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RESEARCH REPORT

End-to-side nerve repair in the upper extremity of rat

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Abstract The end-to-side nerve-repair technique, i.e., when the distal end of an injured nerve is attached end-to-side to an intact nerve trunk in an attempt to attract nerve fibers by collateral sprouting, has been used clinically. The technique has, however, been questioned. The aim of the present study was to investigate end-to-side repair in the upper extremity of rats with emphasis on functional recovery, source, type, and extent of regenerating fibers. End-to-side repair was used in the upper limb, and the radial or both median/ulnar nerves were attached end-to-side to the musculocutaneous nerve. Pawprints and tetanic muscle force were used to evaluate functional recovery during a 6-month recovery period, and double retrograde labeling was used to detect the source of the regenerated nerve fibers. The pawprints showed that, in end-to-side repair of either one or two recipient nerves, there was a recovery of toe spreading to 60–72% of the preoperative value (lowest value around 47%). Electrical stimulation of the end-to-side attached radial or median/ulnar nerves 6 months after repair resulted in contraction of muscles in the forearm innervated by these nerves (median tetanic muscle force up to 70% of the contralateral side). Retrograde labeling showed that both myelinated (morphometry) sensory and motor axons were recruited to the end-to-side attached nerve and that these axons emerged from the motor and sensory neuronal pool of the brachial plexus. Double retrograde labeling indicated that collateral sprouting was one mechanism by which regeneration occurred. We also found that two recipient nerves could be supported from a single donor nerve. Our results suggest that end-to-side repair may be one alternative to reconstruct a brachial plexus injury when no proximal nerve end is available.

Key words: collateral sprouting, end-to-side, immunocytochemistry, morphometry, nerve injury, nerve repair, pawprints, retrograde labeling, tetanic muscle force

Introduction

End-to-side repair is a technique where a distal stump of the injured nerve is attached end-to-side to an intact peripheral nerve trunk. The idea of such a repair method emerged more than 100 years ago *(Despray, 1876; Kennedy, 1899; Balance et al. 1903; cited in Rowan et al., 2000; Al-Qattan, 2001; Rovak et al., 2001; Zhang and Fischer, 2002)*. However, no encouraging results were reported for nearly a century until Viterbo and coworkers demonstrated axons in an end-to-side attached nerve in a rat model *(Viterbo et al., 1992)*. The authors claimed that the end-to-side repair allowed a collateral growth of the intact axons into the attached nerve. They also reported that such axons conducted impulses and that the corresponding muscles were reinnervated *(Viterbo et al., 1994a; 1994b; 1998)*. Since then several experimental and clinical articles reported more or less successful use of end-to-side repair *(Lundborg et al., 1994; Bertelli et al., 1996; Mennen, 1998; Tarasidis et al., 1998; Terzis*

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and Papakonstantinou, 2000; Frey and Giovanoli, 2003a; 2003b). Still, the end-to-side repair method is controversial, and a number of issues remain to be clarified. One concerns the source of the regenerating axons. In some of the experimental designs, it cannot be excluded that axons could have come from the proximal stump of the end-to-side attached nerve rather than from the intact and intended donor nerve. Furthermore, despite several reports of functional recovery *(Tham and Morrison, 1998; Liu et al., 1999; Sanapanich et al., 2002)*, some authors have failed to demonstrate or found poor motor reinnervation of the end-to-side attached nerve *(Bertelli et al., 1996; Bertelli and Ghizoni, 2003)*. The recruitment of motor fibers and the extent to which sensory and motor axons can reinnervate an end-to-side attached nerve therefore deserves attention. Other issues of concern involve the possible denervation of the target area of the donor nerve and rewiring in the central nervous system.

Clinically, the brachial plexus or nerves in the upper extremity are sites where end-to-side repair technique could be suitable in view of the proximity of the nerve trunks. Thus, researchers have recently turned their attention to the brachial plexus of the rat as an experimental model *(Bertelli et al., 1996; Sanapanich et al., 2002)*. In rats, these sites offer several experimental advantages, including relatively rapid regeneration, but also because of the similarities of the rat brachial plexus to that of humans with regard to its components and branches *(Bertelli et al., 1995)*.

The aim of the present study was to investigate end-to-side repair in the upper extremity with special emphasis on functional recovery, source, type, and extent of regenerating fibers from the donor nerve. We also raised the question whether one donor nerve could be used to reinnervate two different target nerves.

Materials and Methods

Animals

Thirty-four female Wistar rats, weighing 180–200 g, were used. The experiments were approved by the local animal committee at Lund University, Sweden. All animals were housed in plastic cages with a 12/12 light/dark cycle, and water and food were available *ad libitum*. At surgery, the rats were initially anesthetized with an intraperitoneal injection (10 ml/kg) of 1:10 solution of pentobarbital (60 mg/ml) and physiologic saline. Supplemental doses of 0.1–0.2 ml were used, over the course of the surgical procedure, to maintain anesthesia.

Surgical procedures

In one set of experiments, an anterior approach was used to expose the left musculocutaneous and radial nerves at the midarm level ($n = 11$). A 3-mm-long epineurial window was made in the musculocutaneous nerve with the tip of a scalpel blade (number 11), without opening the perineurium. The radial nerve was transected, and the distal end was sutured to the window using 3–4 epineurial 11–0 sutures (Ethilon, Ethicon®) in an end-to-side fashion (Fig. 1). The proximal end was ligated and buried into the adjacent muscle. The skin was sutured, and the animals were allowed to recover and move freely for 6 months. The contralateral control side was not explored.

