OPEN

PRS GLOBAL OPEN EXPERIMENTAL

Effect of Axonal Trauma on Nerve Regeneration in Side-to-side Neurorrhaphy: An Experimental Study

Henrikki Rönkkö, MD*
Harry Göransson, MD, PhD†
Hanna-Stiina Taskinen, MD, PhD‡
Pasi Paavilainen, MD†
Tero Vahlberg, MSc§
Matias Röyttä, MD, PhD¶

Background: Side-to-side (STS) neurorrhaphy can be performed distally to ensure timely end-organ innervation. It leaves the distal end of the injured nerve intact for further reconstruction. Despite encouraging clinical results, only few experimental studies have been published to enhance the regeneration results of the procedure. We examined the influence of different size epineural windows and degree of axonal injury of STS repair on nerve regeneration and donor nerve morbidity.
Methods: Three clinically relevant repair techniques of the transected common peroneal nerve (CPN) were compared. Group A: 10-mm long epineural STS windows; group B: 2-mm long windows and partial axotomy to the donor tibial nerve; and group C: 2-mm long windows with axotomies to both nerves. Regeneration was followed by the walk track analysis, nerve morphometry, histology, and wet muscle mass calculations.
Results: The results of the walk track analysis were significantly better in groups B and C compared with group A. The nerve fiber count, total fiber area, fiber density, and percentage of the fiber area values of CPN of the group C were significantly higher when compared with group A. The wet mass ratio of the CPN-innervated anterior tibial muscle was significantly higher in group C compared with group A. The wet mass ratio of the tibial nerve–innervated gastrocnemial muscle was higher in group A compared with the other groups.
Conclusions: All three variations of the STS repair technique showed nerve regeneration. Deliberate donor nerve axotomy enhanced nerve regeneration. A larger epineural window did not compensate the effect of axonal trauma on nerve regeneration. *(Plast Reconstr Surg Glob Open 2016;4:e1180; doi: 10.1097/GOX.0000000000001180; Published online 22 December 2016.)*

In proximal nerve injuries, the main clinical problem is nerve regeneration: how to reach the end organs in sufficient time before muscle atrophy occurs. Distal end-to-end nerve transpositions and end-to-side (ETS) repair have been used in these situations, but with these techniques, the distal end of the nerve is, at least partially, reserved for neurorrhaphy and, thus, cannot be used for further reconstructions. The side-to-side (STS) nerve repair technique, which leaves the distal nerve end free, was introduced by Yüksel et al[1] in 1999. They reported

*From the *Department of Orthopaedics, Hatanpää City Hospital, Tampere, Finland; †Department of Hand and Microsurgery, Tampere University Hospital, Tampere, Finland; ‡Department of Hand Surgery, Turku University Hospital, Turku, Finland; and §Institute of Clinical Medicine, §Department of Biostatistics, and ¶Department of Pathology/Neuropathology, Turku University Hospital, University of Turku, Turku, Finland.*
Received for publication July 6, 2016; accepted October 25, 2016.
Part of the results of this paper have been presented at the following meetings: 5th Vienna Symposium on Surgery of Peripheral Nerves, Vienna, Austria, March 21 to 23, 2014: p 12 (abstract and oral presentation): Axonal Trauma in Side-to-Side Repair of Peripheral Nerve - Histomorphometric Results and World Society for Reconstructive Microsurgery, World Congress Chicago, Ill., July 11 to 14, 2013: p 103 (abstract and oral presentation): Axonal Trauma in Side-to-Side Repair of Peripheral Nerve.

DOI: 10.1097/GOX.0000000000001180

Disclosure: *Dr. Rönkkö has received financial support for this study from the following foundations: Research Foundation of Instrumentarium, Finnish Research Foundation for Orthopaedics and Traumatology, competitive research funding of the Pirkanmaa Hospital District, and Medical Research Fund of Turku University Hospital. None of the other authors has any financial disclosures. The Article Processing Charge was paid for by Medical Research Fund of Turku University Hospital.*

histological regeneration and functional recovery in their experimental study. Also, clinical sensory recovery[2,3] and functional improvement[3,4] have been achieved with STS repair. In our previous study, the morphometric and functional results of the STS repair were comparable with the more commonly used ETS repair technique.[5]

The optimal size of STS neurorrhaphy enabling regeneration of nerve repair is not known. In the present study, we varied the size of the epineural window and performed a deliberate axotomy to examine their influence on nerve regeneration and functional recovery.

MATERIALS AND METHODS

Animals

Twenty-four female young adult Sprague Dawley rats (Central Animal Laboratory, University of Turku, Turku, Finland) weighing 242 to 293 g were used in the present study. The National Animal Experiment Board approved all interventions, the analgesic treatment, and animal care. The animals were fed laboratory chow and allowed to drink tap water freely.

