H. Millesi and
R. Schmidhammer (eds.)

How to Improve the Results of Peripheral Nerve Surgery

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Preface

All over the world research is being undertaken to improve the treatment outcome of peripheral nerve lesions. Questions over questions arise. Is autologous nerve grafting still the gold standard for bridging defects? Have alternative techniques for overcoming defects of peripheral nerves reached a level to replace autografting? To which length are they effective? What is the role of allografting? Are there still indications for vascularized nerve grafts? What can be expected from end-to-side coaptation? Does it exist at all? In what conditions can useful recoveries be achieved? Are there new developments in physical medicine and physiotherapy? Can the quality of recovery be influenced by surgery on muscles to provide a better equilibrium of forces? To what extent may cerebral plasticity be exploited to improve functional results?

If you want an answer to all these questions, look into this book. You will find comprehensive and well founded arguments to make up your own mind.

Hanno Millesi and *Robert Schmidhammer*

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The potential of electrical stimulation to promote functional recovery after peripheral nerve injury – comparisons between rats and humans

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Summary

The declining capacity for injured peripheral nerves to regenerate their axons with time and distance is accounted for, at least in part, by the chronic axotomy of the neurons and Schwann cell denervation prior to target reinnervation. A largely unrecognized site of delay is the surgical suture site where, in rats, 4 weeks is required for all neurons to regenerate their axons across the site. Low frequency stimulation for just 1 h after surgery accelerates this axon crossing in association with upregulation of neurotrophic factors in the neurons. We translated these findings to human patients by examining the number of reinnervated motor units in the median nerve-innervated thenar muscles before and after carpel tunnel release surgery in a randomized controlled trial. Motor unit number estimates (MUNE) in patients with moderate and severe carpal tunnel syndrome were significantly lower than normal. This number increased significantly by 6–8 months after surgery and reached normal values by 12 months in contrast to a non-significant increase in the control unstimulated group. Tests including the Purdue Pegboard Test verified the more rapid functional recovery after stimulation. The data indicate a feasible strategy to promote axonal regeneration in humans that has the potential to improve functional outcomes, especially in combination with strategies to sustain the regenerative capacity of neurons and the support of Schwann cells over distance and time.

Keywords: Electric stimulation; peripheral nerve injury; regeneration; reinnervation.

Introduction

Recovery of function is frequently poor despite the considerable technical improvements in surgical repair of nerve injuries and our enhanced understanding of the biology of axon regeneration [40, 63, 64]. A common mistake frequently made by investigators of nerve regeneration in animal models is that all injured peripheral nerve regenerate successfully. Recovery of both sensory and motor function in the animal models may be very good but the injuries generally require regeneration of axons over relatively small distances to make functional connections with target muscles and sense organs [30, 38, 68]. However, the situation is more complex for transection injuries of larger nerve trunks where functional recovery may be compromised by misdirection of regenerating axons to inappropriate targets including motor axons reinnervating muscles with antagonistic functions [12]. The inability of axons to navigate selectively into their original Schwann cell tubes is a key component that is responsible for the considerable misdirection of regenerating axons and, in turn, generation of inappropriate movements, synkinesias, and abnormal and/or loss of sensations [18, 38]; this problem may be exacerbated by central changes of cortical representation that may or may not be reversible [42].

The problems of misdirection of regenerated axons are readily discernable in humans after surgical repair of large peripheral nerves. In the case of the ulnar or median nerves at the wrist, random reinnervation of the muscles across the hand has been documented [41, 67]. Misdirection after more proximal nerve injuries that include brachial and lumbar plexi nerve injuries, are well recognised [28, 40]. For the latter injuries in humans, the problems of the distance and the time required for axons to regenerate at rates of 1 mm/day or lower [62] culminate in very poor functional recoveries. This is so particularly for the more distally placed muscles and sense organs. These poor outcomes have been attributed to the progressive denervation atrophy of target muscles and their replacement by fat [5, 63].

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However, our experiments in an animal model of prolonged axon regeneration provided strong evidence that it is the long durations over which first the injured neurons remain without target connections (chronic axotomy), and second denervated Schwann cells in the distal nerve stumps lack axon contact (chronic Schwann cell denervation), that account for the progressive failure of neurons to regenerate their axons over time and distance [26, 27, 32–34, 57, 60]. These experiments elucidated the relatively narrow window of opportunity for successful regeneration of axons in the peripheral nervous system [27].

Normally motoneurons and sensory neurons mount a strong regenerative response to injury: regeneration associated genes including tubulin, GAP-43 and neurotrophins are expressed in association with regeneration of axons within the endoneurial tubes of the denervated distal nerve stump that are lined by Schwann cells [3, 7, 11, 65]. The growth response of the axotomized neurons is not sustained, expression of the regeneration associated genes declining with time [66]. This explains the progressive failure in regenerative success whether or not the axon growth of the neurons is frustrated by physical block or the axon growth proceeds over long distances without target contact (Furey *et al.*, 2007). The deterioration of the growth response of the chronically axotomized neurons can be reversed by administration of exogenous neurotrophic factors suggesting that the growth program of the injured neurons is sustained in part by neurotrophic factors which are provided by the Schwann cells in the growth pathway in the distal nerve stumps [9, 10, 11, 33].

The Schwann cells that normally myelinate the intact axons, undergo cell division during Wallerian degeneration of the isolated axons in the nerve stump distal to the injury site [6, 16, 19, 27]. Schwann cells respond to neuregulin and other axon-derived components, dividing and guiding the regenerating axons which they myelinate as the axons increase in diameter in direct proportion to their parent axons in the proximal nerve stump [6, 16, 17, 36]. Schwann cell expression of growth-associated proteins that include glial-derived and brain-derived neurotrophic factors [7, 11, 27], is not sustained if denervation of the distal nerve stump is prolonged [16, 36, 37, 49] and the cells progressively undergo atrophy and attrition by cell death [20, 29, 36, 39, 58, 71, 72]. These processes parallel the progressive reduction in numbers of neurons that succeed in regenerating their axons even though the Schwann cells that remain are reactivated by regenerating axons and successfully remyelinate the axons [36, 57]. Importantly, the small percentage of axons that do regenerate through chronically denervated Schwann cell tubes make functional connections with the chronically denervated skeletal muscle fibers and form enlarged reinnervated motor units [26]. Therefore, it is the chronic denervation of the Schwann cells and not the chronic denervation of skeletal muscle that is a major determinant of the progressive failure of regeneration through the Schwann cell tubes. The challenge is to sustain the population of Schwann cells and their capacity to support axonal regeneration: these include the use of cytokines to promote cell division and reexpression of the growth supportive phenotype of the Schwann cell as well as surgical methods to attenuate the atrophy and loss of Schwann cells [25, 33, 34, 47, 58, 59–61].

