The Laryngoscope
Lippincott Williams & Wilkins, Inc., Philadelphia

End-to-Side Neurorrhaphy and Lateral Axonal Sprouting in a Long Graft Rat Model

B. Goheen-Robillard, MD; T. M. Myckatyn, MD; S. E. Mackinnon, MD; D. A. Hunter, RT

Objective/Hypothesis: **Controversy exists regarding collateral axonal sprouting across an end-to-side neurorrhaphy to provide functional motor reinnervation of a target organ without compromise of the donor nerve. Rat models may be limited in the study of end-to-side repair given potential contamination from the proximal nerve stump of the recipient distal nerve and the use of antagonistic muscle groups for donor and recipient. The current study attempts to address these issues by using a rat model in which an end-to-side coaptation is performed with a long graft interposed between the intact donor tibial nerve and the divided, distal contralateral tibial nerve.** ***Materials and Methods:*** **The graft used in proximal end-to-side coaptation consisted of both sciatic nerves in a donor syngeneic animal. The distal repair to the contralateral tibial nerve was done immediately or in a delayed fashion to allow potential motor axons to transverse the graft before division of the recipient tibial nerve.** ***Results:*** **After 24 weeks, axons were noted to transverse the entire distance of the graft and into the contralateral distal posterior tibial nerve. A significant increase in axonal numbers was observed in the immediate repairs compared with the delayed. No animal recovered functional motor ability on the contralateral side as assessed by walking tracks.** ***Conclusions:*** **These findings suggest the importance of immediate distal neurotrophic factors in encouraging nerve regeneration even in a long graft end-to-side repair. Our model is successful in demonstrating innervation through an end-to-side coaptation but questions its use given the lack of motor recovery.** ***Key Words:*** **End-to-side neurorrhaphy, termino-lateral repair, axonal sprouting.**

Laryngoscope, **112:899–905, 2002**

INTRODUCTION

Recovery of function following a nerve injury frequently yields less than satisfactory results. Prolonged denervation of distal motor targets often leads to irreversible muscle atrophy after such an injury, especially when a primary repair of the injured nerve is not feasible. The novel strategy of end-to-side repair in this scenario could potentially provide a source of motor axons both close to the recipient target muscle and without causing donor deficit. End-to-side repairs have been conclusively shown to facilitate sensory sprouting. If a motor axon is divided, such as in partial hypoglossal to facial nerve transfers, the proximal portion will regenerate through an end-to-side nerve repair to innervate the new recipient target. What remains controversial is whether motor axons from an intact nerve will de novo sprout into an end-to-side neurorrhaphy. In other words, does the transected distal end of the recipient nerve stimulate collateral sprouting from intact motor axons without donor disruption?

Criticism of end-to-side neurorrhaphy models includes the use of ipsilateral nerves for donor and recipient that supply antagonistic muscle groups with little regard for the subsequent disruption of spinal and cortical pathways (i.e., peroneal nerve vs. tibial nerve).[1] Another concern is that axons from the cut proximal stump of the recipient nerve may inadvertently contaminate the repair.[2] Recent studies, primarily in the rat, looking at end-to-side neurorrhaphy have rendered variable results with little consistency in terms of surgical procedure, technique, or subsequent histologic, electrodiagnostic, or functional assessment. Some investigators have suggested that both motor and sensory collateral sprouting can occur with end-to-side repair[3,4] with subsequent physiological recovery of previously denervated muscle[5,6] and meaningful functional recovery.[3] Conversely, others have shown that predominantly sensory regeneration occurs in an end-to-side neurorrhaphy, even at 4 to 6 months postprocedure survival.[7,8]

The current study endeavors to address some of these unresolved issues by coapting a nerve graft end-to-side to an intact tibial nerve. The graft is brought across the midline and anastomosed to the distal end of the contralateral tibial nerve to attempt reinnervation of the corresponding contralateral muscle group. This avoids the use of antagonistic muscle groups necessary in other end-to-side models. Functional recovery was based on regeneration from motor neurons originating in the contralateral spinal column from the target muscle, which will help

From the Departments of Otolaryngology–Head and Neck Surgery (B.G.-R.) and Surgery, Division of Plastic Surgery (T.M.M., S.E.M., D.A.H.), Washington University School of Medicine, St. Louis, Missouri, U.S.A.

Supported by the Peripheral Nerve Research Fund of the Division of Plastic Surgery, Washington University.

Editor's Note: This Manuscript was accepted for publication January 16, 2002.