Operative Procedure

The animals were randomly divided into 3 groups. Anesthesia was carried out with an intraperitoneal injection of 5 μg/kg medetomidine hydrochloride (Domitor; Orion Oyj, Espoo, Finland) and 750 μg/kg ketamine hydrochloride (Ketalar; Pfizer Oy, Helsinki, Finland). The fluid balance was maintained perioperatively with a 5-mL subcutaneous injection of 9 mg/mL sodium chloride (Fresenius Kabi AB, Uppsala, Sweden). The left common peroneal nerve (CPN) was ligated with 2 sequential 8-0 polyamide sutures (Nylon; S&T AG, Neuhausen Switzerland) 5 mm distally to the bifurcation of the left CPN and tibial nerve (TN). The CPN was transected between the ligations. In group A, 10-mm-long epineural windows were performed microsurgically to the distal CPN and to the TN. Neurorrhaphy between the nerves was performed with ten 11-0 polyamide sutures (Monosof; Covidien, Mansfield, Mass.) under a surgical microscope (Wild M3Z; Wild Leitz Ltd, Heerbrugg, Switzerland). In group B, 2-mm long epineural windows were performed similarly to the previous group. In addition, a donor nerve partial axotomy to the extent of one half of the nerve was cut with microscissors. In group C, 2-mm-long epineural windows were performed similarly to the previous group, and axotomies to one half of the nerve were cut to both donor and recipient nerves. In groups B and C, neurorrhaphy was performed with four 11-0 sutures. In all groups, the ligated stumps of the CPN were turned to the opposite direction and fixed to the adjoining muscles with three 10-0 polyamide sutures (Nylon; S&T AG). The muscle and skin were closed with 5-0 polyglycolic acid sutures (Deknatel Bondek Plus; Teleflex Medical, Durham, N.C.). The analgesic treatment was ensured with a subcutaneous injection of 5 mg/kg carprofen (Rimadyl; Vericode Ltd., Dundee, United Kingdom) during 3 postoperative days.

Walk Track Analysis

The walk track analysis was performed preoperatively and 2, 4, 6, 8, and 12 weeks after surgery. The print length (PL; distance between the heel and the third toe) and toe spread (TS; distance between the first and fifth toe) were calculated as a mean of 3 measurements. The following formula was used to determine the peroneal function index: PFI = 174.9 ([EPL − NPL]/NPL) + 80.3 ([ETS − NTS)/NTS) − 13.4 in which "N" refers to the normal, unoperated side and "E" to the experimental side.[6] The investigator was blinded to know the intervention groups when analyzing the footprints. He had passed the self-education test of the walk track analysis.[7]

Sample Preparation

At 12 weeks, the animals were terminal anesthetized with an intraperitoneal injection of 60 mg/kg sodium pentobarbital (Mebunat; Orion Oyj).

Seven of 8 animals per group were perfused intracardially with 4% phosphate-buffered formalin. The operated nerves, the tibial anterior and gastrocnemial muscles of the operated limb, and the corresponding tissues from the contralateral side were carefully excised with microsurgical instruments and operating loupes. The muscles were weighed with a balance (PG403-S DeltaRange; Mettler-Toledo GmbH, Greifensee, Switzerland). Tissue samples for further studies were immersion fixed in phosphate-buffered formalin overnight. Nerve biopsy sites are seen in Figure 1. From the paraffin blocks, 4-μm-thick sections were cut for neurofilament immunohistochemistry or hematoxylin and eosin staining.

Table 1. Results of Morphometric Analyses of Common Peroneal and Tibial Nerve

	Nerve Area (μm²)	Fiber Count	Mean Fiber Area (μm²)	Total Fiber Area (μm²)	Fiber Density (n/mm²)	Percentage of Fiber Area (%)
Common peroneal nerve						
Group A	87,251 (19,648)	1,930 (364)	3.2 (0.51)	6,131 (1,762)	22,991 (7,101)	7.1 (1.6)
Group B	89,815 (13,809)	2,683 (515)	3.4 (0.58)	9,363 (3,043)	29,813 (3,001)	10.2 (2.2)
Group C	85,718 (15,007)	2,866 (310)	3.6 (0.71)	10,257 (2,287)	33,984 (4,743)	12.3 (3.7)
Group B stump	115,489 (14,267)	1,993 (329)	3.4 (0.83)	6,818 (2,071)	17,291 (2,217)	5.8 (1.4)
Group C stump	121,869 (31,720)	2,333 (330)	3.7 (0.81)	8,493 (1,938)	20,054 (5,409)	7.0 (1.0)
Tibial nerve						
Group A	315,264 (59,736)	5,772 (513)	11.3 (2.66)	65,276 (17,784)	18,855 (3,840)	20.4 (2.7)
Group B	295,929 (62,227)	6,188 (759)	9.77 (4.26)	60,154 (25,509)	21,588 (4,094)	20.0 (6.8)
Group C	297,575 (32,041)	5,937 (677)	9.99 (1.40)	59,100 (9,415)	20,232 (3,366)	19.9 (2.2)

Data are expressed in terms of mean (SD).