A latent period of hours to a few days has been repeatedly described for the crossing of regenerating axons into the distal nerve stump. Latent periods and regeneration rates were calculated from measurements of the distance from the crush site where nerve crush elicited an inspiratory reflex contraction [8]. Rate of regeneration is 3 mm/day for the most rapidly regenerating axons and there is a latent period of hours to a few days prior to onset of regeneration [25, 35, 72]. A more direct method of determining the time course of the axon outgrowth across the suture site is to apply a retrograde dye just distal to the site of section and repair [13, 14]. Using this technique, we noted that indeed there was a latent period of a few days before few motor axons had crossed the suture site of the cut and surgically repaired femoral nerve in the rat. It was very dramatic to observe a surprisingly long period of 28 days for all the motoneurons to regenerate their axons across the suture site and 1.5 mm into the distal nerve stump [13]. Hence the regeneration of axons across the suture site is a rate-limiting process. It is only after which the axons regenerate within the distal nerve stumps at rates of 3 mm/day or less. We have recently confirmed this rate-limiting step in the hindlimb after common peroneal nerve section and surgical repair (unpublished data). Brushart *et al.* [70] have just recently visualized yellow florescent protein labelled motor axons as regenerating axons traverse the surgical site to confirm the ''staggering'' of regenerating axons across the surgical gap and their multiple branching to penetrate several endoneurial tubes in the distal nerve stump. The beautiful silver-stained regenerating fibers visualized by Cajal as ''wandering'' across the suture site predated these findings [54]. The extensive collateral branching of the regenerating axons results in the pas-

sage of up to 20 regenerated axons in the distal nerve stumps for every parent axon in the nerve proximal to the injury site [1, 43].

Many attempts have been made to accelerate axonal regeneration. It is only the conditioning lesion which precedes the section of the nerve and resuture that has been shown to accelerate rate of regeneration in concert with acceleration of slow transport of cytoskeletal proteins [8, 45]. Despite a long history of the clinical use of electrical stimulation after nerve injuries to sustain denervated muscle bulk and/or to circumvent contractures that are deleterious to joint movement, scientific investigations of the effects of the stimulation on either axon regeneration or muscle bulk were relatively scarce. In fact, there remain few studies that provide convincing evidence that electrical stimulation prevents denervation muscle atrophy [2, 15, 48, 50, 51, 69]. The research that pursued the question of the role of muscle fiber electrical activity in reducing extrajunctional acetylcholine receptor distribution has established the role of the activity in suppressing the synthesis of the receptors by the nuclei outside of the neuromuscular junction [46, 55]. Only a few studies pursued the question of a role of electrical stimulation of the proximal stump of injured nerves in promoting axon regeneration. These provided enticing evidence of positive effects of electrical stimulation immediately after crush injuries accelerating both the recovery of reflexes and of nerve-elicited muscle contractions in vivo [13, 23, 24, 52] and increasing neurite outgrowth *in vitro* [56]. In order to determine whether electrical stimulation accelerates axon outgrowth and/or rate of axon regeneration and slow axonal transport after nerve section and repair, we undertook a series of experiments to evaluate the effects of low frequency electrical stimulation on the number of motor and sensory neurons that regenerate their axons into and along the distal nerve stumps. We used a rat model of femoral nerve section and resuture and application of retrograde dyes to count the number of motoneurons that regenerated their axons across the suture site and through the distal nerve stump. On the basis of our dramatic findings of accelerated outgrowth of regenerating axons in the animal model [3, 13, 14, 34], we undertook a randomized clinical trial of application of a one hour period of low frequency stimulation after carpal tunnel syndrome release. We used electromyographical methods to evaluate the time course and extent of muscle and sense organ reinnervation by motor and sensory regenerating axons, respectively.

Methods

Animal studies

Surgeries, electrical stimulation, and neuronal backlabelling for counting of neurons that regenerate their axons

Sprague-Dawley rats of 220–260 gm body weight were anesthetized with somnotol (30 mg/kg.i.p.) for exposure of the femoral nerve bilaterally. The nerve was sectioned 20 mm from the bifurcation into the sensory and motor branches for microsurgical repair using 10-0 silk. Silver wires were bared at the tips and positioned proximal to the surgical site for either 20 Hz supramaximal stimulation at 200 μs and 3 V in the experimental group and, for sham stimulation (electrodes connected to the stimulator but not turned on) for 1 h. The skin incisions were sutured closed with 4-0 silk and the rats recovered consciousness a heat lamp.

At time periods of 4 d, 1, 2, 3, and 4 weeks after nerve section and resuture, either the femoral nerve or its motor and sensory branches were exposed for application of retrograde dyes, fluorogold (FG) and/or fluororuby (FR). The nerve was crushed 1.5 mm from the suture site for microinjection of 0.5 μl FR to backlabel motor and sensory neurons that regenerated their axons just across the suture site. The nerve branches were cut in the other set of rats, 5 mm from the branch point, for application of FG and RR via Vaseline pools that isolated the dyes to the cut end of the motor and sensory nerves.

Three to 5 days later, the rats were perfused with 4% paraformaldehyde under surgical anesthesia. The fixed spinal cord at levels of Thoracic 11 to lumbar L1, and the L2–L4 dorsal root ganglia were removed and frozen at −70 °C prior to sectioning at 40 μm to count the number of backlabelled neurons that had regenerated their axons.

Human subjects

Selection, surgery and electrical stimulation

Human subjects were recruited from a university hospital electromyography clinic for a randomized controlled trial of the application of electrical stimulation after carpal tunnel syndrome release, complying with the guidelines of and approved by the Human Research Ethics Board at the University of Alberta. Diagnosis of carpal tunnel syndrome (CTS) and the classification into mild, medium or severe was made based on nerve conduction studies [34]. If the conduction speeds of the median sensory and motor nerve fibers were both abnormal but the action potentials were still present, subjects were categorized as having moderate CTS; if the median sensory nerve action potential was absent, the patients were classified as severe. Patients with moderate and severe CTS who had not responded to conservative treatments were recruited for this study. Presence of other neurological conditions, trauma to wrist or arm and previous carpal tunnel release, were used as exclusion criteria.