Send Correspondence to Susan E. Mackinnon, MD, Washington University, Division of Plastic Surgery, One Barnes Hospital Plaza, Suite 17242, St. Louis, MO 63110, U.S.A. E-mail: mackinnons@msnotes.wustl.edu

to further delineate the use of end-to-side neurorrhaphy in donor nerves not originating in the same dorsal root ganglion or motor neuron pool as the recipient.

MATERIALS AND METHODS

Experimental Design

Adult male Lewis rats (weighing 250–400 g) were housed in a dedicated animal facility and received water and chow ad libitum. Animal care complied with all institutional and National Institutes of Health guidelines. Animals were divided into six groups with 10 animals per group (Fig. 1). Group 1 had an end-to-side neurorrhaphy performed between the left distal peroneal nerve and ipsilateral tibial nerve. In groups 2 to 4, a graft from a syngeneic donor was used to create an end-to-side neurorrhaphy from the donor left tibial nerve to the recipient right tibial nerve. The distal end of the nerve graft was not repaired but left free in the right thigh. Group 3 animals underwent a second staged procedure at 8 weeks in which the distal end of the graft was sutured to the divided distal stump of the contralateral tibial nerve. Group 4 animals had the repair to the contralateral distal tibial nerve immediately performed at the initial procedure. Animals were weighed and walked before surgery and every 2 weeks before being killed at 24 weeks. A final group of 20 animals were included in group 1 and 3 but were killed after 2 weeks to assess for signs of early Wallerian degeneration of the distal donor nerve.

Surgical Procedure

Animals were anesthetized for surgery through a subcutaneous injection of 75 mg/kg ketamine (Fort Dodge Animal Health, Fort Dodge, IA) and 1 mg/kg medetomidine hydrochloride (Pfizer Animal Health, Exton, PA). A standard hind limb muscle splitting approach was used to expose the left sciatic and tibial nerves, using a Wild M651 operating microscope to aid in identification (Leica Microsystems, Deerfield, IL). In group 1 animals, the peroneal nerve was transected 1 to 3 mm distal to the sciatic nerve trifurcation. A 0.5-mm slit was made in the epineurium and perineurium of the tibial nerve. The cut end of the distal peroneal nerve was mobilized and coapted with four 10-0 microepineurial sutures end-to-side to the slit in the tibial nerve. Care was taken to enclose all proximal nerve stumps in silastic tubing to control for contamination.

Groups 2 to 4 had similar exposure of their left tibial nerve. A graft was prepared from a donor syngeneic animal at the same time by harvesting both sciatic nerves. The nerves were joined end to end to create the 8-cm graft. After creation of the perineural window in the donor tibial nerve, an end-to-side neurorrhaphy was performed with the nerve graft. The graft was tunneled subcutaneously across the back of the animal and allowed to rest in proximity to the contralateral tibial nerve. The contralateral hind leg of animals from group 3 was re-explored after an 8-week period, and the distal portion of the nerve graft was released from the surrounding fibrous capsule. It was then sutured to the transected distal end of the contralateral tibial nerve. Group 3 animals were not chosen until after the initial surgical procedure, thus blinding the surgeon to animals that were to have a subsequent distal repair. Group 4 had the same distal repair performed at the time of initial surgery. The proximal divided end of the tibial nerve was similarly enclosed in silastic tubing to prevent contamination.

Walking Track Analysis

Walking track analysis was used to evaluate rate hindfootprint patterns as previously described.[9] Walking tracks were performed before any intervention and every 2 weeks thereafter until the animals were killed. The footprint length was hand-measured on the left side to assess pre- and postoperative walking tracks for any evidence of loss of function related to the donor nerve. Walking track analysis was also performed on animals after the graft was repaired to the contralateral tibial nerve. The investigator performing the walking tracks was blinded from the animal groups by an additional investigator (T.M.) who selected the animals without identifying them to the first investigator (B.G.).

Histomorphometric Analysis

Donor left tibial nerve, including the end-to-side repair, the recipient distal or graft nerve, and a portion of the contralateral tibial nerve in groups 5 and 6, were obtained from the animals for histologic examination. Immediately after harvest, nerves were fixed and stored *en bloc*. Half of the nerves were fixed in a 3% glutaraldehyde solution and washed in 0.1 mol/L phosphate buffer (Fischer Scientific, Fair Lawn, NJ) and postfixed in 1% buffered osmium tetroxide (Polysciences, Warrington, PA), rinsed in 0.1 mol/L phosphate buffer, and dehydrated in a graded series of ethanol solutions. Nerve segments were then infiltrated with a graded Araldite-propylene oxide (Fischer Scientific) mixture and embedded in Araldite 502 (Polysciences). Nerves were cut into 1-μm cross sections using a LKB III Ultramicrotome (LKB Produkter A.B., Broma, Sweden). Sections were stained using 1% toluidine blue and visualized under light microscopy to assess nerve architecture, extent of myelination and nerve innervation, and the presence of Wallerian degeneration.

