Recovery of cutaneous pain sensitivity after end-to-side nerve repair in the rat

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Object. The hypothesis that collaterally sprouting axons from an uninjured donor nerve may provide recovery of pain sensitivity in the skin after end-to-side nerve repair was investigated in rats. In addition, the effect of this technique on the donor nerve was examined.

Methods. The distal stump of the transected peroneal nerve was sutured end to side to the intact sural nerve. No epineurial window or perineurial slit was made in the sural nerve at the site of coaptation. Other nerves in the leg were transected and ligated. Eighteen weeks later, the sural nerve was transected at a site distal from the coaptation site. The residual pain sensitivity in the peroneal innervation field in the instep was documented using the skin pinch test in three of 11 animals. The area of sensitivity encompassed 19 to 40% of the maximum nociceptive innervation area of the normal peroneal nerve. The nerve pinch test revealed functional sensory axons in all communicating peroneal nerves, in which \( 277 \pm 119 \) myelinated axons (mean \( \pm \) standard deviation) were found by histological investigation.

Conclusions. The authors conclude that at least partial recovery of sensory function due to collateral sprouting of axons after end-to-side nerve repair is possible in principle. However, the presence of functional sensory axons in the peroneal nerve stumps did not guarantee the recovery of skin sensitivity to pain in all animals. No functional or morphological evidence of an untoward effect of collateral sprouting into the end-to-side communicating nerve was detected in the axons of the donor nerve itself.

Key Words • surgically created end-to-side nerve communication • peripheral nerve • collateral sprouting • rat
Surgical Procedures

In accordance with national guidelines in Slovenia, the animal experiments were approved by the Veterinary Administration of the Ministry for Agriculture, Forestry, and Food (Permit No. 526-07-26/98). Experiments were performed in male albino Wistar rats each weighing 260 to 300 g at the time of surgery. All surgical procedures were performed after deep anesthesia had been induced in the animals by intraperitoneal administration of dihydrothiazine and a ketamine cocktail (8 mg/kg Rompun and 60 mg/kg Ketalar). In 11 rats, the peroneal, tibial, and sural nerves were exposed in the right thigh. The peroneal nerve was transected and its proximal stump was ligated and deflected. The distal stump of the peroneal nerve was coapted end to side to the intact sural nerve by epineurial sutures.

Materials and Methods

Estimation of Recovery of Skin Sensitivity to Pain

The nociceptive skin pinch test was used to estimate the extent of recovery of pain sensitivity in the hind foot skin. The animals were lightly anesthetized by a 25-mg/kg dose of intraperitoneal pentobarbital (Vetanarcol). The skin of the instep was pinched with a fine forceps (diameter of the tip 0.2 mm) in 1-mm intervals from the toes to the ankle. The reflex withdrawal response on the treated side was compared to that elicited by pinching the corresponding spot on the nontreated foot. The area of positive response was depicted in the schematic drawing of the foot. These drawings were then used to estimate the percentage of innervated skin surface by computer-based planimetry. Recovery of sensitivity to pain was tested in the skin of the instep, which is normally innervated by the sural and peroneal nerves. The test was first performed 1 day after surgery and was repeated after 5, 10, and 14 weeks. Skin sensitivity in the peroneal innervation field in the skin of the instep of the foot was finally examined after 18 weeks of recovery. The test was performed before and 24 hours after transecting the sural nerve 1.5 cm distal from the site at which the nerve communication was surgically created. The second test was performed to reveal residual sensitivity in the skin due to sensory axons that grew through the communicating peroneal nerve.

Presence of Sensory Axons in the Communicating Peroneal Nerve

Sprouting of nociceptive axons into the communicating distal stump of the peroneal nerve was revealed using the nerve pinch test. Rats were lightly anesthetized by administration of pentobarbital, as described earlier. The distal stump of the peroneal nerve and the site of surgically created end-to-side nerve communication were carefully cleaned and dissected from nearby tissue. The nerve pinch test was performed at the site where the peroneal nerve penetrates through the fascia of the peroneal muscles. A light pinch was delivered to the peroneal nerve with fine-tipped watchmaker forceps and the animal’s response was recorded.