Open carpal tunnel release was performed under local anesthesia and below a forearm inflated cuff. Via a 3 cm long longitudinal skin incision, the transverse carpal ligament was divided with a scalpel along the ulnar side of the incision. In the randomly assigned patients of the stimulation group, 2 sterile 30 gauge Cooner wires insulated except for 1 cm at the tip, were placed over the medial nerve above the site of compression. The wire electrodes were attached to a Grass (C9) stimulator for 1 h electrical stimulation at maximum tolerance level for 1 h at 20 Hz limit (4–6 V, 0.1–0.8 ms duration), which was initiated 30 min following closure of the incision with 5-0 nylon. Two surface electrodes (TECA, Oxford Instruments), were placed to record compound muscle action potential (CMAP) during the post-surgical electrical stimulation, one over the motor point on the thenar eminence muscles and the other over the dorsal aspect of the first metacarpophalangeal joint. The site was covered by a soft dressing and the hand was elevated above heart level

for 24 h after surgery to prevent subsequent swelling and discomfort. Finger movement and gentle use of hand after surgery was encouraged. The dressing over the incision site was removed a week later, the sutures were removed 2 weeks after the surgery, and patients were allowed to return back to work 2–4 weeks later.

Outcome measures

In 2 pre-operative recording sessions, 1 week apart prior to surgery, and 3 post-operative time periods of 3, 6–8 and 12 months, median motor and sensory nerve conduction studies and motor unit number estimates (MUNE) were carried out. The third month was selected for the first assessment based on the assumptions that the most optimal axons growth rate is 1 mm/day and the distance between the compression site and the thenar muscles is approximately 70–80 mm depending on the size of the hand.

Disposable, self-adhesive surface strip silver/silver chloride surface electrodes (Nicolet VIASYS Healthcare), measuring 1×2.5 cm were used to record surface-detected potentials. For sensory conduction measurements, the recording electrode was placed on the proximal interphalangeal joint and the reference electrode was placed on the distal interphalangeal joint of the third digit. The median nerve was stimulated in mid-palm and also just proximal to the distal wrist crease to record the sensory nerve action potentials (SNAP). Maximum M-wave and surface-detected motor unit potentials (S-MUAP) were recorded from a recording electrode placed over the motor point on the thenar eminence muscles and a reference electrode placed over the dorsal aspect of the first metacarpophalangeal joint. A 3×3 cm metal plate on the back of the hand served as a ground. The bandpass filter was set at 5–2000 Hz. The position of the thumb was standardized by taping it to the side of the palm in an adducted position. For measurement of motor conduction, a maximum M-wave was elicited by supramaximal stimulation of the median nerve (10% above maximal intensity with a duration of 0.01 ms) at the wrist 8 cm proximal to the recording electrode. A hand-held constant-current bipolar surface bar stimulator was used for surface stimulation of the nerves.

The motor unit number estimation (MUNE) used the multiple point stimulation technique to determine the number of motoneurons that regenerate their axons and innervate thenar muscles, as described by Doherty *et al.* [68]. S-MUAPs with the lowest stimulus thresholds were elicited by stimulating the median nerve at multiple sites at the wrist and between the elbow and the axilla (Fig. 1). The nerve was stimulated at 1 Hz with gradually increasing intensity until the first reproducible, "all-or-none" S-MUAP was evoked. Using the template subtraction method, the lowest threshold S-MUAP was obtained by subtracting the "all" response from the baseline. To increase the yield, the next higher threshold S-MUAP could sometimes be obtained through template subtraction. A collected sample of at least 12 S-MUAPs was stored in computer memory. The mean peak-to-peak amplitude of this sample of S-MUAPs was calculated using "datapoint-by-datapoint" summation. All S-MAUPs

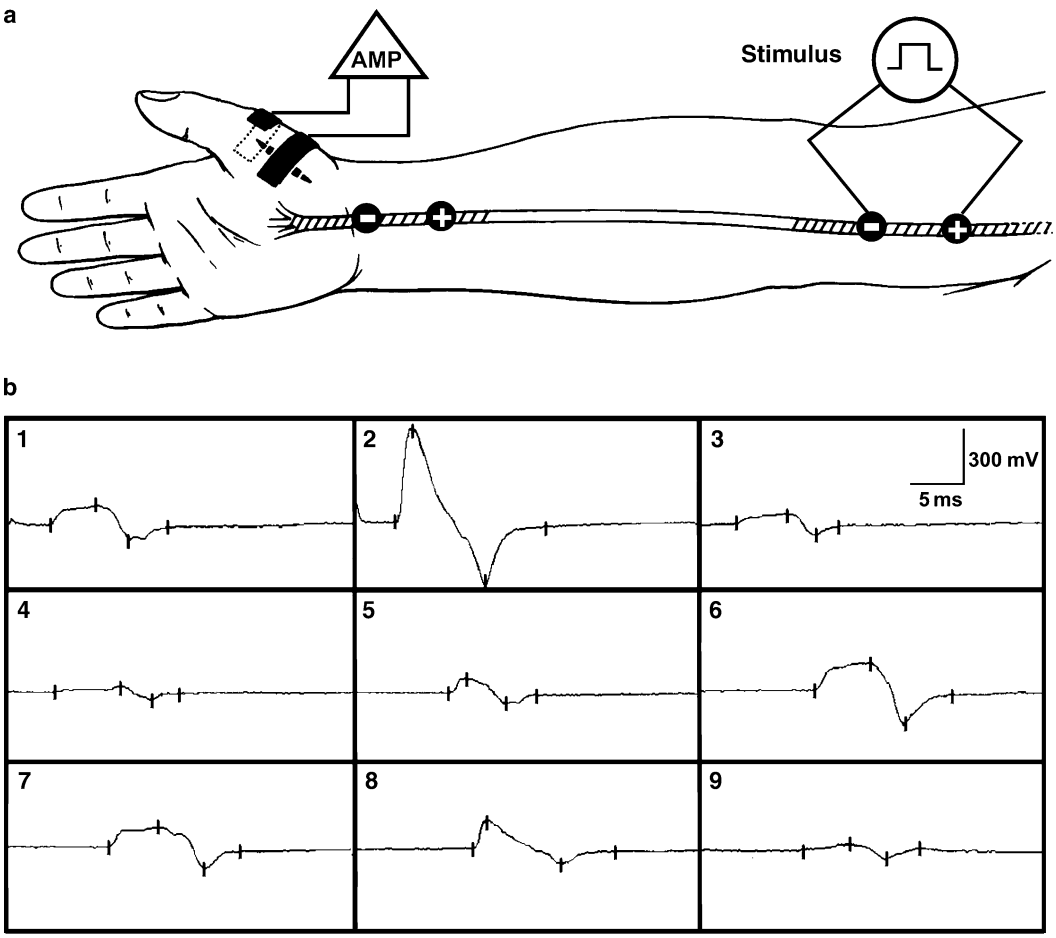

Fig. 1. (a) Figurative illustration of the electromyographic recording from the thenar muscles of the median eminence in response to stimulation of the median nerve at progressively more proximal sites along the nerve in the forearm. (b) Examples of the electromyographic signals elicited in an all-or-none fashion at the in response to progressive increase in stimulus voltage

were temporally aligned at the same onset latency before they were averaged. The MUNE was obtained using the following equation:

$$\frac{\text{Peak-to-peak amplitude of the maximum M-wave}}{\text{Peak-to-peak amplitude of the average S-MUAP}} = \text{MUNE}$$