In the second set of experiments ($n = 11$), the same procedure was performed, but the median and ulnar nerves (not the radial nerve) were both sectioned and sutured at the same site and time, within a 5-mm epineurial window, end-to-side to the musculocutaneous nerve with the same technique as described above (Fig. 1). The proximal ends were ligated and buried into the adjacent muscle. The skin was sutured, and the animals were allowed to recover. These rats were also followed for 6 months. The contralateral side was not explored.

In a third set of experiments, the radial ($n = 2$) or the median/ulnar ($n = 3$) nerves were exposed as described above and transected, but the distal nerve ends were not attached end-to-side to the musculocutaneous nerve. Hence, no type of repair was performed. Again, the proximal ends were ligated and

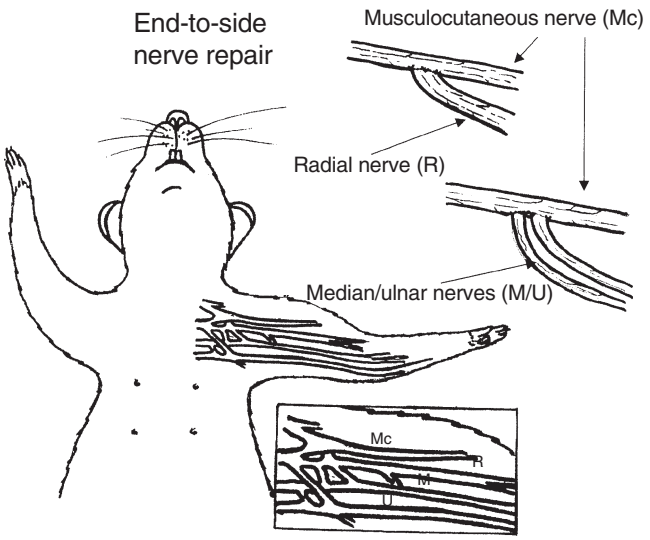


Figure 1. Schematic drawing of the experimental design. The musculocutaneous nerve was utilized as a donor nerve. The distal stump of either the radial or the median/ulnar nerve (attached to the same site and at the same time) was attached end-to-side to the musculocutaneous nerve. Modified from Bontioti *et al. (2003)*.

buried into the adjacent muscle. These rats were also followed for 6 months, and the contralateral side was not explored.

In a fourth set of experiments, rats were used to trace the sensory and motor neuronal pool in the spinal cord and the dorsal root ganglia (DRG) of the musculocutaneous, radial, median, and ulnar nerves where no end-to-side nerve repair was performed. To that end, crystals of the neuronal tracer Fast Blue (FB; Sigma) (see below) were applied to the freshly cut end of the radial, median, ulnar, or musculocutaneous nerves ($n = 4$). In other rats ($n = 3$), the freshly cut ends of the musculocutaneous and the radial or median/ulnar nerves, again without any prior end-to-side nerve repair, were labeled with FB and diamidino yellow (DY; Sigma), respectively (see below). The skin was sutured, and the rats were allowed to recover for 7 days. Then, the rats were anesthetized and sacrificed by an overdose of pentobarbital. The spinal cord and the DRG were harvested and processed as described below.

Evaluation

Pawprints

Functional assessment in all operated rats, except those intended for tetanic muscle force and those used for identifying the neuronal pools (see below), was investigated (i.e., $n = 5$ from each group) using pawprints *(Bontioti et al., 2003)* on days 0, 3, 6, 14, 30, 45, 60, 90, 120, 150, and 180. The forefoot on the rats was marked with ink, and the animals were allowed to walk freely across a corridor on a sheet of paper. The toe spread during walking was defined as the distance between the first to fourth and the second to third toes. The pawprint measured was the one obtained from a non-interrupted step out of at least three walking tracks. The individual values were expressed in percent of the pre-operative (day 0) control value of the specific toes and expressed as mean $\pm$ SEM. Distances were measured both on the experimental side and on the uninjured control side.

In the rats where the distal nerve segments were not attached in any fashion to the musculocutaneous nerve or the original proximal segment, the surgical site was re-explored after 6 months.

Retrograde labeling

On day 180, the end-to-side operated animals were re-anesthetized, and the most distal parts of the radial, median, ulnar, and musculocutaneous nerves were exposed. Two-millimeter-long segments were harvested from each nerve and prepared for morphometry (see below). Then, the distal ends of the remaining nerves were prepared for retrograde tracing. Thus, the nerve ends were isolated from the surroundings with a piece of parafilm. Crystals of DY were carefully placed on the cut end of the radial or the median and the ulnar nerves, while crystals of FB were placed on the cut end of the musculocutaneous nerve. The parafilm was removed, and the labeled ends were isolated and buried in the surrounding tissue. The skin was sutured, and the animals were allowed to recover.

After another 7 days, the rats were sacrificed with a lethal dose of pentobarbital. A laminectomy was performed, and DRG from C6-Th1 were dissected and removed together with the spinal cord segments from C3-Th3.

Morphometry

The most distal 2-mm nerve segment from each nerve was fixed in 2.5% glutaraldehyde and then transferred to 0.1 M sodium cacodylate buffer. The nerve pieces were treated with 2% osmium tetroxide, soaked again in sodium cacodylate buffer, and dehydrated in serial alcohol solutions. The preparations were then immersed in propylenoxide, followed by propylenoxide-Agar Resin 1:1 solution, and embedded in Agar Resin and polymerized. One-micrometer-thick cross sections were cut with a microtome. The sections were stained with methylene blue and Azure II or paraphenylene-diamine and examined by light microscopy.

Digital photomicrographs of each nerve section were analyzed with Adobe Photoshop and NIH Image *(Scherman et al., 2001)*, and total number of fibers (TNFe), mean myelin area per fiber (MMA), and myelination ratio (M-ratio = total axonal area/total myelin area) were calculated. From these available data, the mean axonal diameter in each nerve was calculated.