Microscopic images were studied using a digital image-analysis system linked to morphometry software (Leco Instruments, St. Joseph, MI) using a video monitor calibrated to 0.125 μm/pixel. Cross sections throughout the grafts were assessed by the computer for total fascicular area and total fiber numbers through digitized information based on gray and white scales. Six randomly selected fields per nerve (or a minimum of 500 myelin-

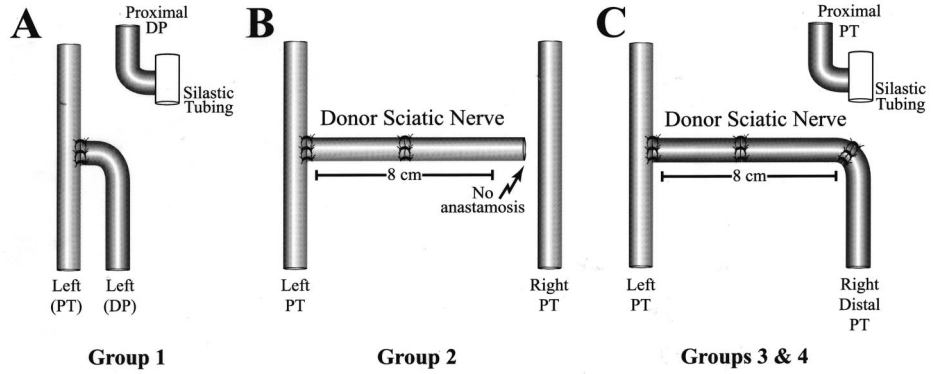

Fig. 1. Schematic diagram outlining the surgical procedures performed within each animal group.

ated fibers) were evaluated under 1000× magnification for axon width, myelin width, and fiber diameter. These primary values were used to calculate the total number of myelinated fibers, percentage of neural tissue (100× neural area/intrafascicular area), and percentage of neural debris (100× area of debris/intrafascicular area). The investigator performing the analysis (D.H.) was unaware of the group from which each nerve was obtained while performing this analysis.

The remaining harvested nerves were embedded in paraffin blocks and 4-μm sections were taken longitudinally at the site of repair. The sections were subsequently stained with neurofilament protein antibody (Dako Corp., Carpinteria, CA) that was conjugated using the AEC Chromagen stain (Dako Corp.) for visualization under light microscopy.

Fast blue retrograde tracer (Sigma, St. Louis, MO) was used on two animals from group 4 at 24 weeks survival time to assess origin of axons within the spinal cord. Injections were performed with a microglass pipette into the graft distal to the repair site. After 4 days, the animals were deeply anesthetized and transcardially perfused with 100 mL of 0.9% saline, followed by 500 mL of 4% paraformaldehyde/0.25% glutaraldehyde in 0.1 mol/L sodium acetate buffer (pH 6.5), followed by 4% paraformaldehyde in 0.1 mol/L sodium borate buffer (ph 9.5), and then 10% sucrose in 0.1 mol/L Sorenson's phosphate buffer. The spinal cords were carefully removed at levels L4 to L5. Longitudinal serial frozen sections (15 μm) were cut with a cryostat and mounted on slides. Epifluorescence microscopy with an ultraviolet filter was used to examine sections for labeled motoneurons.

RESULTS

Histologic examination of the distal donor tibial nerve beyond the end-to-side repair showed mild evidence of Wallerian degeneration involving from 5% to 20% of the nerve in the 2-week survival groups (Fig. 2). These animals showed some degenerating fibers proximal to the site of repair as well. In the 2-week groups, the recipient nerve or graft showed extensive Wallerian degeneration on examination without evidence of ingrowth of axons.

Animals in the 24-week survival groups showed no evidence of donor axonal degeneration proximal or distal to the neurorrhaphy (Fig. 3). Group 1 showed abundant axonal growth across the end-to-side repair between the tibial and the distal peroneal nerve (Fig. 3). The number of fibers in cross sections averaged 2630 ± 502 (Fig. 4). There was a normal distribution of smaller than average nerve fibers without evidence of Wallerian degeneration up to 2 cm distal to the end-to-side repair. Walking tracks of group 1 showed a footdrop on the operated side, as would be expected after division of the peroneal nerve. This group showed the most evidence of regeneration into the donor of the experimental groups, as evidenced by the greatest width and density of fibers, as well as having the largest percentage of nerve fibers within the nerve.