Results

Stimulation-induced acceleration of staggered axon regeneration across a suture site in rats

A 1 h period of low frequency electrical stimulation (20 Hz) of the proximal nerve stump of the cut and resutured femoral nerve in the rat accelerated axon regeneration across the repair site. The time taken for all the axotomized motoneurons to regenerate their axons across the surgical site was accelerated by a week, the motoneurons requiring 28 and 21 days in the unstimulated control and electrically stimulated groups of nerves, respectively (Fig. 2). The number of motoneurons that regenerated axons across the repair site was significantly higher for the stimulated neurons within 4 days after nerve repair and stimulation and continued to be significantly higher for the next 3 weeks at which point in time, all motoneurons have regenerated their axons. The accelerated outgrowth of axons across the surgical site was seen both for motor and sensory neurons. Stimulation significantly increased the number of motor and sensory neurons that regenerated their axons across the suture line: at 4 days after surgery, a mean of 460 sensory neurons and of 40 motoneurons regenerated their axons across the suture line and were backlabeled with FR

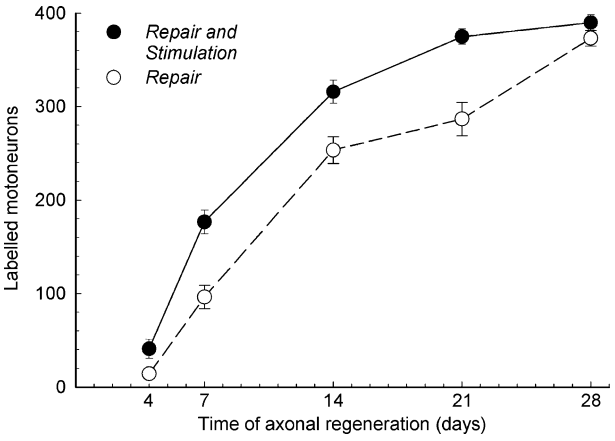

Fig. 2. The mean number (±S.E.) of axotomized femoral motoneurons that regenerated their axons 1.5 mm into the distal nerve stump across the suture line of the transected and surgically repaired nerve. The number was significantly elevated at all times measured from 4 to 21 days after the surgical repair when the proximal nerve stump was electrically stimulated at 20 Hz for 1 h after the surgical repair. The motoneurons were backlabeled with the retrograde dye, fluororuby

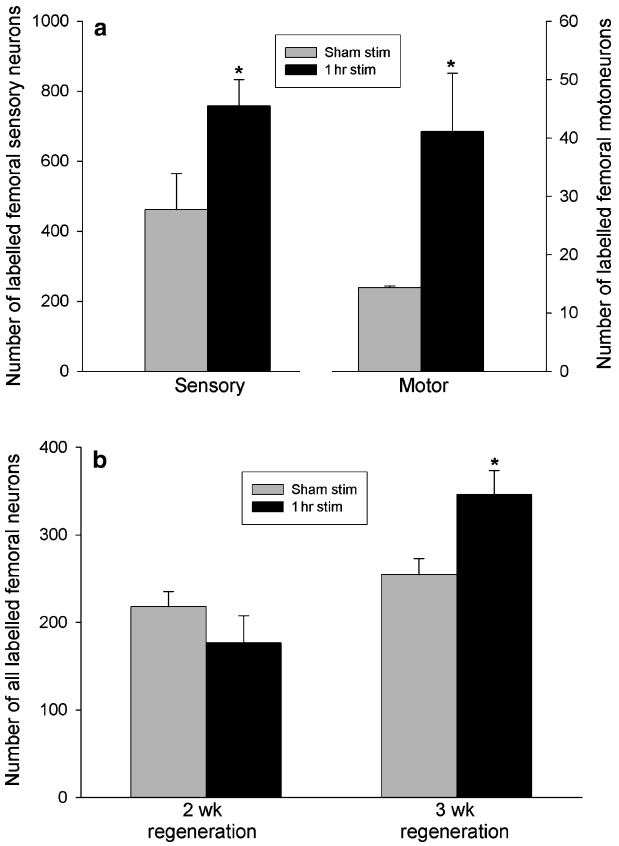

Fig. 3. A 1 h period of 20 Hz stimulation of the nerve immediately after surgical repair of the transected and repaired femoral nerve significantly elevated the number of (a) sensory and motor neurons that regenerated their axons 1.5 mm into the distal nerve stump across the surgical repair site in 4 days, and (b) the motoneurons that regenerated their axons a distance of 25 mm into the saphenous and quadriceps nerve branches of the femoral nerve 2 and 3 weeks after surgical repair

applied 1.5 mm distal to the suture line (Fig. 3a). Electrical stimulation accelerated the axon outgrowth across the suture line with mean values of 760 and 13 sensory and motor neurons regenerating their axons, an increase of 1.7 for the sensory neurons and 3.1 times for the motoneurons due to the electrical stimulation. Stimulation dramatically increased the number of sensory and motor neurons that regenerated their axons a distance of 25 mm by 3 weeks after the surgical repair of the femoral nerve: the number of motoneurons that had regenerated their axons corresponding to the entire motoneuron pool of the intact femoral nerve (Fig. 3b). Since the stimulation did not alter the rate of slow axon transport [13], we conclude that the increased number of neurons that regenerated their axons 25 mm from the suture line after stimulation likely reflects this accelerated axon outgrowth across the suture site (Fig. 2). We did not record the force of contraction of the reinner-

vated quadriceps muscle but we observed the evoked muscle contraction 3 weeks after nerve repair, the contraction being visibly stronger in the stimulation group of rats.

Stimulation-induced acceleration of axon regeneration and muscle reinnervation in a human model of nerve crush injury and surgical release of pressure

We then asked whether electrical stimulation could accelerate axon regeneration after crush injury in human subjects. Patients diagnosed with moderate and severe carpal tunnel syndrome, based on nerve conduction studies [53] were divided into stimulation and no stimulation groups. The mean $\pm$ SE age of the patients in the two groups was not significantly different, being 53 ± 18 year and 61 ± 16 years, respectively. Motor unit number estimates were made from the ratio of the maximum M wave, evoked by median nerve stimulation at the wrist, and the averaged S-MUAP, obtained through ''data-point-by-datapoint summation''of S-MUAPs that were evoked in an all-or-none manner by stimulation at multiple sites at the wrist and between the elbow and the axilla. Presurgical MUNEs are the motor axons that have not been injured by the compression at the wrist and retain their connection with muscle fibers in the median eminence. The mean number was significantly lower than