Retrograde tracer evaluation

The spinal cord and the DRG were fixed in Stefanini's fixative [2% paraformaldehyde and 1.9% picric acid in 0.1 M phosphate-buffered saline (PBS), pH 7.2] for 2 h and then cryoprotected in 20% sucrose in PBS. The spinal cords and the DRG were mounted in Tissue Tek (Sakura) and cut into 10- and 8-μm-thick sections, respectively. Labeled DY and FB DRG neurons were counted in every fourth section of a total of 25–30 from each DRG. In the spinal cord, every twelfth section between C3-Th1 was counted. A neuron was counted only if its nucleus was visible.

Tetanic muscle force and muscle weight

Tetanic muscle force was measured in the extensor carpi radialis brevis/longus muscles or the flexor carpi radialis muscle in the rats, where the radial nerve ($n = 6$) or the median/ulnar nerves ($n = 6$) were attached end-to-side to the musculocutaneous nerve, respectively. Six months post-operatively, the above-mentioned muscles were exposed, the tendon was

cut as distally as possible and ligated. The wrist, the elbow, and the shoulder joints were transfixed by wires. The ligature was connected to a force transducer (Grass Medical Instruments). The tetanic muscle force in the above-mentioned muscles was measured at supramaximal stimulation (6 V at 100 Hz) of the end-to-side attached nerve as previously described *(Scherman et al., 2001)*. The force was measured on the experimental and the control side and expressed in percentage of the control side. The wet weight of the muscles was registered using an electronic balance and again expressed in percentage of the contralateral side.

Statistics

All values for the pawprints (percentage of preoperative value) are expressed as mean ± SEM. Repeated ANOVAs, followed by Bonferroni–Dunn post-hoc test, were used to compare the data from the contralateral and ipsilateral side and the time pattern within and between the experimental groups. For the morphometry, the tetanic muscle force and the muscle weight, the values are presented as median (IQR) for each group. Mann–Whitney was used to detect differences between groups concerning these variables. A significant value was accepted at a p-value of <0.05.

Results

Pawprints

On the third day after the end-to-side procedure, all rats from either the radial end-to-side or the median/ulnar end-to-side groups showed no or decreased willingness to use their paws on the operated side. All animals in the radial end-to-side group had their elbow, wrists, and fingers flexed, and therefore, no values were obtained from that group. In the group where the median/ulnar nerves were connected to the musculocutaneous nerve, the animals used their paws better. The values (first to fourth toes) for the latter group of rats were 61 ± 3% [mean ± SEM] on day 3 (data not shown). The toe spread between the first and fourth toes on day 6 was 56 ± 3% of the pre-operative value for the radial end-to-side group and 49 ± 3% for the median/ulnar end-to-side group. The lowest value was obtained on day 45 for the radial nerve group and on day 14 for the median/ulnar nerve groups [46 ± 3% and 48 ± 2%, respectively]. The values at the endpoint on day 180 for toes 1–4 were 72 ± 4% and 60 ± 8% for the radial end-to-side and median/ulnar end-to-side groups, respectively (Fig. 2A,B). The repeated ANOVA showed a significant difference from the contralateral (uninjured) control side ($p < 0.0001$ for both groups),

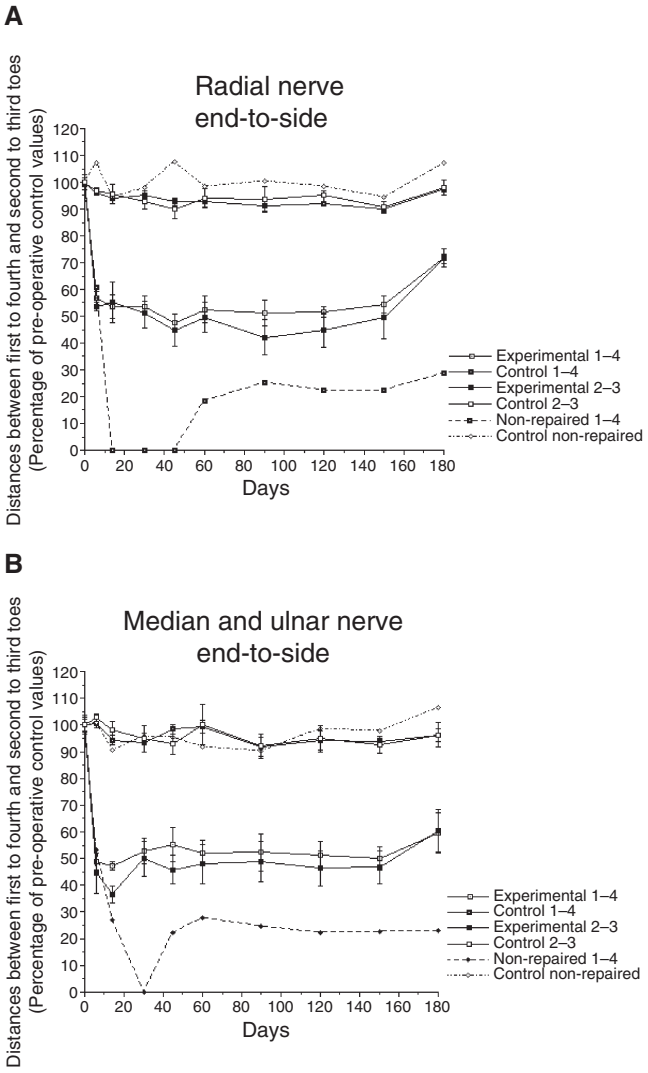


Figure 2. Pawprints, distances between first to fourth and second to third toes (percent of pre-operative control value at day 0) vs. time, after a distal radial nerve (A) or distal median/ulnar nerves (B) end-to-side attachment to the musculocutaneous nerve, are shown. There was a significant difference between the contralateral control side and the experimental side ($p < 0.0001$; repeated ANOVA) and a significant change over time ($p < 0.001$ and 0.0001; repeated ANOVA in both experimental setups). There was no difference between the two control groups or between the two experimental groups. Non-repaired radial and median/ulnar nerves, which were not attached to the musculocutaneous nerve, showed no recovery (lower dotted lines in both figures). The control side from the non-repaired group is also shown and was not different from the other control groups.

and there was a significant change over time ($p < 0.001$) in both groups.