Group 2 with a graft but no distal repair also showed minimal evidence of damage to the donor tibial nerve, with a mild amount of distal donor nerve Wallerian degeneration, as well as no difference in tibial walking track function pre- and post-procedure. There was regeneration noted across the end-to-side neurorrhaphy into the graft (1300 ± 993 fibers) (Fig. 4). On neurofilament staining of sections, regeneration was noted to occur across the entire graft, but with decreasing density of fibers in the more distal portions of the graft. Histologically, the graft had

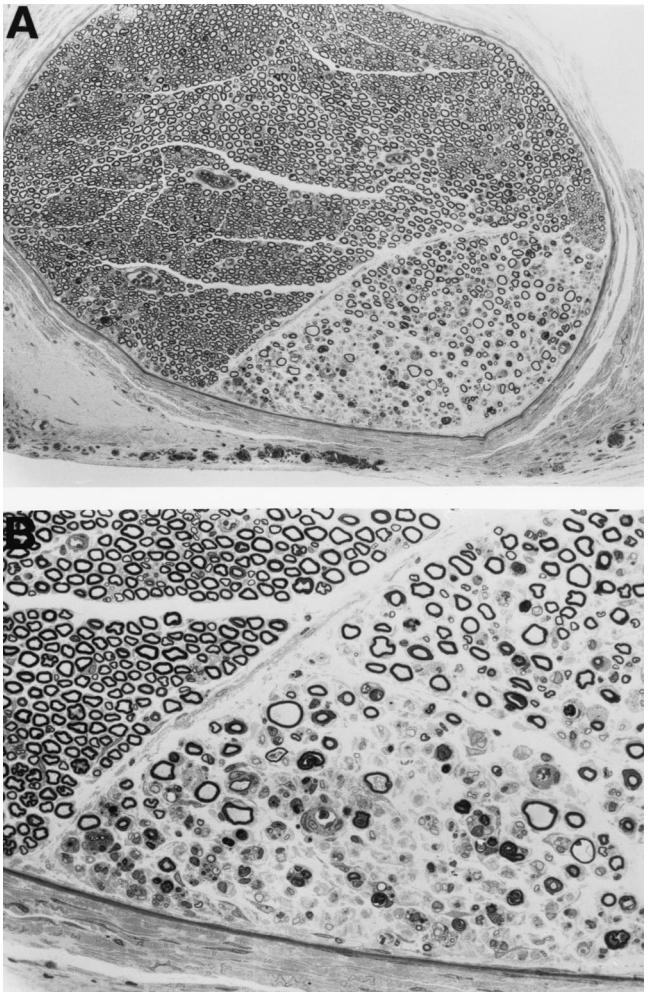

Fig. 2. Light micrographs demonstrating evidence of Wallerian degeneration in distal donor tibial nerve at 2 weeks post-procedure. (**A**) Low-power micrograph showing focal degeneration within the donor nerve. (Toluidine blue stain; x53). (**B**) Higher-power magnification demonstrating clear demarcation between normal nerve architecture and focal injury (Toluidine blue stain; x537).

evidence of ischemia and edema throughout, with the layers of the perineurium markedly thickened, and increased collagen and fibroblast infiltration between the perineurial cells. The endothelium of the vessels within the graft was markedly thickened. There was a gradual decline of the number of fibers noted within the graft progressing from proximal to distal from the repair site (Fig. 5).

Group 3 animals with the delayed repair also displayed intact functioning of the donor tibial nerve after graft placement, as demonstrated by consistent walking tracks. On re-exploration of the contralateral hind leg, the distal end of the graft was encased in dense scar tissue from which it had to be mobilized. Axons were again noted on neurofilament staining to traverse the entire length of the graft, but were fewer in number than group 1. These grafts contained 1326 ± 892 fibers, similar to that observed within group 2 (Fig. 4). There was also histologic evidence of ischemic damage, with edema of the peri-