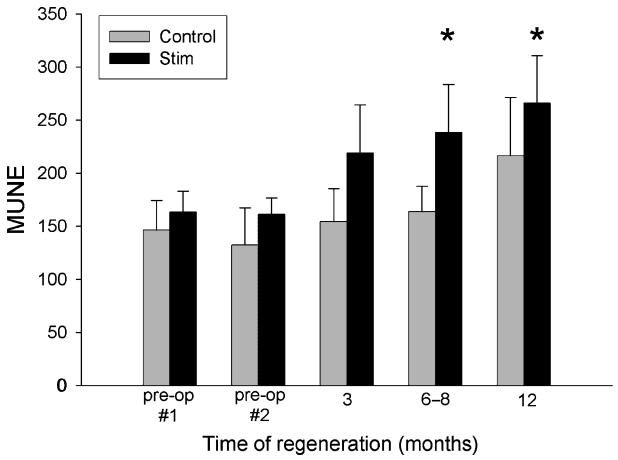

Fig. 4. A 1 h period of 20 Hz stimulation of the median nerve immediately after carpal tunnel release surgery in human patients with moderate and severe carpal tunnel syndrome effectively increased the number of motoneurons that regenerated and reinnervated the muscles of the thenar eminence. The motor unit number estimate (MUNE) ($\pm$S.E) was similar in the stimulation and the control groups of patients prior to surgery. After surgery, the trend for the MUNE to rise over a period of a year after the surgery was not significant in contrast to the significant increase in the number of motor units counted in the thenar eminence after electrical stimulation following the surgery

288 ± 95 (mean $\pm$ SD), the normal number of intact motor units in healthy individuals [21].

There was a trend for the mean number of motor units (MUNE) to increase at 3, 6–8 and 12 months after carpal tunnel release without stimulation (Fig. 4). However, this increase was not statistically significant. The upward trend progressed more rapidly in the patients in which electrical stimulation was carried out immediately after the carpal tunnel release surgery. The number of intact motor units increased significantly compared to the numbers at baseline within the same subjects in the stimulation group and was significantly higher than the number of motor units in the non-stimulation group. By 12 months, MUNE had increased to a level that was not significantly different from the MUNE of 288 ± 95 intact motor units in the normal hand [21]. Hence, carpal tunnel release surgery did not afford a recovery of normal numbers of motor units in the median eminence within a year. In combination with electrical stimulation however, the release surgery resulted in an increase of 2.1 from mean values of 140 to 290 motor units after stimulation. This increase compares with an increase of 1.4 in the number of motoneurons that regenerate their axons a distance of 25 mm towards the denervated targets after femoral nerve section and surgical repair in rats (Fig. 3b). In both cases, electrical stimulation promoted the regeneration of all motor axons, the regenerating axons effectively reinnervating the median eminence within one year over a distance of about 60–70 mm in the human patients.

Discussion

With the conventional assumptions of a latent period of hours to days and a regeneration rate of 3 mm/day, one would predict that all the axotomized rat femoral motoneurons would only require less than 3 weeks to regenerate their axons a distance of 25 mm into the distal nerve stump. However, our findings that all the motoneurons required $\sim$8 weeks to regenerate their axons over this distance suggested to us that the outgrowth of sprouts and their passage across the site of surgical suture of the proximal and distal stumps may be regarded as staggered axon regeneration [4]. Cajal had reported the ''wandering'' of regenerating axons across the suture site [54], a finding that has been supported and extended by the analysis of single fluorescent axon outgrowth at the site and the penetration of the distal Schwann cell tubes [70]. Indeed, our analysis of the number of motoneurons that regenerated their axons just 1.5 mm into the

distal nerve stump, using retrograde FR uptake, demonstrated that the number was less than 20, ~10% of the total number of axotomized motoneurons 4 days after the surgical repair and electrical stimulation. The number increased progressively over a period of 4 weeks, a period of time that was quite consistent with the suggested staggering of regeneration across the suture site (Fig. 2). The 4 week period of time necessary for regenerating axons to cross the surgical gap includes the previously measured latent periods. Once the axons cross the suture line, a regeneration rate of 3 mm/day predicts that all femoral motoneurons would regenerate the distance of 25 mm from the suture site within 6–7 weeks, a reasonable correspondence with the time that was observed by Al-Majed *et al.* [4].

A period of just 1 h electrical stimulation at 20 Hz was effective in accelerating the regeneration of axons across the surgical gap. The acceleration across the surgical gap was a little less than expected from the findings that all motoneurons had regenerated their axons 25 mm into the distal nerve stump by 3–4 weeks after surgical repair and the stimulation immediately after the repair. Some of the discrepancy could be accounted for by variations in the time of application of the retrograde dyes. Nonetheless, the effect of the electrical stimulation clearly accelerated the crossing of regenerating axons. In light of the findings that the slow rate of axon transport was not altered by electrical stimulation [13], the effectiveness of the stimulation is clearly localized at the suture site. We are presently visualizing the coursing of the regenerating axons across the suture line in fluorescent motoneurons in transgenic mice with the expectation that the electrical stimulation should be associated with less ''wondering'' of the regenerating axons across the surgical site. To observe the crossing of regenerating axons across the suture site, Brushart *et al.* [70] has used this transgenic mouse to clarify and extend Cajal's original observations of the complex course that the regenerating axons traverse in the suture line before they enter into the endoneurial sheaths of the distal nerve stump.

We have extended these very promising findings in rats to human patients who underwent a carpal tunnel release surgery to promote axon regeneration after compression injury. The trend for a progressive increase in reinnervated motor units up to 1 year after the release was not significant. In contrast, a 1 h period of 20 Hz electrical stimulation of the median nerve some 15 min after the surgical release of the median nerve from the carpal tunnel, led to a dramatic and significant progressive increase in the number of reinnervated motor units (Fig. 4).

Compare and contrast between the animal and human models

In the human, electrical stimulation affected the same proportional increase in reinnervated motor units within 26 and 52 weeks of stimulation while electrical stimulation in the rat had affected the regeneration of all motor axons within 3–4 weeks of stimulation [4]. In the human case, median nerve axons regenerate over a distance of 60–70 mm to reinnervate the thenar muscles in the median eminence. In the rats, femoral motor axons regenerate over a distance of ~30 mm to reinnervate the quadriceps muscle. The data obtained in the human and rats compares well, the principle delay of outgrowth of axons and their crossing the surgical site to enter the distal nerve stumps being longer in the human. In addition, the rate of regeneration is known to be 3 times slower in the human than in the rat. Taken in the light of the animal findings of the stimulation-induced axon outgrowth from the proximal nerve stump across a suture site, this positive finding for the effectiveness of electrical stimulation to promote axon regeneration after a compression injury in human subjects, indicates that the electrical stimulation is effective in accelerating axon outgrowth whether or not the continuity of the nerve sheath is sustained prior to surgery. Hence at a regeneration rate of 1 mm/day for the fastest regenerating axons, our finding of a 6 month period before there were significantly more reinnervated motor units in the stimulation group is consistent with our findings in animals of the substantial delay that normally occurs at the injury site and the effectiveness of the stimulation in accelerating the axon outgrowth across this site. In humans where the movement of the Schwann cells is recognized to be more sluggish, a longer delay in axon outgrowth across the suture site would not be unexpected.