Histomorphometrical Evaluation of Myelinated Axons in the Communicating Peroneal Nerve and the Donor Sural Nerve

Myelinated axons were visualized in cross sections of surgically treated nerves by staining their myelin sheaths with azure blue dye. At the end of the experiment (18 weeks after surgical creation of nerve communication), a short segment of the peroneal nerve lying 4 mm distal from the site of coaptation was excised. At the same time, small segments of the sural nerve were removed from sites 4 mm proximal to and 4 mm distal from the site of communication. Sural nerve segments from control animals not subjected to surgery were excised at comparable sites. Excised segments were fixed in 2% glutaraldehyde and 2% paraformaldehyde in veronal-acetate buffer, pH 7.4. For 12 hours at 4°C. Afterward, the nerve samples were dehydrated and embedded in Epon. Semithin cross sections, 1.5 to 2 μm thick, were cut from the middle of the nerve segment and stained with azure blue dye. Myelinated axons were counted in whole-nerve cross sections and their cross-sectional areas were measured using a light microscope and a computer-based image analysis system. In addition, longitudinal histological sections were cut in the region of the surgically created end-to-side nerve communication and the myelinated axons in the sections were stained with azure blue, as described earlier.

Sources of Supplies and Equipment

The Rompun was obtained from Bayer AG (Leverkusen, Germany), the Ketalar from Parke-Davis GmbH (Berlin, Germany), and the Vetanarcol from C. Richter & Co. (Wels, Austria). The Ethilon epineurial sutures were purchased from Ethicon (Edinburgh, UK). The light microscope was purchased from Opton Feintechnik GmbH (Oberkochen, Germany) and the Microcomputer Imaging Device program from Imaging Research Inc. (Brock University, St. Catharines, Ontario, Canada).

Results

Recovery of Skin Sensitivity to Pain in the Innervation Field of the Communicating Peroneal Nerve

After transecting the peroneal, tibial, and saphenous nerves, the nociceptive skin pinch test was used to estimate the extent of recovery of pain sensitivity in the hind foot skin. The animals were lightly anesthetized by a 25-mg/kg dose of intraperitoneal pentobarbital (Vetanarcol). The skin of the instep was pinched with a fine forceps (diameter of the tip 0.2 mm) in 1-mm intervals from the toes to the ankle. The reflex withdrawal response on the treated side was compared to that elicited by pinching the corresponding spot on the nontreated foot. The area of positive response was depicted in the schematic drawing of the foot. These drawings were then used to estimate the percentage of innervated skin surface by computer-based planimetry. Recovery of sensitivity to pain was tested in the skin of the instep, which is normally innervated by the sural and peroneal nerves. The test was first performed 1 day after surgery and was repeated after 5, 10, and 14 weeks. Skin sensitivity in the peroneal innervation field in the skin of the instep of the foot was finally examined after 18 weeks of recovery. The test was performed before and 24 hours after transecting the sural nerve 1.5 cm distal from the site at which the nerve communication was surgically created. The second test was performed to reveal residual sensitivity in the skin due to sensory axons that grew through the communicating peroneal nerve.
End-to-side nerve repair

nerves and suturing the site of end-to-side nerve communication between the peroneal and sural nerves, the area of residual pain sensitivity in the instep skin, 16 ± 3% (mean ± standard deviation [SD], 11 animals) corresponded to the maximum nociceptive innervation area of the uninjured sural nerve (Fig. 2). The area of sensitivity in the instep skin began to spread and reached 27 ± 6% (mean ± SD, 11 animals) of the total during the 5th week after surgery. The difference in the size of the pain sensitive area between the 1st day and the 5th week after surgery was statistically significant (p < 0.02). Thereafter, the area of peroneal innervation remained constant until the end of the 18th week. At that time, the sural nerve was transected 1.5 cm distal from the site of the surgically created nerve communication. Pain sensitivity in the innervation field of the peroneal nerve in the instep skin was unequivocally documented in three of 11 animals. The reflex withdrawal response on the treated side, however, was not as quick as that elicited by pinching the corresponding spot on the contralateral (nontreated) foot in all three animals. The areas of residual pain sensitivity encompassed 12%, 21%, and 25% of the instep skin of the foot (Fig. 2). This is between 19% and 40% of the maximum nociceptive innervation area of the normal peroneal nerve. There was no residual pain sensitivity in the instep skin after sural nerve transection in the rest of the animals.