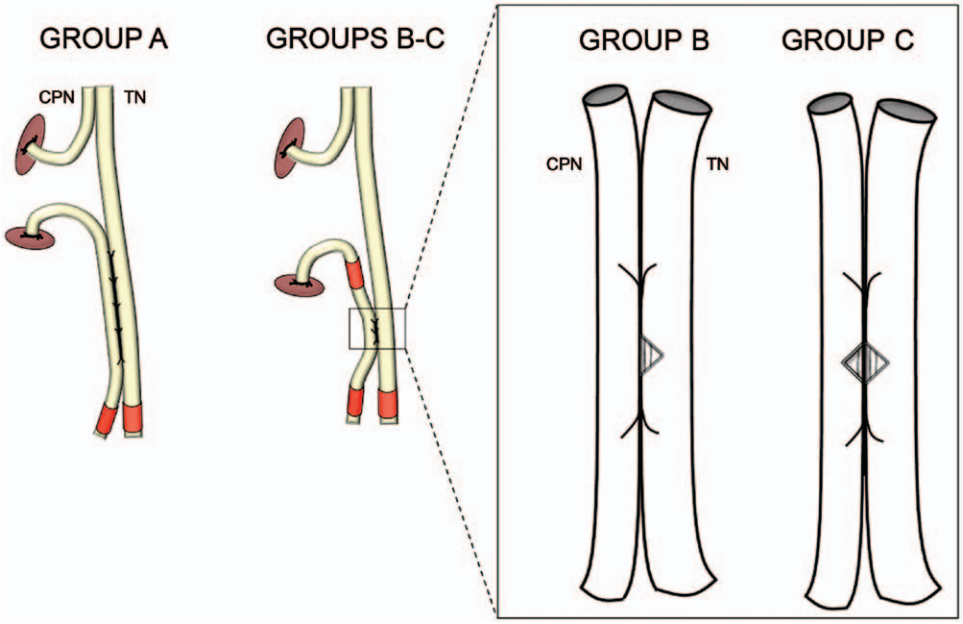

Fig. 1. Schematic diagram of experimental groups. Side-to-side repair between the distal end of transected CPN and parallel TN was performed in 3 different ways. In group A, 10-mm long epineural window was performed to both nerves. In group B, 2-mm long epineural windows were performed with tibial nerve axotomy. In group C, 2-mm long epineural windows and axotomies to both nerves inside epineural windows were performed. Red marks show the biopsy sites for morphometry.

One of 8 animals per group was perfused intracardially with 4.4 mL 0.1 M Millonig phosphate buffer and 0.6 mL 25% glutaraldehyde. The samples were postfixed with osmium tetroxide, dehydrated, and embedded in epon. One-micrometer sections were stained with toluidine blue. The qualitative analysis was performed with toluidine blue–stained sections.

Neurofilament Protein Immunocytochemistry

Morphometry was performed with immunohistochemically stained samples of 7 animals of 8 per group. The stainings were performed with a biotin-free Poly-HRP-Anti-Mouse kit (BrightVision; Immunologic BV, Duiven, The Netherlands) according to the instructions of the manufacturer. Mouse monoclonal neurofilament (200 and 68 kDa) Ab1 (Clone 2F11) antibody (Thermo Fisher Scientific, Fremont, Calif.) was applied and incubated. Normal antibody diluent (Immunologic BV) was used to dilute and stabilize HRP conjugates. The sections were then incubated with peroxidase-compatible chromogen (Bright-DAB; Immunologic BV).

Morphometry

The whole-nerve cross-sections of immunohistochemically stained samples were photographed with an AxioCam HRc camera (Carl Zeiss, Göttingen, Germany) connected to an AxioVert 200M microscope (Carl Zeiss). The images were stitched as a mosaic image by using AxioVision software (Carl Zeiss). The digitalized images of subperineural, transsectional areas of the nerves were processed by using imaging software (Graphics Suite X6/Photo-Paint; Corel Corp., Ottawa, Ontario, Canada). From the final grayscaled images, the nerve area (mm^2), nerve fiber count, and areas of the nerve fibers (μm^2) were measured with BioImageXD.[8] The following outcomes were calculated: total nerve fiber area (sum of nerve fiber areas [μm^2]), nerve fiber density (nerve fiber count/nerve area [number/mm^2]), mean nerve fiber area (total nerve fiber area/fiber count [μm^2]), and percentage of the nerve fiber area (total nerve fiber area/nerve area × 100; Table 1).

Statistical Analysis

The statistical analyses were done with SPSS (version 21; IBM Corp., Armonk, N.Y.) and SAS System for Windows (version 9.4; SAS Institute Inc., Cary, N.C.). On the basis of the power analysis, the sample size of 7 animals per group gives 90% power and a type I error rate of no more than 5% to detect a difference of 15 or more in the mean PFI values between the groups. This presumption is based on our previous study in which the STS repair group reached a PFI value of −40.3±12.2 SD in a 12-week follow-up.[5] In the present study, a sample size of 8 animals was selected. An experienced statistician supervised the statistical analysis. Differences with P values less than 0.05 were considered statistically significant. Type 3 tests of fixed effects were used to reveal a significant difference between the intervention groups.

The comparisons between the groups of the results of the walk track analysis were analyzed with the analysis of covariance for repeated measurements, which was adjusted for baseline PFI values. Heterogeneous autoregressive covariance structure was used to consider the correlation between repeated measurements in these longitudinal data. The Tukey–Kramer adjustment was used to control the effect of multiple comparisons.

The nerve fiber count, nerve fiber density, total nerve fiber area, percentage of the nerve fiber area, and nerve area outcomes are expressed as mean values (SD). The groups were compared using the two-way analysis of variance analysis with Tukey–Kramer adjustment for multiple comparisons. Comparison of the distal site of neurorrhaphy of CPN to the distal stump of CPN (Fig. 1) was performed with the paired *t* test.