Clinical implications

Carpal tunnel syndrome is one of the most common nerve injuries. Surgical decompression is currently the treatment of choice for moderate and severe cases. However, even with surgery, axonal regeneration in severe cases remains poor. Even though a great number of carpal tunnel release operations are carried out in Canada annually, more direct methods of assessing their success in inducing motor axonal regeneration have generally been extremely limited. In this longitudinal study, we used quantitative methods of motor unit number estimation by electromyographic recordings developed originally by McComas and extended here to recruit motor units selectively [44]. The develop-

ment of the selective recruitment by progressively moving the stimulating electrodes along the arm from the wrist to the shoulder allows for a more accurate counting of single motor units, providing a strong quantitative measure of regenerative success. An advantage over the method used in the animal experiments to count the number of neurons that regenerated their axons into the distal stump, the enumeration of reinnervated motor units provided the first measure of functional recovery.

In this study, we demonstrated the feasibility of applying electrical stimulation post-surgically to patients to accelerate axon regeneration. The procedure was well tolerated with no acute or long term complications. There is a narrow window of opportunity for axon regeneration after nerve injury afforded by the failure of axotomized neurons to sustain their growth potential and for the denervated Schwann cells to provide support for regenerating axons. Hence, the very significant improvement in the number of motoneurons that regenerated their axons to reinnervate denervated target muscles provides exciting possibilities to further explore this method of accelerating axon regeneration after new injuries in humans.

Acknowledgements

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References

1. Aitken JT, Sharman M, Young JZ (1947) Maturation of peripheral nerve fibres with various peripheral connections. J Anat 81: 1–22
2. Al-Amood WS, Lewis DM, Schmalbruch H (1991) Effects of chronic electrical stimulation on contractile properties of long-term denervated rat skeletal muscle. J Physiol 441: 243–256
3. Al-Majed AA, Brushart TM, Gordon T (2000) Electrical stimulation accelerates and increases expression of BDNF and trkB mRNA in regenerating rat femoral motoneurons. Eur J Neurosci 12: 4381–4390
4. Al-Majed AA, Neumann CM, Brushart TM, Gordon T (2000) Brief electrical stimulation promotes the speed and accuracy of motor axonal regeneration. J Neurosci 20: 2602–2608
5. Anzil AP, Wernig A (1989) Muscle fibre loss and reinnervation after long-term denervation. J Neurocytol 18: 833–845
6. Atanasoski S, Scherer SS, Sirkowski E, Leone D, Garratt AN, Birchmeier C, Suter U (2006) ErbB2 signaling in Schwann cells is mostly dispensable for maintenance of myelinated peripheral nerves and proliferation of adult Schwann cells after injury. J Neurosci 26: 2124–2131
7. Bisby MA, Pollock B (1983) Increased regeneration rate in peripheral-nerve axons following double lesions – Enhancement of the conditioning lesion phenomenon. J Neurobiol 14: 467–472
8. Bisby MA, Tetzlaff W (1992) Changes in cytoskeletal protein synthesis following axon injury and during axon regeneration. Mol Neurobiol 6: 107–123
9. Boyd JG, Gordon T (2002) A dose-dependent facilitation and inhibition of peripheral nerve regeneration by brain-derived neurotrophic factor. Eur J Neurosci 15: 613–626
10. Boyd JG, Gordon T (2003) Glial cell line-derived neurotrophic factor and brain-derived neurotrophic factor sustain the axonal regeneration of chronically axotomized motoneurons in vivo. Exp Neurol 183: 610–619
11. Boyd JG, Gordon T (2003) Neurotrophic factors and their receptors in axonal regeneration and functional recovery after peripheral nerve injury. Mol Neurobiol 27: 277–324
12. Brushart TM, Mesulam MM (1980) Alteration in connections between muscle and anterior horn motoneurons after peripheral nerve repair. Science 208: 603–605
13. Brushart TM, Hoffman PN, Royall RM, Murinson BB, Witzel C, Gordon T (2002) Electrical stimulation promotes motoneuron regeneration without increasing its speed or conditioning the neuron. J Neurosci 22: 6631–6638
14. Brushart TM, Jari R, Verge V, Rohde C, Gordon T (2005) Electrical stimulation restores the specificity of sensory axon regeneration. Exp Neurol 194: 221–229
15. Carraro U, Rossini K, Mayr W, Kern H (2005) Muscle fiber regeneration in human permanent lower motoneuron denervation: relevance to safety and effectiveness of FES-training, which induces muscle recovery in SCI subjects. Artif Organs 29: 187–191
16. Carroll SL, Miller ML, Frohnert PW, Kim SS, Corbett JA (1997) Expression of neuregulins and their putative receptors, ErbB2 and ErbB3, is induced during Wallerian degeneration. J Neurosci 17: 1642–1659
17. Cheng L, Esch FS, Marchionni MA, Mudge AW (1998) Control of Schwann cell survival and proliferation: autocrine factors and neuregulins. Mol Cell Neurosci 12: 141–156
18. Choi D, Raisman G (2002) Somatotopic organization of the facial nucleus is disrupted after lesioning and regeneration of the facial nerve: the histological representation of synkinesis. Neurosurg 50: 355–362
19. Clemence A, Mirsky R, Jessen KR (1989) Non-myelin-forming Schwann cells proliferate rapidly during Wallerian degeneration in the rat sciatic nerve. J Neurocytol 18: 185–192
20. Dedkov EI, Kostrominova TY, Borisov AB, Carlson BM (2002) Survival of Schwann cells in chronically denervated skeletal muscles. Acta Neuropathol (Berl) 103: 565–574
21. Doherty TJ, Brown WF (1993) The estimated numbers and relative sizes of thenar motor units as selected by multiple point stimulation in young and older adults. Muscle Nerve 16: 355–366
22. Doherty T, Simmons Z, O'Connell B, Felice KJ, Conwit R, Chan KM, Komori T, Brown T, Stashuk DW, Brown WF (1995) Methods for estimating the numbers of motor units in human muscles. J Clin Neurophysiol 12: 565–584
23. Eberhardt KA, Irintchev A, Al-Majed AA, Simova O, Brushart TM, Gordon T, Schachner M (2006) BDNF/TrkB signaling regulates HNK-1 carbohydrate expression in regenerating motor nerves and promotes functional recovery after peripheral nerve repair. Exp Neurol 198: 500–510
24. Eberstein A, Pachter BR (1986) The effect of electrical stimulation on reinnervation of rat muscle: contractile properties and endplate morhpometry. Brain Res 384: 304–310
25. Fenrich K, Gordon T (2004) Canadian association of neuroscience review: axonal regeneration in the peripheral and central nervous systems – current issues and advances. Can J Neurol Sci 31: 142–156
26. Fu SY, Gordon T (1995) Contributing factors to poor functional recovery after delayed nerve repair: prolonged denervation. J Neurosci 15: 3886–3895
27. Fu SY, Gordon T (1997) The cellular and molecular basis of peripheral nerve regeneration. Mol Neurobiol 14: 67–116