As for the toe spread between the second and the third toes, the obtained curves were similar to those for the first and the fourth toes. At the endpoint at day 180, the values were 72 ± 2% for the radial and

$60 \pm 8\%$ for the median/ulnar end-to-side groups regarding the distance between second and third toes. The ANOVA for the values for second and third digits showed a significant difference from the contralateral (uninjured) control side ($p < 0.0001$), and repeated ANOVA also showed a change over time ($p < 0.0001$).

ANOVA was also used to compare the two experimental groups, i.e., end-to-side repair with the radial and median/ulnar nerves, but no statistical differences were seen regarding either first and fourth ($p = 0.70$) or second and third ($p = 0.68$) toes. ANOVA showed an interaction between the experimental groups and time ($p < 0.01$). There was no difference between the control sides from the two groups of animals.

In injured but not repaired rats ($n = 2$ and 3 in each group), there were no signs of recovery but rather a compensation in walking tracks after 6–8 weeks (dotted lines in Fig. 2A,B). There were no obvious differences between the contralateral control values of these rats and the control side of the repaired rats.

Routine morphology and morphometry

Cross sections of the end-to-side attached distal radial, median and ulnar nerves showed myelinated nerve fibers with different thickness of myelin in the entire nerve area. The number of axons varied between different rats. The nerves were predominated by smaller axons that were assembled into clusters (Fig. 3). The morphological analysis [presented as median (IQR)] showed that the TNFe in the median/ulnar nerves [3799 ± 2026] was significantly higher ($p = 0.03$) than that in the radial nerve [137 ± 444] (Fig. 4A). The MMA (Fig. 4B) was significantly higher ($p = 0.02$) in the radial nerve [15.1 ± 6 vs. 6.6 ± 2.6 μm^2/fiber], and the M-ratio (total axonal area/total myelin area) [radial 0.42 ± 0.24 vs. median/ulnar 0.48 ± 0.14] (Fig. 4C) was not different ($p = 0.35$). The axonal diameter in the radial nerve [3.2 ± 0.6 μm] was not statistically different ($p = 0.08$) from the diameter in the median/ulnar nerves [2.0 ± 0.5 μm] (Fig. 4D).

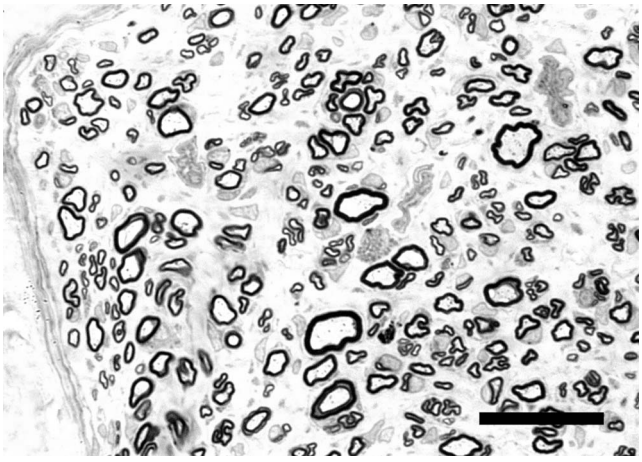


Figure 3. Cross section of a median nerve where that nerve had been attached end-to-side to a musculocutaneous nerve for 6 months. Bar = 25 μm.

Retrograde labeling

Retrograde labeling was used to detect the origin of axons in the musculocutaneous, radial, and median/ulnar nerves and whether collateral sprouting was present. Both motor and sensory neurons, identified as FB labeled, showed an intense blue fluorescence predominantly in the cytoplasm (Fig. 5A,D). Motor neurons also exerted blue fluorescence in the nucleus (Fig. 5D). DY-labeled motor and sensory neurons showed intense yellow/green fluorescence in the nucleus and less staining in the cytoplasm (Fig. 5B,E). Some satellite cells were also labeled in the DRG. Double-labeled motor and sensory neurons demonstrated both characteristics (Fig. 5C,F). No motor or sensory neurons were labeled on the contralateral side, indicating that no leakage occurred.

Motor neuron pool

The retrogradely labeled neurons, including doubled-labeled ones, in spinal cord (C4-Th2) after end-to-side repair are presented in Figure 6A,B. In total, 146 DY-labeled motor neurons and 234 FB-labeled cells were found, but only three double-labeled cells were observed in five rats that had the radial nerve attached end-to-side to the musculocutaneous nerve. After end-to-side repair with median/ulnar nerves, 199 DY-labeled and 179 FB-labeled motor neurons were found. Again, only a few double-labeled cells (9) at the different spinal cord levels in the five animals were observed.

Sensory neuron pool

In DRG C6-C8 (distal radial nerve attached, Fig. 7A) and C6-Th1 (distal median/ulnar nerves attached, Fig. 7B), around 27% were stained with DY and/or FB. In the five rats where the distal radial nerve was attached to the musculocutaneous nerve, around 15,000 cells were counted. Of these cells, up to 20% were FB-labeled (mainly C6) and up to 11% were DY-labeled (mainly C7-C8). However, only up to 1.9% was double-labeled in C6-C8 in the five rats (Fig. 7A).