In comparisons of the nerve fiber area, the data were well correlated because of the thousands of values from each animal. This was taken into account using the linear mixed model with random intercept for animal. The fiber area values were normally distributed after $\log_{10}$-transformation. The effect of multiple comparisons was taken into account with Tukey–Kramer and Dunnett adjustments.

The wet mass ratios were compared using the Kruskal–Wallis test and the Mann–Whitney *U* test with Bonferroni adjustment for multiple comparisons.

RESULTS

Walk Track Analysis

The change in the PFI values was different between the groups (group by time interaction effect, $P < 0.001$). At 12 weeks, groups B and C showed significantly higher values than group A (PFI: A, −48.8 [10.7]; B, −35.7 [9.1]; C, −37.0 [6.3]; Fig. 2). The values of groups B and C did not differ significantly. The intervention groups reached better results than the unrepaired group (PFI, −75.8 [12.0]). The result of the unrepaired group is derived from our previous study.[5]

Morphometry

CPN

The nerve fiber count values of groups B and C were significantly higher compared with group A (both $P < 0.007$). The total nerve fiber area, nerve fiber density, and percentage of the nerve fiber area of group C were significantly higher when compared with group A (Fig. 3). The mean nerve fiber area values did not differ between the 3 groups.

In groups B and C, nerve biopsies of CPN were taken from both sides of neurorrhaphy (Fig. 1). The nerve fiber count, nerve fiber density, and percentage of the nerve fiber area were significantly higher distal to the neurorrhaphy compared with the distal stump in both groups. The nerve area of the distal stump was larger compared with the distal site in group B. The values of the distal stump did not differ between groups B and C (Fig. 3).

TN

The nerve area, nerve fiber count, total nerve fiber area, nerve fiber density, and percentage of the nerve fiber areas did not differ significantly between the groups. The mean values of the nerve fiber area of group A were higher compared with the values of groups B and C (both $P < 0.0001$), but there was no difference between groups B and C.

Light Microscopy

The distal stump of CPN (proximal to the side of neurorrhaphy; Fig. 1): there were no differences between the groups. Several axons and plenty of fibrosis were seen in the specimens of all groups.

Distal CPN: in all groups, a number of axons could be seen, part of them well myelinated. A small amount of fibrous tissue was present, and in more distal sections, the amount of fibrosis was further decreased. In groups B and C, the axonal regeneration seemed better compared with group A (Fig. 4).

Distal TN: there were very few signs of nerve injury in group A. Occasionally, in groups B and C, the axons seemed to be smaller in the lateral zones compared with the central zones.

Anterior tibial muscle: the size of the muscle fiber was slightly decreased, and some angular-shaped cells could be seen in all groups. The changes were focal in groups B and C but wider in group A. The histology of the specimens of the contralateral unoperated side looked normal (Fig. 5).

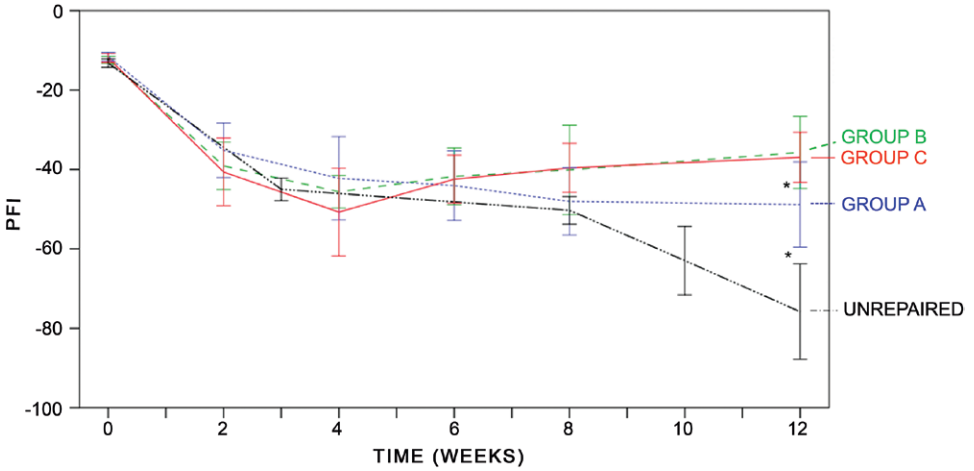

Fig. 2. Results of the walk track analysis. At 12 weeks, groups B and C showed significantly better results than group A. All experimental groups had significantly better values than the unoperated controls. The unrepaired group was derived from our previous study.[5] The data were analyzed with the analysis of covariance with Tukey–Kramer adjustment for multiple comparisons (*$P < 0.05$; error bar, ± 1 SD).