28. Gentili F, Hudson AR, Midha R (1996) Peripheral nerve injuries: types, causes, and grading. In: Wilkins RH, Rengachary SS (eds) Neurosurgery, McGraw-Hill, pp 3105–3114
29. Giannini C, Dyck PJ (1990) The fate of Schwann cell basement membranes in permanently transected nerves. J Neuropathol Exp Neurol 49: 550–563
30. Gillespie MJ, Gordon T, Murphy PR (1986) Reinnervation of the lateral gastrocnemius and soleus muscles in the rat by their common nerve. J Physiol 372: 485–500
31. Gordon T (1994) Mechanisms for functional recovery of the larynx after surgical repair of injured nerves. J Voice 8: 70–78
32. Gordon T, Fu SY (1997) Long-term response to nerve injury. Adv Neurol 72: 185–199
33. Gordon T, Boyd JG, Sulaiman OAR (2005) Experimental approaches to promote functional recovery after severe peripheral nerve injuries. Eur Surgery 37: 193–203
34. Gordon T, Sulaiman OAR, Boyd JG (2003) Experimental strategies to promote functional recovery after peripheral nerve injuries. J Peripher Nerv Syst 8: 236–250
35. Gutmann E, Guttmann L, Medawar PB, Young JZ (1942) The rate of regeneration of nerve. J Exp Biol 19: 14–44
36. Hall SM (1999) The biology of chronically denervated Schwann cells. Ann NY Acad Sci 883: 215–233
37. Hoke A, Gordon T, Zochodne DW, Sulaiman OAR (2002) A decline in glial cell-line-derived neurotrophic factor expression is associated with impaired regeneration after long-term Schwann cell denervation. Exp Neurol 173: 77–85
38. Kuffler DP (1986) Accurate reinnervation of motor end plates after disruption of sheath cells and muscle fibers. J Comp Neurol 250: 228–235
39. Li H, Terenghi G, Hall SM (1997) Effects of delayed re-innervation on the expression of c-erbB receptors by chronically denervated rat Schwann cells in vivo. Glia 20: 333–347
40. Lundborg G (1988) Nerve injury and repair. Churchill Livingstone, Edinburgh
41. Lundborg G (2000) Brain plasticity and hand surgery: an overview. J Hand Surg [Br] 25: 242–252
42. Lundborg G (2003) Richard P. Bunge memorial lecture. Nerve injury and repair – a challenge to the plastic brain. J Peripher Nerv Syst 8: 209–226
43. Mackinnon SE, Dellon AL, O'Brien JP (1991) Changes in nerve fiber numbers distal to a nerve repair in the rat sciatic nerve model. Muscle Nerve 14: 1116–1122
44. McComas AJ, Fawcett PR, Campbell MJ, Sica RE (1971) Electrophysiological estimation of the number of motor units within a human muscle. J Neurol Neurosurg Psychiatry 34: 121–131
45. McQuarrie IG (1986) Structural protein-transport in elongating motor axons after sciatic-nerve crush – Effect of a conditioning lesion. Neurochem Pathol 5: 153–164
46. Mejat A, Ramond F, Bassel-Duby R, Khochbin S, Olson EN, Schaeffer L (2005) Histone deacetylase 9 couples neuronal activity to muscle chromatin acetylation and gene expression. Nat Neurosci 8: 313–321
47. Midha R, Munro CA, Chan S, Nitising A, Xu QG, Gordon T (2005) Regeneration into protected and chronically denervated peripheral nerve stumps. Neurosurg 57: 1289–1299
48. Mokrusch T, Engelhardt A, Eichhorn KF, Prischenk G, Prischenk H, Sack G, Neundorfer B (1990) Effects of long-impulse electrical stimulation on atrophy and fibre type composition of chronically denervated fast rabbit muscle. J Neurol 237: 29–34
49. Nave KA, Schwab MH (2005) Glial cells under remote control. Nat Neurosci 8: 1420–1422
50. Nemoto K, Williams HB, Nemoto K, Lough J, Chiu RC (1988) The effects of electrical stimulation on denervated muscle using implantable electrodes. J Reconstr Microsurg 4: 251–255, 257
51. Nix WA, Dahm M (1987) The effect of isometric short-term electrical stimulation on denervated muscle. Muscle Nerve 10: 136–143
52. Nix WA, Hopf HC (1983) Electrical stimulation of regenerating nerve and its effect on motor recovery. Brain Res 272: 21–25
53. Padua L, Lo Monaco M, Monaco M, Padua R, Gregori B, Tonali P (1997) Neurophysiological classification of carpal tunnel syndrome: assessment of 600 symptomatic hands. Ital J Neurol Sci 18: 145–150
54. Ramon y Cajal S (1991) Cajal's degeneration and regeneration of the nervous system
55. Sanes JR, Lichtman JW (2001) Induction, assembly, maturation and maintenance of a postsynaptic apparatus. Nat Rev Neurosci 2: 791–805
56. Schmidt CE, Shastri VR, Vacanti JP, Langer R (1997) Stimulation of neurite outgrowth using an electrically conducting polymer. Proc Natl Acad Sci USA 94: 8948–8953
57. Sulaiman OAR, Gordon T (2000) Effects of short- and long-term Schwann cell denervation on peripheral nerve regeneration, myelination, and size. Glia 32: 234–246
58. Sulaiman OAR, Gordon T (2002) Transforming growth factor-beta and forskolin attenuate the adverse effects of long-term Schwann cell denervation on peripheral nerve regeneration in vivo. Glia 37: 206–218
59. Sulaiman OAR, Gordon T (2003) TGF-beta reverses the deleterious effect of long-term Schwann cell denervation on nerve regeneration by inducing erbB3 receptor expression. Glia 24 Suppl 2
60. Sulaiman OAR, Boyd JG, Gordon T (2005) Axonal regeneration in the peripheral system of mammals. In: Kettenman H, Ransom BR (eds) Neuroglia 2nd edn. Oxford University Press, Chapter 36, pp 454–466
61. Sulaiman OA, Voda J, Gold BG, Gordon T (2002) FK506 increases peripheral nerve regeneration after chronic axotomy but not after chronic schwann cell denervation. Exp Neurol 175: 127–137
62. Sunderland S (1947) Rate of regeneration in human peripheral nerves: analysis of interval between injury and onset of recovery. Arch Neurol Psychiat 58(3): 251–295
63. Sunderland S (1978) Nerve and nerve injuries. Livingstone, Edinburgh
64. Sunderland S (1991) Nerve injuries and their repair. Churchill Livingstone, Edinburgh
65. Tetzlaff W, Bisby MA, Kreutzberg GW (1988) Changes in cytoskeletal proteins in the rat facial nucleus following axotomy. J Neurosci 8: 3181–3189
66. Tetzlaff W, Leonard C, Krekoski CA, Parhad IM, Bisby MA (1996) Reductions in motoneuronal neurofilament synthesis by successive axotomies: a possible explanation for the conditioning lesion effect on axon regeneration. Exp Neurol 139: 95–106
67. Thomas CK, Stein RB, Gordon T, Lee RG, Elleker MG (1987) Patterns of reinnervation and motor unit recruitment in human hand muscles after complete ulnar and median nerve section and resuture. J Neurol Neurosurg Psychiatry 50: 259–268
68. Verdu E, Navarro X (1997) Comparison of immunohistochemical and functional reinnervation of skin and muscle after peripheral nerve injury. Exp Neurol 146: 187–198
69. Williams HB (1996) The value of continuous electrical muscle stimulation using a completely implantable system in the preservation of muscle function following motor nerve injury and repair: an experimental study. Microsurgery 17: 589–596
70. Witzel C, Rohde C, Brushart TM (2005) Pathway sampling by regenerating peripheral axons. J Comp Neurol 485: 183–190
71. Wood PM, Cuervo EF, Bunge RP, Gordon T (1998) Functional capacities of long-term denervated Schwann cells. Soc Neurosci 24: 690.8
72. You S, Petrov T, Chung PH, Gordon T (1997) The expression of the low affinity nerve growth factor receptor in long-term denervated Schwann cells. Glia 20: 87–100