In the rats where the median/ulnar nerves were attached to the musculocutaneous nerve, 25,000 cells were evaluated. Of these cells, up to 22% were FB positive, mainly located in the C6 DRG. The corresponding value for DY was 21%, where C7-Th1 was predominantly labeled. Only up to 3.3% were double-labeled, and most of them were located at C8-Th1 (Fig. 7B).

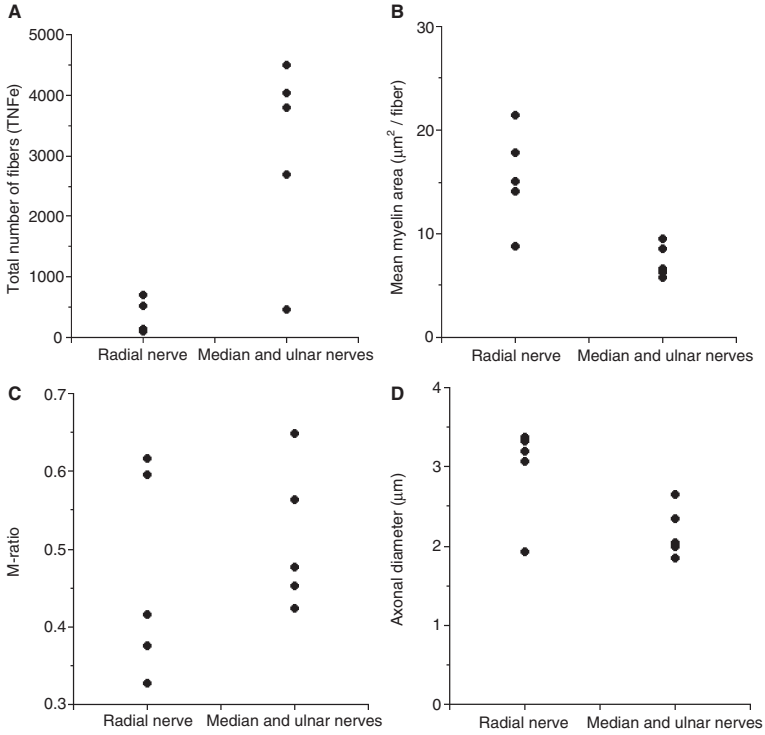


Figure 4. Scattergram showing the (A) total number of nerve fibers (TNFe) ($p = 0.03$), (B) mean myelin area per fiber (MMA) ($p = 0.02$), (C) myelination ratio (M-ratio; total axonal area/total myelin area; $p = 0.35$), and (D) axonal diameter ($p = 0.08$) of radial and median/ulnar nerves 6 months after end-to-side nerve repair.

Normal motor and sensory neuronal distribution

In the separate group of rats where no injury was made before labeling, we used retrograde tracing to study the location of the normal neuronal pools of the nerves in the upper extremity. The motor neuron pool for the musculocutaneous nerve was mostly at C5-C6 spinal cord level, and for the radial, median, and ulnar nerves, it was at C4-Th2 (mainly C7-C8), C5-Th1 (mainly C8), and C6-Th1 (mainly C8) spinal cord level, respectively. In the same rats, the sensory neuron pool for the musculocutaneous nerve was at C5-C6 DRG, and for the radial, median, and ulnar nerves, it was mainly at C6-T1, C5-Th1, and C6-Th1 DRG levels, respectively.

We compared the values of the retrogradely labeled motor and sensory neurons from the end-to-side repaired nerves and expressed these in percentage of those observed in single-traced uninjured control rats. The percentage of motor neurons for the end-to-side-attached radial nerve was 9.5 and 15.3 for the median/ulnar nerves. The corresponding values for sensory neurons were 15.2% for the attached radial nerve and 49.7% for the median/ulnar nerve.

Tetanic muscle force and muscle weight

Electrical stimulation of the end-to-side attached radial or median and ulnar nerves resulted in muscle contraction of the forearm muscles innervated by the attached nerves (wrist/toe extension and wrist/toe flexion, respectively). Measurement of the tetanic muscle force in the extensor carpi radialis muscle and the flexor carpi radialis muscle, respectively, showed that the ipsilateral side had values of 63% (30) and 70% (23) ($n = 6$ in each group; $p = 0.63$) of the contralateral side, after the radial and the median/ulnar nerves, respectively, were attached end-to-side to the intact musculocutaneous nerve. The corresponding values of the wet muscle weight were 58% (25) and 76% (24) ($n = 5$ in each group; $p = 0.17$), after radial and median/ulnar nerves were attached to the musculocutaneous nerve, respectively.

Exploration of the brachial plexus of the rats, where no repair was done after transection, revealed no nerve structure in the gap between the distal nerve segment and the proximal transected nerve (buried in muscle) or the musculocutaneous nerve. Furthermore, no pinch reflex or muscle contraction could be elicited from forearm muscles in such non-repaired rats.