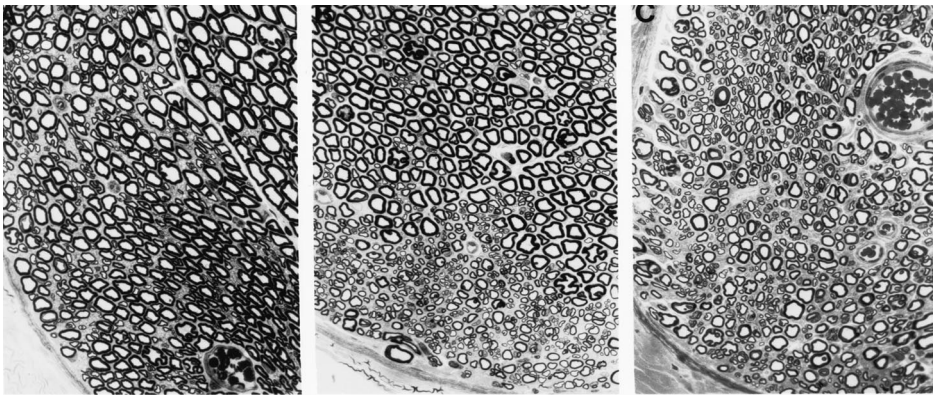

Fig. 3. Photomicrograph illustrating donor tibial nerve and recipient distal peroneal nerve in group 1 at 24 weeks. (**A**) Proximal donor nerve demonstrating absence of Wallerian degeneration. (**B**) Distal donor nerve in which a number of axons show decreased diameter, indicating regeneration secondary to axonal injury at the time of repair. (**C**) Distal peroneal nerve showing extensive regeneration across the repair site and into the nerve (Toluidine blue stain; x537).

neurium and a small number of blood vessels within the graft (Fig. 5). Walking tracks on these animals after contralateral division of the tibial showed the expected lengthening of the print length resulting from loss of gastrocnemius function.[9] No animal showed any subsequent significant change in walking tracks at any survival point.

Group 4 animals with immediate end-to-end repair of the graft to the contralateral tibial nerve also showed minimal evidence of Wallerian degeneration of the distal donor axons. Histologic examination of these grafts demonstrated a significant number of regenerating axons (6507 ± 2983) along the length of the graft and across the end-to-end repair into the contralateral tibial nerve. This group had increased fiber density when compared with the other graft groups and was equal to group 1. The fiber width was similar to that of groups 2 and 3 (Fig. 4). The walking tracks showed initial elongation of the contralateral print. Despite the large number of nerve fibers, the prints remained elongated and at no point showed shortening, which would indicate return of contralateral gastrocnemius function.

There was significantly less edema of the perineurium in the group 4 grafts when compared with groups without or delayed distal repair. A greater number of blood vessels were also noted between the fascicles, suggesting increased angiogenesis. There was also less collagen and fibroblast infiltrate within the graft, correlating with the increased fiber density (Fig. 5). Neurofilament staining of these nerves demonstrated axonal growth originating from the donor tibial nerve across the full length of the graft and entering into the distal contralateral tibial nerve (Fig. 6). Retrograde labeling performed on two ani-

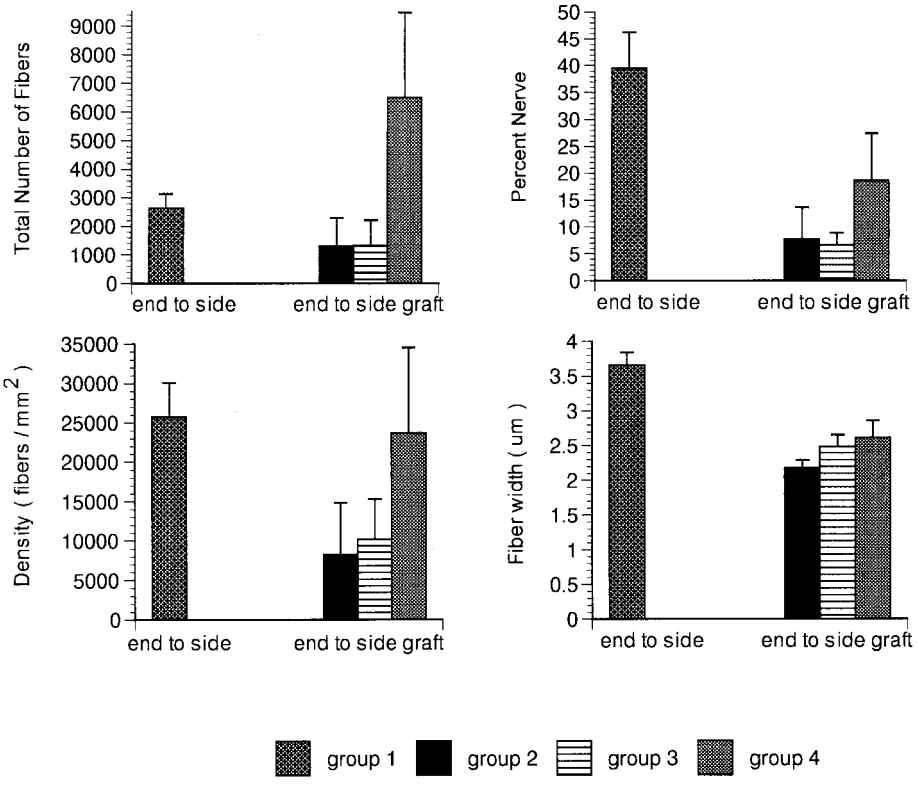

Fig. 4. Histomorphometry data demonstrating a comparison of number of nerve fibers, percent nerve within the perineural sheath, density of fibers, and width of fibers between the different groups.