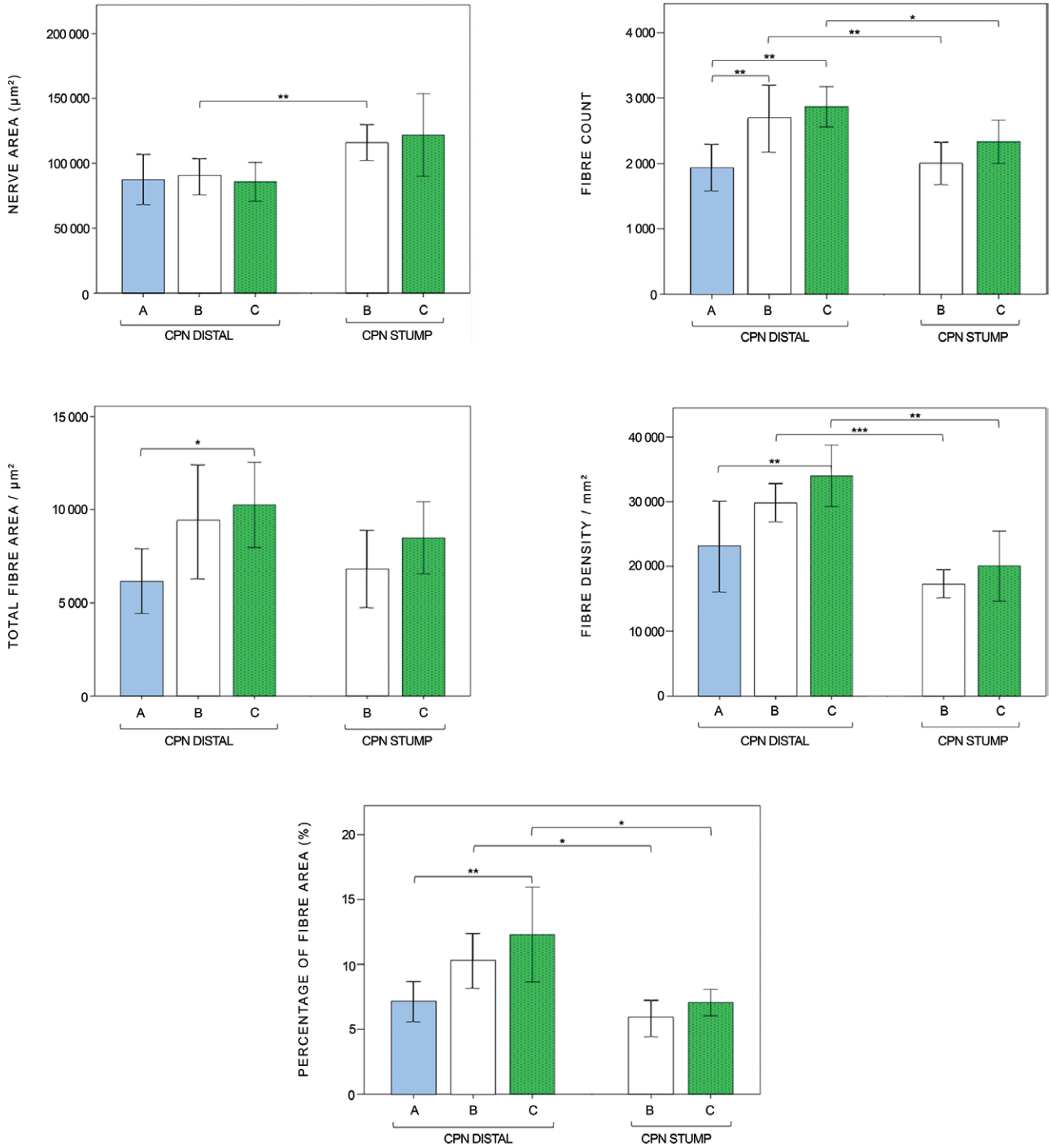

Fig. 3. Morphometric results of the CPN. A, The nerve areas of the groups did not differ. B, The values of the nerve fiber count of groups B and C were significantly higher compared with group A (both $P < 0.007$). The total nerve fiber area (C), nerve fiber density (D), and percentage of the nerve fiber area values (E) of group C were significantly higher compared with group A. The nerve fiber count, nerve fiber density, and percentage of the nerve fiber area values were significantly higher in the CPN distal site compared with the distal stump in groups B and C. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$. Error bar, ± 1 SD.

Gastrocnemial muscle: the general appearance was normal in group A. There were focal areas of atrophy in groups B and C, but as a whole, these findings seemed mild (Fig. 5).

Muscle Mass Calculations

In group C (57.2% [3.5]), the anterior wet mass ratio of the tibial muscle was significantly higher compared with group A (46.1% [6.3]). There was no difference between