Discussion

In this study, we have demonstrated that end-to-side nerve repair can be used for nerve repair in the upper extremity of the rat and that axons grow into the

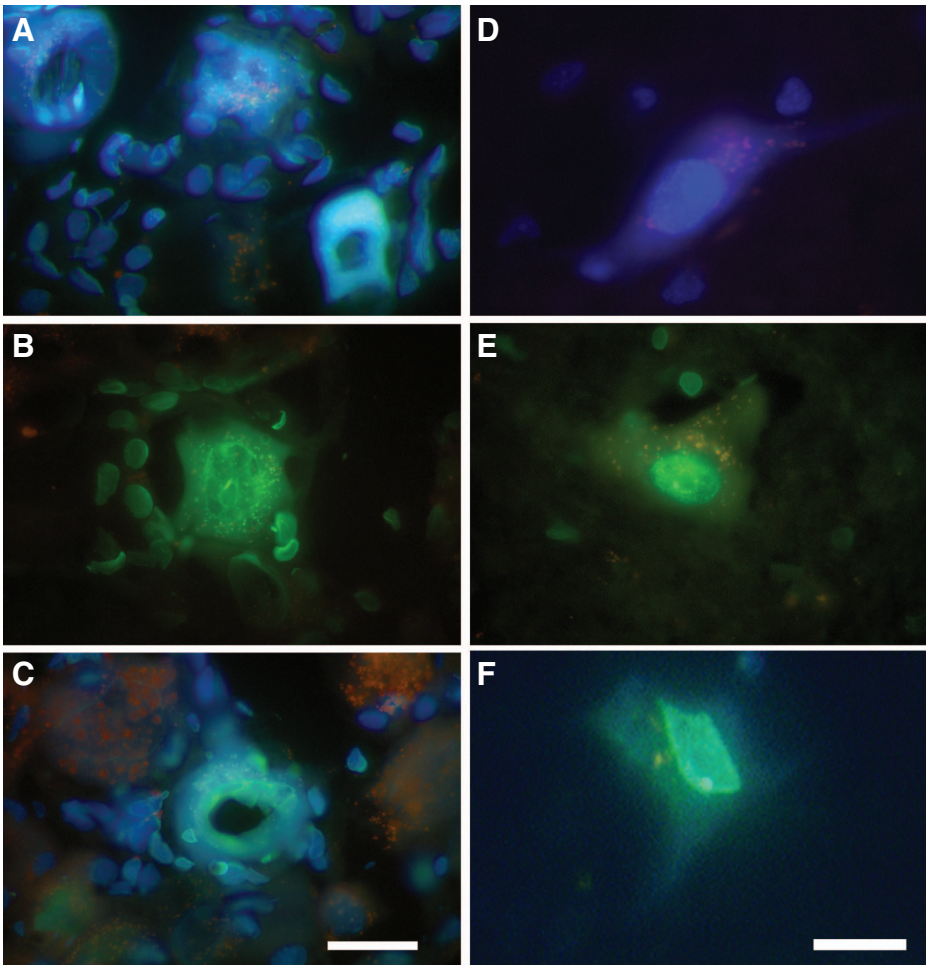


Figure 5. Sensory (A–C) and motor (D–F) neurons labeled with Fast Blue (FB) (A, D), diamidino yellow (DY) (B, E), and double-labeled (C, F) 180 days after end-to-side repair with the distal radial nerve attached to the musculocutaneous nerve. The neurons, which were double-labeled with both FB and DY, indicate a collateral sprouting at the site of end-to-side repair. Bar = 25 μm.

radial, the median, or the ulnar nerves when these nerves are attached to the intact musculocutaneous nerve (Fig. 8). Retrograde labeling revealed that these axons emanated from the neuronal pool of the brachial plexus and in some cases through collateral sprouting. At 6 months, the end-to-side repair technique had restored toe spreading to 60–72% of its pre-operative value (lowest value around 47%), and functional reconnection of muscles in the paw and forearm were seen.

The merits of the end-to-side repair technique have been extensively discussed. Advocates of the technique refer to papers describing recovery of both motor and sensory functions *(Viterbo et al., 1992; 1994a; 1994b; 1998; Lundborg et al., 1994; Noah et al., 1997; Tham and Morrison, 1998; Liu et al., 1999; Matsumoto et al., 1999; Sanapanich et al., 2002)*, whereas others refer to articles where poor motor recovery has been observed *(Bertelli et al., 1996; Bertelli and Ghizoni, 2003)*. We have previously demonstrated a 90–100% recovery of pre-operative value (pawprints) following a conventional end-to-end repair of the radial or the median/ulnar nerves and an 80% recovery (of pre-operative value; pawprints) following conventional nerve-graft interposition of defects in these nerves *(Bontioti et al., 2003)*. Despite the lower recovery values of the present end-to-side repair, still a recovery up to 60–72% of function may merit attention if no alternative repair method is available. The present study, therefore, does not lend support to the idea that there is a poor motor recovery. Electrical stimulation of the end-to-side attached nerves elicited contractions in the muscles normally innervated by these recipient nerves with a median tetanic muscle force of 63–70% of the contralateral side. In addition, no response was found upon stimulation of the distal segment of the radial or median/ulnar nerves in non-repaired rats. We conclude that motor fibers from the donor nerve must have entered the end-to-side attached nerve segment to innervate muscles, which were formerly supplied by motor fibers from the recipient nerve. The magnitude of the tetanic muscle force was up to 70% (median value) of the