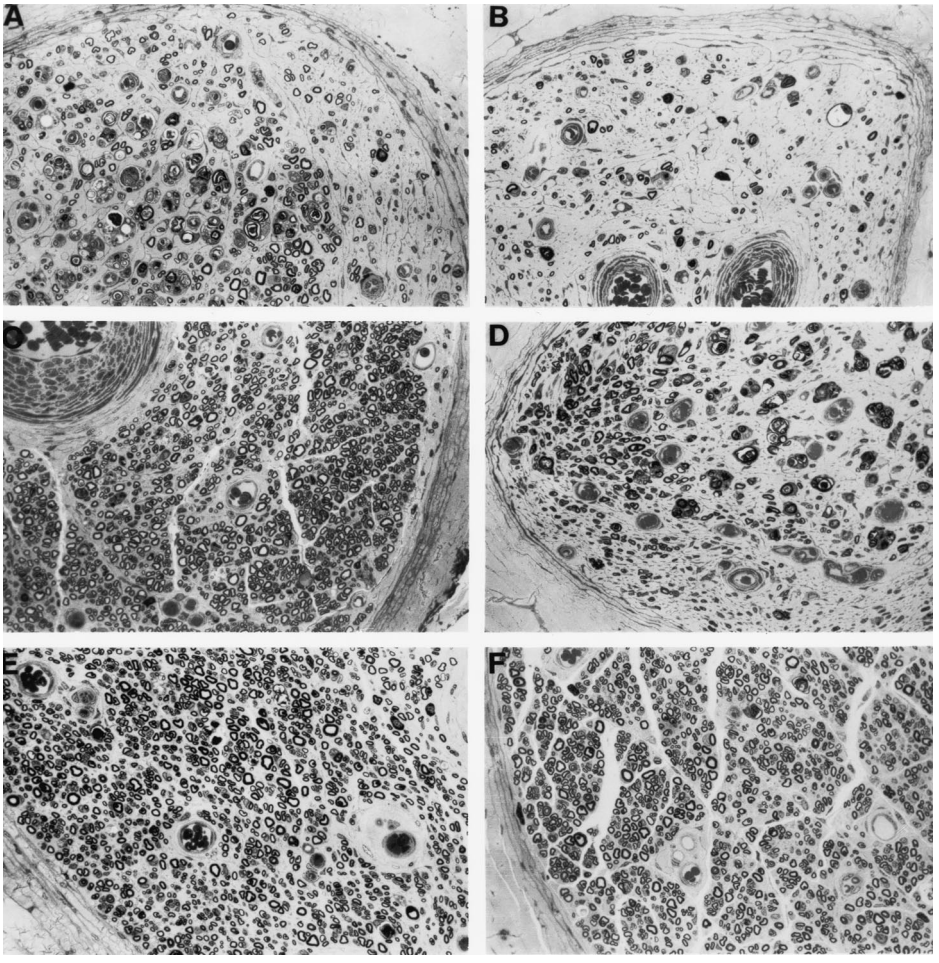

Fig. 5. Photomicrograph showing proximal and distal grafts in groups 2, 3 and 4. (**A** and **B**) Group 2. Evidence of ischemia demonstrated by perineurial thickening and collagen infiltration of graft. Note even fewer fibers in the distal graft as compared with the proximal graft, along with continued signs of injury. (**C** and **D**) Group 3. Ischemic damage is evident in the graft, with thickened endothelial lining of the blood vessels and fibroblast infiltration. (**E** and **F**) Group 4. Proximal and distal graft demonstrates increased axonal fibers as compared with groups 2 and 3. There is decreased evidence of ischemia and collagen in the graft (Toluidine blue stain; x537).

mals from group 4 demonstrated scattered, sparse motor neuron labeling of the ipsilateral spinal cord at the L4 to L5 level. Four motoneurons were identified in the first animal, and five motoneurons were labeled in the second. There was no staining observed within the contralateral cord at this level.