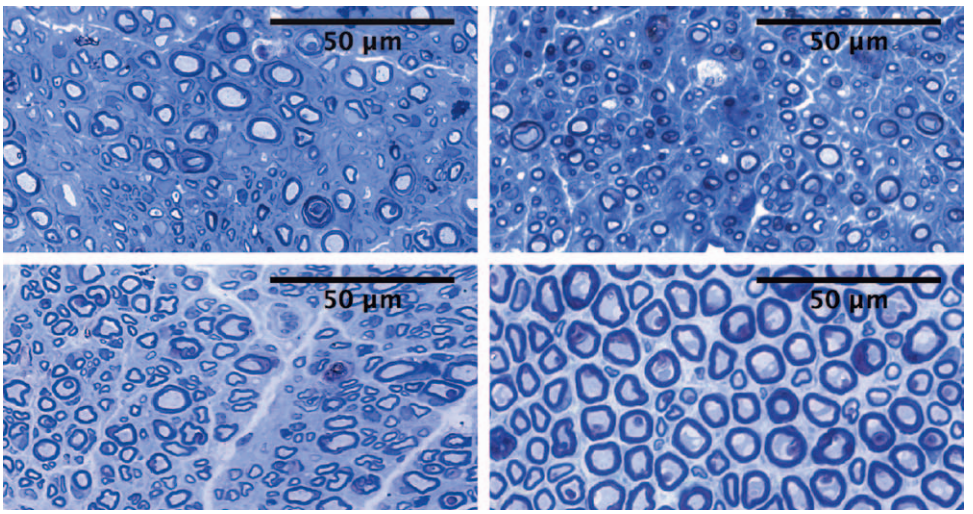

Fig. 4. Regenerating axons of the CPN distal to the neurorrhaphy. STS repair with 10-mm epineural windows (A), STS repair with 2-mm epineural windows and donor nerve axotomy (B), STS repair with 2-mm epineural windows and axotomies to both nerves (C), and control specimen from the contralateral side (D). Axons, some of them well myelinated, can be seen in all STS groups: toluidine blue staining.

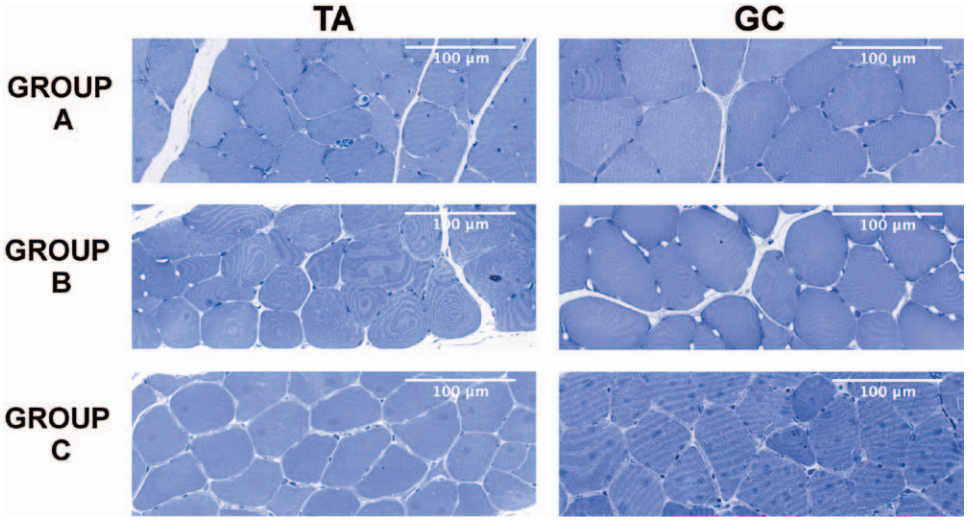

Fig. 5. In the anterior tibial muscle (TA) specimens, the fiber size was slightly decreased in the experimental groups. Focal changes of mild atrophy are more clearly seen in group A than in groups B and C. In the gastrocnemial muscle (GC), the general appearance was normal in group A, whereas in groups B and C, there were focal areas of atrophy: toluidine blue staining.

groups A and B (51.8% [4.3]) and B and C, respectively (Fig. 6).

In group A (79.0% [5.4]), the wet mass ratio of the gastrocnemial muscle was significantly higher compared with the other groups. No difference was seen between groups B (67.6% [9.3]) and C (70.2% [4.6]; Fig. 6).

DISCUSSION

Regeneration through neurorrhaphy of peripheral nerve repair is a topic of continuous interest among scientists and surgeons. The mechanism of regeneration after STS and ETS repairs is not, so far, clearly understood. There is controversy to what an extent nerve regeneration occurs without axonal injury of the donor and recipient nerves. Previously, it was reported that nerve regeneration occurs through neurorrhaphy of ETS repair even without a window[9–12] through collateral sprouting, and intact axons were presumed to produce branches from nodes of Ranvier.[11] On the other hand, it has been reported that axonal injury in the donor nerve is a prerequisite for axonal sprouting.[13]

Various techniques have been introduced with ETS repair in attempt to enhance nerve regeneration.[14–17] Yan et al[14] reported that the number of regenerating nerve fibers increased when the size of the epineural window was enlarged. In the present study, STS repair with 10-mm epineural windows (group A) was compared with a 2-mm window with deliberate axonal injury in the donor

