 10 ps pps stimulated animals showed minimal reinnervation by foreign RGC motoneurons, while the nonstimulated control showed complete crossover in this erroneous reinnervation pattern relative to the adductor muscle by PCA inspiratory motoneurons accounted for this dynamic paradoxical closure.

Figure 6. Endoscopic measurements of dynamic airway during hypercapnea after muscle reinnervation. Following 60 second exposure to CO2, non-stimulated and 40 pps stimulated animals showed a significantly greater dynamic phase of global closure which only exacerbated the compromise from their reduced starting passive airways. Misdirected reinnervation of the adductor muscles by PCA inspiratory motoneurons accounted for this dynamic paradoxical closure.

Figure 7. Images of the glottal airway for each animal at rest and following CO2 administration.

Figure 8. Functional outcome evaluated by treadmill performance. Only the 10 pps stimulated animals ran the entire treadmill course throughout the 8-20 month study period. The exercise tolerance in the other two groups dropped to less than 3 minutes at 100 days for the 40 pps stimulated animals and at 150 days for the nonstimulated animals.

CONCLUSION

In summary, low-frequency stimulation of a denervated muscle simulating endogenous motor activity, can direct reinnervation of foreign RGC motoneurons, improves functional recovery, and controls muscle fiber histochemistry.

DISCLOSURE

This study is supported by NIH Grant R01-DC001149 and R01-DC008429.