DISCUSSION

In 1903, Ballance first reported using end-to-side neurorrhaphy for reconstruction of the facial nerve damaged by ear disease.[10] The technique fell into disuse because of conflicting and disappointing results following this report. Interest in this repair technique was renewed by the use in rat models and facial palsy patients by Viterbo and colleagues in 1992 with reports of good preliminary results.[3] However, end-to-side neurorrhaphy remains controversial because of inconsistent findings with regard to motor innervation. Given the variability of findings in the current studies, it is imperative to continue to address the question of functional motor recovery after such a repair.

Recovery of peripheral nerves after injury is the culmination of a series of complicated molecular, cellular, biochemical, and electrophysiological mechanisms. These occur at multiple levels within both the peripheral and central nervous system.[11] The complexity of this process may account for the inconsistency of findings within various experimental models of end-to-side neurorrhaphy.

Like in other studies of end-to-side repairs, the current model demonstrates that regeneration of axons across the repair occurs with minimal deficits to the donor nerve. Creation of a perineural window in the donor nerve did cause some mild axonal injury in our study, as evi-

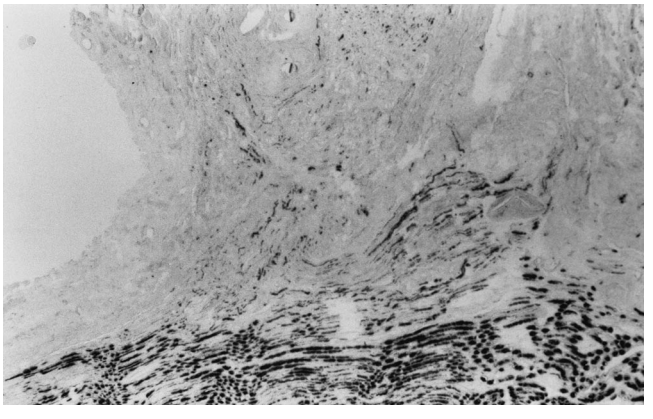

Fig. 6. Light micrograph of neurofilament staining in a graft from group 4 at the site of repair. Fibers entering the graft from the donor nerve are observed (x134).

denced by the observed Wallerian degeneration. However, walking track analysis failed to demonstrate any evidence of functional impairment to the donor nerve. Walking track analysis may not be sensitive enough to detect subtle changes in motor function, but this does suggest that the level of injury did not significantly impact on the performance of the donor muscle group. Prior studies have suggested an intact epineurium and perineurium acts as a barrier to collateral sprouting,[4,12–14] but the process of removal may create axonal injury that accounts for at least a portion of the collateral sprouting at an end-to-side repair. Other end-to-side models have also demonstrated degenerating axons within the donor nerve after repair,[4,15] but the literature is conflicting regarding the amount and impact of axonal injury occurring with end-to-side repair. This study again questions whether lateral sprouting occurs from intact donor axons or only after injury to these axons and will need to be addressed in further studies.

Another matter complicating our model and proposed clinical uses of end-to-side repair is the atrophy of the target muscle during the time interval it takes for regeneration to occur. Most models examine regeneration over a short distance in which prompt innervation of the muscle occurs.[3,5,6,8] However, in many clinical applications for peripheral nerve injury, loss of muscle fibers before innervation is a concern. The animals in our study did not regain functional use of the contralateral gastrocnemius muscle despite the demonstration of axonal growth through the graft and into the distal tibial stump at prolonged survival times. Whether this was the result of lack of motor axons within the graft, inability of central pathways to elicit a response in contralateral muscle groups, need for even greater regenerative times, or an irreversible atrophy of the target muscle itself is not clear and will need to be examined in future studies.

We designed our model to prejudice in favor of demonstrating motor recovery. The group in which the distal repair was delayed was intended to minimize the duration of denervation of the target muscles. This was to increase the likelihood of any motor axons present in the graft to be able to reinnervate these muscles before irreversible atrophy. Similarly, if there was contamination from the proximal tibial nerve on the contralateral side it should result in functional tibial recovery. Even with these maneuvers, no recovery was seen with walking track analysis. Also, examination of the origin of axons within the graft using retrograde tracing methods demonstrated few motor cell bodies, suggesting primarily sensory axons available to innervate the distal target. Perhaps our method of detecting motor reinnervation was not sensitive enough to observe a positive recovery, but these findings do question if end-to-side repair can provide enough motor axons to have clinical use.

The increased regeneration noted with immediate distal repair also suggests that the presence of a distal target is important in encouraging axonal growth from the onset of repair, even when a significant distance separates the donor and recipient nerves. Neuronal growth is enhanced by neurotrophic factors emanating from the degenerating distal nerve stump in all types of repairs.[16] Although the graft underwent degeneration as well, our findings demonstrated that the immediate neurorrhaphy of the graft to a distal nerve provided a dramatic improvement in axonal growth when compared with the grafts with delayed or no distal contact.