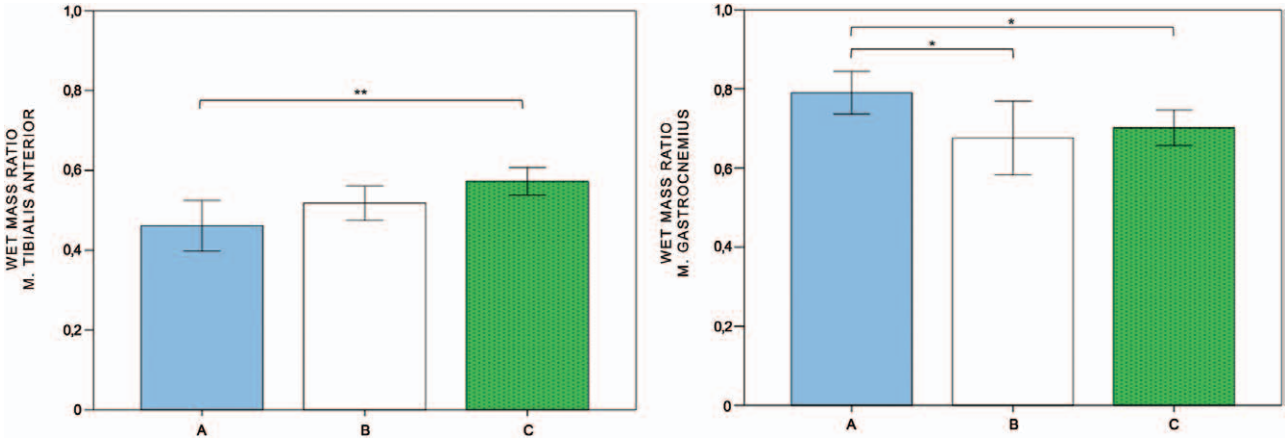

Fig. 6. Results of the muscle wet mass ratio calculations. A, The anterior tibial muscle (recipient CPN innervated) mass of group C was significantly higher compared with group A. B, The gastrocnemial muscle (donor TN innervated) mass of group A was significantly higher compared with groups B and C. The data were analyzed using Mann–Whitney test *U* test with Bonferroni adjustment for multiple comparisons. **P* < 0.05 and ***P* < 0.01. Error bar, ± 1 SD.

nerve (group B) and to 2-mm window with axonal injury in both the donor and recipient nerves (group C). All groups showed regeneration when compared with the unrepaired nerve. The results are in accordance with previous studies with STS repair.[5] The mean nerve fiber counts of the groups with donor-side axotomy were significantly higher when compared with the bare window group. Thus, a larger epineural window did not compensate the effect of donor nerve axotomy on axonal flow to the recipient nerve. Furthermore, the nerve fiber count distal to the repair was not significantly increased when the axotomy was added also to the recipient nerve. The results of the walk track analysis are in relation to the number of regenerating nerve fibers as PFI was significantly higher in the groups with donor-side axotomy compared with the mere epineural window group.

In the present study, the wet muscle mass ratio of the CPN –innervated anterior tibial muscle was significantly higher in group C (57 %) with axotomies on both nerves compared with group A (46 %). The results are in accordance (or better) with those of the previous ETS repair studies: Ozmen et al[16] (2004): 47% at 12 weeks and Tarasidis et al[18] (1998): 26% at 24 weeks. Yan et al[14] (2002) found a correlation between the size of the epineurial window and the muscle mass. Also in the present study, the signs of denervation were wider in muscles with mere epineural windows compared with the muscle specimens of the axonal injury groups.

The present results indicate that in STS repair, the donor-side degree of axonal trauma was decisive to the recruitment of the number of nerve fibers to the regenerating nerve. In ETS neurorrhaphy, collaterally regenerated axons are said to be able to form functional connections with new end organs after relinquishing their original connections.[19] If this conclusion is correct, every axon forming a functional connection via the recipient nerve is ultimately from the donor nerve. This is in accordance with the present results as the nerve fiber areas were significantly smaller in donor TNs with axotomy (Fig. 5). Furthermore, the muscle mass ratio of the donor nerve–innervated gastrocnemius was significantly lower (B: 68% and C:70 % vs A: 79%), and focal signs of denervation could be seen microscopically in muscle specimens in groups with axotomy. Essential in optimizing the size and depth of the donor-side window is to find out if the achievable benefits of recipient nerve regeneration exceed the possible donor-site morbidity.

The present results increase the understanding of STS repair and elucidate the possibilities and drawbacks of deliberate axotomy in neurorrhaphy. In this study, the axotomy was intentionally relatively large, a half of the nerve, and it is not acceptable in humans. Besides comparable repair results with ETS neurorrhaphy,[5] STS neurorrhaphy can be combined with other reconstructive techniques as it leaves the end of the distal nerve stump available. Although STS repair alone may not have enough regenerative potential for sufficient functional recovery, it may have potential to reduce muscle atrophy[20,21] and maintain a growth-supportive atmosphere for further reconstructions. Thus, we aim to study STS neurorrhaphy as part of the treatment of proximal nerve injuries.

The present results together with those of our previous study[5] provide a scientific basis for the regeneration capacity of the STS repair technique. In the present study, regeneration was followed by morphometry and wet muscle mass calculation at 12 weeks. Although the walk track analysis was repeated 2, 4, 6, 8, and 12 weeks postoperatively, another shorter follow-up period for morphometry would have ensured the results. In further studies, more detailed information about axonal regeneration will be gained if unmyelinated axons are assessed with electron microscopy, and sensory and motor axons are distinguished with retrograde labeling studies.

CONCLUSIONS

The present study on STS nerve repair was conducted to examine the requirements for the increase of axonal supply to the recipient nerve and to improve the func-

tional results. We conclude that STS repairs both with and without deliberate axonal injury showed regeneration of the nerve and functional improvement. A larger epineural window could not compensate the result of better regeneration, which was achieved with partial donor nerve axotomy.