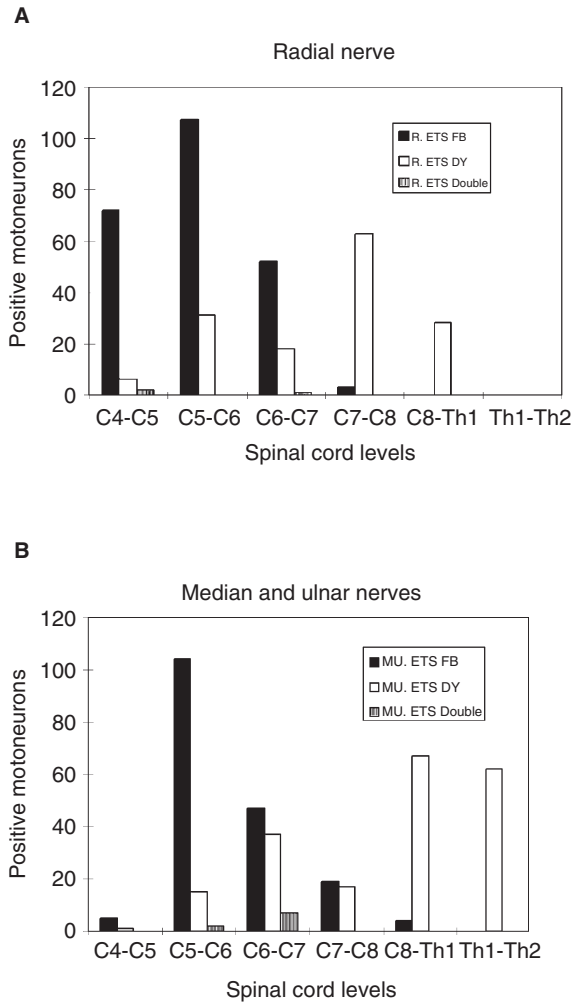


Figure 6. Labeled motor neurons in spinal cord levels C4-Th2 after retrograde labeling of the musculocutaneous [donor nerve; Fast Blue (FB)] and the radial (A) or median/ulnar (B) nerves [attached recipient; diamidino yellow (DY)]. Double = double-labeled neurons. The values are expressed as the sum of positive neurons in five rats.

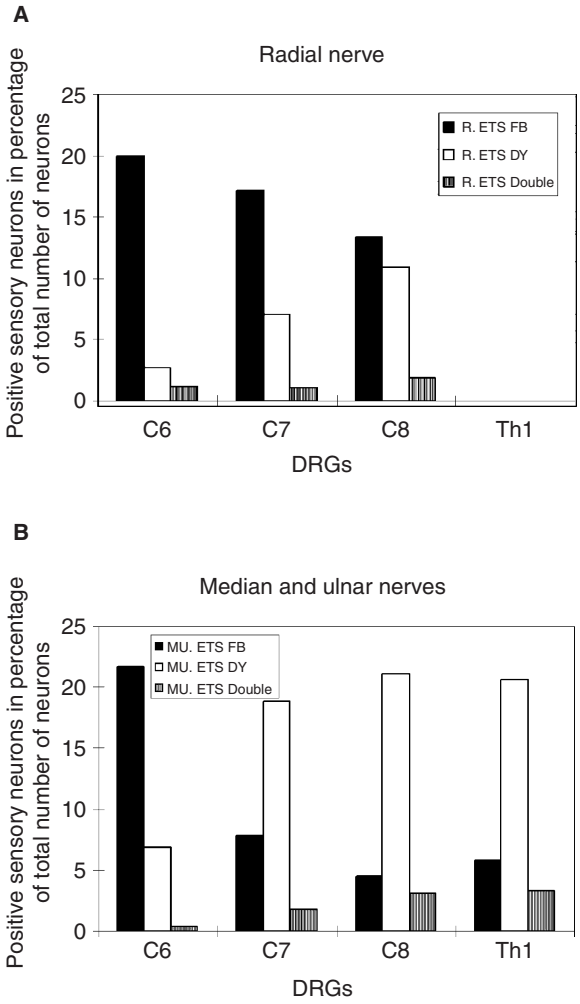


Figure 7. Labeled sensory neurons in dorsal root ganglia (DRG) C6-Th1 after retrograde labeling of the musculocutaneous [donor nerve; Fast Blue (FB)] and the radial (A) or median/ulnar (B) nerves [attached recipient; diamidino yellow (DY)]. Double = double-labeled neurons. The values are expressed as positive neurons in percentage of total number of neurons in each DRG from five rats. Data from Th1 not available for the radial nerve (A).

contralateral side. This is in accordance with what has been reported for end-to-side repair in the lower extremity of rats (60–100%) *(Lundborg et al., 1994)* but higher than those reported for a similar model (musculocutaneus end-to-side to ulnar) in the upper extremity of rats (45%) *(Sanapanich et al., 2002)*. However, the follow-up time was extensively longer in our experiments, which may explain the higher value for both tetanic muscle force and muscle weight in our study in accordance with others (muscle weight 85%) *(Papalia et al., 2003a)*. In contrast, the latter authors reported a low recovery of muscle force using the grasping test (23%; compare end-to-end repair 57%) *(Papalia et al., 2003a; 2003b)*. By retrograde labeling, we also found that both motor and sensory neurons sent axons into the end-to-side attached nerves. In a limited number of animals, we could estimate the extent of motor and sensory reinnervation, which appeared to be higher on the sensory side than on the motor side in accordance with other reports *(Matsumoto et al., 1999; Sanapanich et al., 2002)*. It should be borne in mind that these calculations were "crude estimates." First, the values were derived from tracing experiments with their inherent limitations of the technique, e.g., the values depend on the extent to which the tracer is picked up by the axons *(Haase and Payne, 1990; Molander and Aldskogius, 1992)*, and secondly, for each value only one control animal was used.

It has been suggested that the main explanation for the regeneration into the end-to-side attached nerve is collateral sprouting *(e.g., Viterbo et al., 1992; 1994a; 1994b; 1998; Lundborg et al., 1994; Matsumoto*