Sensory Axons in the Communicating Distal Peroneal Stump

The presence of sensory axons in the communicating distal peroneal stump was determined using the nerve pinch test 18 weeks after surgery. The nerve pinch test was positive in all animals as far as it was possible to test, that is, the site at which the peroneal nerve penetrated the fascia of the peroneal muscles, which is approximately 25 mm from the site of coaptation. The nerve pinch test in the communicating peroneal stump became negative—the animal’s response could not be elicited anymore—after the sural nerve had been transected proximal to the site of the surgically created nerve communication. The total number of myelinated axons in the communicating distal stump of the peroneal nerves was determined in whole nerve cross sections obtained 4 mm distal from the site of surgically created nerve communication. Myelinated axons were present in all nine surgically reconstructed peroneal nerves that were histologically examined (Fig. 3). We found 277 ± 119 (mean ± SD) myelinated axons in these nerves. Analysis of cross-sectional areas of myelinated axons in the communicating peroneal nerve stump showed that the cross-sectional areas of 272 ± 108 axons (mean ± SD) were smaller than 10 \( \mu \text{m}^2 \) (diameter < 4 \( \mu \text{m} \)).

Collateral sprouting of intact sensory axons from the sural nerve into the communicating distal peroneal nerve stump could be demonstrated in longitudinal sections through the site of coaptation (Fig. 4).

Histomorphometrical Analysis of Axons in the Sural Nerve

Eighteen weeks after surgery, light microscopy of sural nerve cross sections revealed no qualitative differences between donor nerves and control (normal) sural nerves.
There was no evidence of wallerian degeneration in the donor nerves.

The total numbers of myelinated nerve fibers in the donor sural nerve proximal to and distal from the site of nerve communication were 1159 ± 62 and 1122 ± 43 (nine animals), respectively. These numbers were not significantly different from the total number of myelinated axons in normal sural nerves at corresponding sites (1179 ± 62 in four animals; p > 0.05).

In transverse sections of control (normal) as well as donor sural nerve sections obtained proximal to and distal from the surgically created end-to-side nerve communication, two groups of myelinated axons were observed. Myelinated axons with small (0–20 μm²) and medium (20–60 μm²) cross-sectional areas contributed to two distinct peaks in the frequency-distribution histograms (Fig. 5). In donor sural nerve sections obtained proximal to and distal from the site of nerve coaptation, there was a slight tendency toward thin axons at the expense of thicker axons. However, when average numbers of axons in both groups were statistically evaluated, there was no significant difference in the number of axons between donor and control (normal) nerves (two-way analysis of variance, p > 0.05).

**Discussion**

Recovery of Skin Sensitivity to Pain After End-to-Side Nerve Repair

Recovery of skin sensitivity to pain due to collateral sprouting of sensory axons from the intact sural nerve into the communicating peroneal nerve was monitored in the instep skin. Early spreading of the pain-sensitive area into the adjacent denervated instep skin, observed during the first 5 weeks after surgery, was most probably due to collateral sprouting of cutaneous axon terminals of the intact sural nerve. However, after transection of the sural nerve distal from the site of coaptation at the end of the experiment, a certain area of the instep skin still remained sensitive to pain in three of 11 animals. This sensitive area encompassed 19 to 40% of the peroneal nerve innervation territory. Recovery of pain sensitivity in the instep skin was obviously achieved by axonal sprouts growing from the sural nerve through the communicating peroneal nerve, because all other nerves innervating the foot were cut and ligated, and there were plenty of sensory axons sprouting into the communicating peroneal nerve.

Our results confirm earlier observations that sensory axons from an intact nerve can sprout collaterally into the end-to-side communicating recipient nerve.5,9,18,19,25 At the end of our experiment, the nerve pinch test in the communicating distal peroneal stump was positive in all animals as far as it was possible to test, that is, 25 mm distal from the site of coaptation. We proved that sensory axons in the communicating peroneal nerve originated from the sural nerve. Any axons that had eventually regenerated into the communicating peroneal stump from the ligated proximal stumps of transected adjacent nerves (although no axon fascicles were observed by the naked eye) were cut off during dissection before the pinch test. Accordingly, the sensitivity to pinch in the coapted peroneal nerve was lost if the sural nerve was transected proximal to the site of communication. In sural nerve cross sections, no axons could be observed outside the perineurium. Therefore, the possibility that some small foreign regenerating axon fascicles adhered closely to the sural nerve trunk and were not transected during dissection of the site of communication can be excluded. On the other hand, collateral sprouts emanating from axons of the intact sural nerve were clearly demonstrated by histological examination of the longitudinal sections through the coaptation site.