This would indicate that the delay in repair of the distal end of a graft after end-to-side neurorrhaphy is more deleterious to the growth of axons across the graft than is benefited by attempting late repair to avoid muscle atrophy. Not all muscle systems undergo atrophy after denervation, which is thought to be secondary to the effect of accessory neuronal input such as the autonomic system. Further studies will be important to understand the mechanism behind these findings of increased regeneration with the use of an immediate distal target in the end-to-side graft repairs.

Our model expands the study of nerve regeneration after an end-to-side repair by designing an animal model that may be more clinically applicable, given the distance that nerves typically need to regenerate after damage within humans. A graft allows the use of contralateral donor and recipient nerves, decreasing the possibility of collateral contamination and allowing synergistic muscle groups to be studied. The ability of axons to regenerate through a graft from the side of an intact tibial nerve to the contralateral distal tibial nerve stump demonstrates the potential for collateral sprouting axons to traverse long distances. However, further investigation of end-to-side neurorrhaphy is warranted given the findings of this study before widespread clinical use.

BIBLIOGRAPHY

1. Lutz B, Chuang C, Hsu J, Ma S, Wei F. Selection of donor nerves—an important factor in end-to-side neurorrhaphy. *Br J Plast Surg* 2000;53:149–154.
2. McCallister WV, Tang P, Trumble TE. Is end-to-side neurorrhaphy effective? A study of axonal sprouting stimulated from intact nerves. *J Reconstr Microsurg* 2001;15: 598–604.
3. Viterbo F, Trindade JC, Hoshino K, Mazzon A. Two end-to-side neurorrhaphies and nerve graft with removal of the epineural sheath: experimental study in rats. *Br J Plast Surg* 1994;47:75–80.
4. Lundborg G, Zhao Q, Kanje M, Danielsen N, Kerns J. Can sensory and motor collateral sprouting be induced from intact peripheral nerve by end-to-side anastomosis? *J Hand Surg* 2001;19B:277–282.
5. Tham S, Morrison W. Motor Collateral sprouting through an end-to-side nerve repair. *J Hand Surg* 1998;23A:844–851.
6. Kalliainen L, Cederna P, Kuzona W. Mechanical function of muscle reinnervated by end-to-side neurorrhaphy. *Plast Reconstr Surg* 1999;103:1919–1927.
7. Tarasidis G, Watanabe O, Mackinnon S, Stausberg S, Haughey B, Hunter D. End-to-side neurorrhaphy resulting in limited sensory axonal regeneration in a rat model. *Ann Otol Rhinol Laryngol* 1997;106:506–512.
8. Tarasidis G, Watanabe O, Mackinnon S, Stausberg S, Haughey B, Hunter D. End-to-side neurorrhaphy: a long-term study of neural regeneration in a rat model. *Otolaryngol Head Neck Surg* 1998;119:337–341.
9. Hare G, Evans P, Mackinnon S. Walking-track analysis: a long-term assessment of peripheral nerve recovery. *Plast Reconstr Surg* 1992;89:251–258.
10. Ballance C, Ballance H, Stewart P. Remarks on the operative treatment of chronic facial palsy of peripheral origin. *BMJ* 1903;2:1009–1013.
11. Lundborg G, Dahlin L, Danielsen N, Zhao Q. Trophism, tro-

pism, and specificity in nerve regeneration. *J Reconstr Microsurg* 1994;10:345–354.
12. Bertelli J, Soares dos Santos A, Calixto J. Is axonal sprouting able to transverse the conjunctival layers of the peripheral nerve? A behavioral, motor, and sensory study of end-to-side nerve anastomosis. *J Reconstr Microsurg* 1996;12: 559–563.
13. Okajima S, Terzis J. Ultrastructure of early axonal regeneration in an end-to-side neurorrhaphy model. *J Reconstr Microsurg* 2000;16:313–326.
14. Al-Qattan MM, Al-Thumyan A. Variables affecting axonal regeneration following end-to-side neurorrhaphy. *Br J Plast Surg* 1998;51:238–242.
15. Giovanoli P, Koller R, Meuli-Simmens C, et al. Functional and morphometric evaluation of end-to-side neurorrhaphy for muscle reinnervation. *Plast Reconstr Surg* 2000;106: 383–392.
16. Seckel B, Ryan S, Gagne R, Chiu T, Watkins E. Target specific nerve regeneration through a nerve guide in the rat. *Plast Reconstr Surg* 1986;78:793–798.