INTRODUCTION

The ability to reliably restore physiologic movement to a paralyzed vocal fold is not yet in hand. Muscle-nerve-muscle (M-N-M) neurotization is a process whereby axons sprouting from within an innervated muscle are directed toward a denervated muscle via a neural conduit (see Figure 1). Given the paired laryngeal musculature, M-N-M neurotization holds promise for restoring physiologic vocal fold movement in cases of unilateral paralysis. This technique of reinnervation has been successfully demonstrated in the feline thyroarytenoid (TA) muscle. This study is aimed at exploring the effectiveness of M-N-M neurotization in the rat larynx.

RESULTS

All surgical and endoscopic procedures were completed without complications. The TA muscle nerve-motor endplate contact immunohistochemical data demonstrates increased reinnervation of the paralyzed vocal fold in experimental M-N-M graft animals versus controls. The average ratio of nerve-motor endplate contacts was 0.28 in the control group and 0.51 (P<0.01) in the grafted group (see Figure 3 and 4). Endoscopy data were not definitive in separating experimental versus control groups, but confirmed initial vocal fold paralysis (see Figure 5). Myelinated axons were demonstrated within the RLN M-N-M graft used to bridge the innervated and denervated TA muscles (see figure 6). Sections of the trachea showed the resected left RLN did not regenerate spontaneously and, therefore, did not contribute to the reinnervation seen within the paralyzed vocal fold (see Figure 7).

Figure 1. Schematic of the muscle-nerve-muscle neurotization concept.

METHODS & MATERIALS

Twelve male Sprague-Dawley rats were divided equally into two groups: 1) Control and 2) Autologous M-N-M graft. The rats were placed under general anesthesia using inhaled isoflurane. In both groups, the left recurrent laryngeal nerve (RLN) was exposed and a 6mm segment was resected and the proximal and distal ends ligated with silk sutures. In the M-N-M group, the resected segment of RLN was interposed between the innervated right TA muscle and the denervated left TA muscle via a bilateral laryngeal window (see Figure 2). The wound was closed and left vocal fold paralysis was documented using rigid endoscopy. Vocal fold mobility was serially documented bi-weekly via video-assisted rigid endoscopy. The rats were sacrificed at week 12 and the larynges collected and sectioned every 20μm in an axial plane through the TA muscles. The sections were stained for acetylcholinesterase and neurofilament using immunohistochemical techniques in order to define motor endplates and the presence or absence of neural elements at an endplate. Every 7th section was selected and the percent nerve-motor endplate contacts within the left (cut RLN) and right (intact RLN) TA muscles were determined by a blinded observer using light microscopy. Innervated endplates were divided by total number of endplates to determine these numbers. A ratio of left-to-right-sided percent nerve-motor endplate contacts was determined as a measure of reinnervation for control and experimental groups. A two sample t-test was used to calculate statistical significance of difference in ration with an alpha value of 0.05. The RLN’s interposed as nerve grafts in the M-N-N rats were harvested, sectioned and stained for myelin to determine if regenerating axons were present in the grafts. The tracheas were harvested along with the adjacent right and left thyroid lobes and cartilage windows.

CONCLUSIONS

The rat larynx can be used to study the M-N-N neurotization technique of reinnervation. It is logistically easier than a previously employed feline model, but more technically demanding . Current data support the hypothesis that reinnervation can be induced via an autologous nerve graft interposed between the innervated and denervated TA muscles, but the degree of TA innervation is reduced compared to normal control muscle. Physiologic movement of the paralyzed vocal fold was not reliably restored in this study. Future research will focus upon enhancing the efficiency of M-N-N neurotization in this model through the use of nerve growth factors, extra-cellular matrix proteins and nanoscaffolding.