Therefore, we can conclude that, in principle, at least partial recovery of sensory function can be achieved by collateral sprouting of axons from an uninjured donor nerve through the end-to-side coapted distal stump of a transected peripheral nerve. However, although there were functional sensory axons in the communicating peroneal nerve stumps in all surgically treated animals, the majority of animals were obviously not able to recover skin sensitivity to pain during our observation period.
End-to-side nerve repair

There are at least two possible reasons that may have prevented or greatly delayed such a recovery. First, the majority of sensory axonal sprouts in the communicating peripheral nerve might have been misrouted to other less relevant targets. If most axonal sprouts from the intact (donor) sural nerve entered more numerous neurilemmal tubes of the communicating peroneal nerve by chance and invaded inappropriate sensory or motor neurilemmal tubes (deep branches of the peroneal nerve), no recovery of skin sensitivity in the instep would take place, in spite of axonal sprouting from the donor nerve.

Second, ingrowth of terminal collateral sprouts of uninjured adjacent peripheral nerves into the denervated skin territory might hinder successful reinnervation of this territory if the axonal sprouts grew through the communicating peripheral nerve. After transection of the peroneal nerve at the beginning of our experiment, intact sensory cutaneous axons of the uninjured sural nerve began to sprout collaterally into the adjacent denervated skin of the peroneal nerve territory. Therefore, axonal pathways in the skin could have been physically occupied or a trophic factor such as nerve growth factor might have been used up by terminal collateral sprouts of the donor nerve.

Effect of End-to-Side Nerve Repair on the Histomorphological Characteristics and Function of the Donor Nerve

In our experiment, extreme care was taken not to damage the donor nerve during surgical creation of the end-to-side nerve communication. No window in the donor nerve epineurium was made, all sutures were epineurial, and the perineurium of the sural nerve was left intact at the site of coaptation.

Noah, et al., observed only minimum penetration of motor axons into the communicating nerve graft when the perineurium of the donor nerve was left intact, in contrast to donor nerves with epineurial or perineurial windows. Thus, removal of the perineurium at the site of coaptation has been strongly recommended to enhance motor axon invasion into the distal stump of a recipient nerve. Histological examination, however, suggested that injury to some nerve fibers in the donor nerve could not be avoided after longitudinal incision in the donor nerve sheath. Thus, better axonal growth into the communicating distal nerve stump in nerves with a perineurial window could be due to inadvertent axonal injury and regeneration and not just to enhanced collateral sprouting of uninjured axons. In this case, partial denervation of donor nerve targets is expected.

On the other hand, even though no epineurial window had been made, abundant sprouting of sensory axons into the communicating nerve stump was observed in several studies and confirmed by the results of our experiments (280 myelinated axons in the communicating peroneal stump, which is approximately 23% of the number of the myelinated axons in the normal sural nerve and 15% of the normal peroneal nerve).

Using our technique we found no significant functional injury to the donor nerve. After suturing the site of end-to-side communication between the peroneal and sural nerves and transecting the adjacent peripheral nerves, the pain-sensitive area of the sural nerve did not diminish during the recovery period. On the contrary, it increased because of collateral sprouting in the skin. We could also detect no morphological evidence of an untoward effect of collateral sprouting into the end-to-side communicating...
nerve on the axons of the donor nerve itself. After histological examination of sural nerve cross sections, no signs of degeneration and/or regeneration of axons were detected and the number and diameter of myelinated axons did not change significantly.

Conclusions

Fairly abundant collateral sprouting of intact sural sensory axons through the end-to-side communication into the distal nerve stump of the transected peroneal nerve is possible even without creating an epineural or perineurial window in the donor nerve. Following this procedure, recovery of sensory function in a transected peripheral nerve is possible in principle; however, some factors, at present poorly defined, can delay or prevent functional recovery under these conditions in spite of successful collateral sprouting through the end-to-side nerve communication. The results suggest that after a careful surgical procedure, nociceptive supply to the skin by the donor nerve is not perceptibly downgraded after the recipient nerve had been neuromatized. Therefore, the idea of achieving reinnervation of a distal denervated nerve stump and its targets without sacrifice of the donor nerve remains extremely attractive in peripheral nerve surgery and deserves further investigation.

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References

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