Henrikki Rönkkö, MD
Hatanpää City Hospital
P.O. Box 437
FIN-33101 Tampere, Finland
E-mail: ronkko.j.henrikki@student.uta.fi

ACKNOWLEDGEMENT

We are thankful to Pasi Kankaanpää, PhD, for his technical assistance in morphometric analysis. We also owe our gratitude to Mrs. Sinikka Collanus and other laboratory staff of the University of Turku.

REFERENCES

1. Yüksel F, Karacaoğlu E, Güler MM. Nerve regeneration through side-to-side neurorrhaphy sites in a rat model: a new concept in peripheral nerve surgery. *Plast Reconstr Surg.* 1999;104: 2092–2099.
2. Yüksel F, Peker F, Celiköz B. Two applications of end-to-side nerve neurorrhaphy in severe upper-extremity nerve injuries. *Microsurgery* 2004;24:363–368.
3. Zhang S, Ji F, Tong D, et al. Side-to-side neurorrhaphy for high-level peripheral nerve injuries. *Acta Neurochir (Wien).* 2012;154:527–532.
4. Cage TA, Simon NG, Bourque S, et al. Dual reinnervation of biceps muscle after side-to-side anastomosis of an intact median nerve and a damaged musculocutaneous nerve. *J Neurosurg.* 2013;119:929–933.
5. Rönkkö H, Göransson H, Taskinen H, et al. Comparison of peripheral nerve regeneration with side-to-side, end-to-side and end-to-end repairs: an experimental study. *Plast Reconstr Surg Glob Open.* 2016; [epub ahead of print].
6. Bain JR, Mackinnon SE, Hunter DA. Functional evaluation of complete sciatic, peroneal, and posterior tibial nerve lesions in the rat. *Plast Reconstr Surg.* 1989;83:129–138.
7. Brown CJ, Mackinnon SE, Evans PJ, et al. Self-evaluation of walking-track measurement using a Sciatic Function Index. *Microsurgery* 1989;10:226–235.
8. Kankaanpää P, Paavolainen L, Tiitta S, et al. BioImageXD: an open, general-purpose and high-throughput image-processing platform. *Nat Methods* 2012;9:683–689.
9. Kim JK, Chung MS, Baek GH. The origin of regenerating axons after end-to-side neurorrhaphy without donor nerve injury. *J Plast Reconstr Aesthet Surg.* 2011;64:255–260.
10. Akeda K, Hirata H, Matsumoto M, et al. Regenerating axons emerge far proximal to the coaptation site in end-to-side nerve coaptation without a perineurial window using a T-shaped chamber. *Plast Reconstr Surg.* 2006;117:1194–1203; discussion 1204.
11. Hayashi A, Yanai A, Komuro Y, et al. Collateral sprouting occurs following end-to-side neurorrhaphy. *Plast Reconstr Surg.* 2004;114:129–137.
12. Lundborg G, Zhao Q, Kanje M, et al. Can sensory and motor collateral sprouting be induced from intact peripheral nerve by end-to-side anastomosis? *J Hand Surg Br.* 1994;19:277–282.
13. Hayashi A, Pannucci C, Moradzadeh A, et al. Axotomy or compression is required for axonal sprouting following end-to-side neurorrhaphy. *Exp Neurol.* 2008;211:539–550.
14. Yan JG, Matloub HS, Sanger JR, et al. A modified end-to-side method for peripheral nerve repair: large epineurial window helicoid technique versus small epineurial window standard end-to-side technique. *J Hand Surg Am.* 2002;27:484–492.
15. Kayikçioğlu A, Karamürsel S, Ağaoğlu G, et al. A new epineural nerve repair technique: oblique nerve coaptation. *Ann Plast Surg.* 1999;43:506–512.
16. Ozmen S, Latifoglu O, Ayhan S, et al. Impact of epineurial excision of the distal recipient nerve in terminolateral neurorrhaphy. *J Reconstr Microsurg.* 2004;20:385–397.
17. Liu HF, Chen ZG, Shen HM, et al. Efficacy of the end-to-side neurorrhaphies with epineural window and partial donor neurectomy in peripheral nerve repair: an experimental study in rats. *J Reconstr Microsurg.* 2015;31:31–38.
18. Tarasidis G, Watanabe O, Mackinnon SE, et al. End-to-side neurorraphy: a long-term study of neural regeneration in a rat model. *Otolaryngol Head Neck Surg.* 1998;119:337–341.
19. Rovak JM, Cederna PS, Macionis V, et al. Termino-lateral neurorrhaphy: the functional axonal anatomy. *Microsurgery* 2000;20:6–14.
20. Shea JE, Garlick JW, Salama ME, et al. Side-to-side nerve bridges reduce muscle atrophy after peripheral nerve injury in a rodent model. *J Surg Res.* 2014;187:350–358.
21. Ladak A, Schembri P, Olson J, et al. Side-to-side nerve grafts sustain chronically denervated peripheral nerve pathways during axon regeneration and result in improved functional reinnervation. *Neurosurgery* 2011;68:1654–1665.