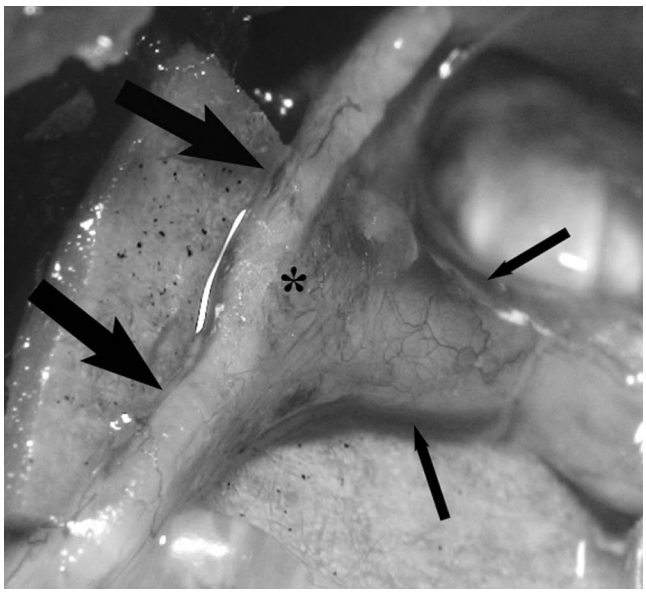


Figure 8. Macroscopic picture from a rat showing a transected distal radial nerve (thin arrows) attached end-to-side (*) to an intact musculocutaneous nerve (thick arrows) 6 months previously.

et al., 1999; Kanje et al., 2000; Rowan et al., 2000; Yamauchi et al., 2000; Rovak et al., 2001) of the intact axons in the uninjured nerve trunk. Such neurons with collateral sprouts can be visualized using double-retrograde tracing *(Chen and Brushart, 1998)*. It has been shown that, for the rat sciatic nerve system, 0.2% of the neurons send axons to both the tibial and sural nerve branches *(Haase and Payne, 1990; Molander and Aldskogius, 1992)*. However, after 6 months in the present study, we found only 2–7% double-labeled neurons. Most of the previous studies have not quantified the number of double-labeled neurons *(Matsumoto et al., 1999; Sanapanich et al., 2002)*. Our data, with 2–7% double-labeled cells, indicate that collateral sprouting exists. Furthermore, the data suggest that either collateral sprouting is not the only mechanism by which fibers could be recruited to the end-to-side attached nerve or pruning of the axons in the donor nerve (musculocutaneous) has occurred. Interestingly, Cederna *et al. (2001)* found degeneration in the donor nerve distal to the side of an end-to-side nerve attached and denervation of muscle fibers in the donor nerve target area. Other authors have also shown degeneration of nerve fibers in the donor nerve when a median nerve was attached end-to-side to an ulnar nerve using an epineurial window *(Papalia et al., 2003a)*. Per se, these findings could be interpreted as pruning, but it could also be that the end-to-side attached nerve, or the procedures involved in attaching it, induces degeneration of fibers in the donor nerve. These fibers could then regenerate into the end-to-side-attached nerve. In contrast, Lutz *(2004)* found no degeneration in the distal part of the donor nerve several months after the end-to-side repair. Further experimentation is required to resolve this issue. However, independent of the mechanism by which regenerating nerve fibers are recruited, it appears that the final outcome is that the majority of axons of motor or sensory neurons end up in only one target area. Therefore, recovery of function at one site must, at least for a period, result in the loss of function at the donor nerve territory. Functionally (muscle force), this denervation, however, is not measurable *(Kalliainen et al., 1999; Liu et al., 1999; Cederna et al., 2001; Sanapanich et al., 2002)*, although denervated muscle fibers (5%) have been found in the donor nerve territory. However, it is likely that collateral sprouting mechanism reinnervates muscle fibers at the level of that target on the donor site. One may also speculate that the size of some of the motor units at the donor site may increase *(Gordon et al., 1993; Tam and Gordon, 2003)*. Simultaneously, at the target of the end-to-side attached nerve, there is reinnervation and recovery of function, but foreign motor and sensory neurons reinnervate these targets. Therefore, the recovered motor function must also involve rewiring of connections at central levels, i.e., the animal has to learn how to utilize the neurons which had found a new target area. While denervation on the motor side appears not to affect the function *(Cederna et al., 2001; Sanapanich et al., 2002)*, neither this study nor others give any clues to the effect of sensory denervation which should accompany the end-to-side repair in the sensory target area of the donor nerve *(Bajrovic et al., 2002)*.

A novel finding in our study was that, with end-to-side nerve repair, the donor nerve could nurture more than one nerve. At 6 months, evaluation of functional recovery (pawprints and muscle tetanic force) showed no statistically significant differences between the two experimental groups, where the radial and median/ulnar nerves were attached to the musculocutaneous nerve. In addition, there were no obvious differences in retrograde labeling. However, there were significantly more nerve fibers in the median/ulnar nerve-attached group than in the radial one, but the former fibers appeared less mature (significantly lower MMA). Even if more nerve fibers were present in the median/ulnar nerves than in the radial nerve, it did not result in significantly higher muscle force or muscle weight. The data of a lower value of myelin area per fiber indicate that the maturation of the nerve fibers has not been accomplished in the end-to-side attached nerve even 6 months after the operation, although we could not see any difference regarding axonal diameter which may also reflect maturation. Furthermore, although the number of fibers was significantly lower in the radial nerve attached group, there was no significant difference concerning pawprints or muscle

force. These findings indicate that the functional recovery in different experimental models does not always reflect the number of regenerating fibers *(Scherman et al., 2001)*.

In conclusion, the present study has shed light on some questions pertinent to the end-to-side repair method. It showed that an end-to-side attached nerve in the upper extremity attracts both motor and sensory axons and that this results in recovery of function. It also showed that one donor nerve could be used to reinnervate the territory of two end-to-side attached nerves. The end-to-side attached targets are reinnervated by fibers from the neuronal pools of the brachial plexus, but very few of these neurons project to more than one site, i.e., most of the axons recruited from the donor nerve lose their contact with their original targets. Because the end-to-side repair method resulted in recovery of function up to 72% of pre-operative value (lowest value 47%) of pawprints and up to 70% of contralateral side median tetanic muscle force, it is reasonable to suggest that this repair method could be considered in selected clinical cases when no other repair options are possible.

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