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Surgical technique for the treatment of C5 and C6 root avulsion

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Summary

C5 and C6 root avulsion is generally treated by neurotization of musculocutaneous (M.C.) nerve by spinal accessory (S.A.) or intercostal nerve, and neurotization of supra-scapular nerve by spinal accessory. For the last few years, permanent paralysis of C5 and C6 root has been treated by neurotization of musculocutaneous nerve by one or two fascicles of the ulnar or median nerve, and axillary nerve by a few fascicles of the radial nerve.

Eighteen patients with M.C. nerve paralysis were treated by end-to-end suture of one or two fascicles of the ulnar nerve. Patients were followed for 4 years.

Neurotization of M.C. nerve by a few fascicles of ulnar or median nerve, and axillary nerve by two fascicles of radial nerve were performed by end-to-end suture. The operative technique is easy and results are good. However, with previous procedures, neurotization of the mentioned nerves usually requires a 6–8 cm nerve graft. With this length of graft, the recovery period is longer than with end-to-end suture. Furthermore, if more than 9 months have passed since the onset of paralysis, especially for axillary nerve, usually good functional results are not obtained. Also, both axillary and radial nerves are the branches of posterior cord, and hence CNS adaptation is more easily attained. Therefore, we recommend the use of this new technique for the treatment of C5 and C6 root avulsion, since the operative time is shorter and procedure is easier for the surgeon. Also recovery period is shorter.

Keywords: Brachial plexus plasy; C5 and C6 root avulsion; M.C. nerve neurotization.

Introduction

The usual treatment of C5 and C6 root avulsion has been neurotization of musculocutaneous (M.C.) nerve by spinal accessory (S.A.) or intercostal nerve, and neurotization of supra-scapular nerve by spinal accessory. For the last few years, permanent paralysis of C5 and C6 root has been treated by neurotization of musculocutaneous nerve by one or two fascicles of the ulnar or median nerve, and axillary nerve by a few fascicles of the radial nerve.

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Material and method

Eighteen patients with M.C. nerve paralysis were treated by end-to-end suture of one or two fascicles of the ulnar nerve. We chose the fascicles of the ulnar nerve specialized for flexi carpi ulnaris muscle by electrostimulation.

Five M.C. nerve paralyses were treated by neurotization by two fascicles of the median nerve, and four axillary nerve paralyses were treated by two fascicles of the radial nerve. All these procedures were performed by end-to-end suture.

All the patients were male, with an average age of 25, ranging from 18 to 35 years.

Results

Patients were followed for 4 years. Elbow flexion usually clearly recovered after 9 months, and reached M4. Even in cases treated 11 months after the onset of paralysis, recovery took place, and we had no failed cases.

Shoulder joint stability was recovered in all patients with axillary nerve paralysis, and in one case, a professional athlete and body builder, the deltoid muscle became very strong and reached nearly normal levels. For the remaining three axillary paralysis cases, abduction movement reached 60 degrees.

Conclusion

Neurotization of M.C. nerve by a few fascicles of ulnar or median nerve, and axillary nerve by two fascicles of radial nerve were performed by end-to-end suture. The operative technique is easy, and the obtained results are good. However, with the previous procedures, neurotization of the mentioned nerves usually requires a 6–8 cm nerve graft. With this length of graft, the recovery pe-

riod is longer than with end-to-end suture. Moreover, if more than 9 months have passed since the onset of paralysis, especially for axillary nerve, usually good functional results are not obtained. Also, both axillary and radial nerves are the branches of posterior cord, and hence CNS adaptation is more easily attained. Therefore, we recommend the use of this new technique for the treatment of C5 and C6 root avulsion, since the operative time is shorter and procedure is easier for the surgeon. Also the recovery period is shorter.

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Lumbosacral plexus lesions

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Summary

Background. Aim of the present study was to analyse the main causes of lumbosacral plexus lesions together with the best diagnostic and therapeutic options for better patient outcome.

Methods. We report our surgical experience with eight patients in whom lesion mechanisms consisted of high-energy trauma (4 pts), firearm injuries (2 pts), spontaneous retroperitoneal haematoma in anticoagulant therapy (1 pt) and schwannoma (1 pt).

The diagnosis was not straightforward and included clinical aspects, electrophysiological studies, magnetic resonance and CT myelography.

Surgery was performed by lateral extraperitoneal approach for the lumbar plexus, transperitoneal approach on the midline to reach the sacral plexus, and neuronavigation was used in the schwannoma case.

Conclusions. Lumbosacral plexus lesions require a challenging multidisciplinary approach to diagnose and treat; the outcome, even if delayed, was very encouraging. In all our patients pain was controlled, and six patients returned to unaided walking.

Keywords: Lumbosacral plexus injury; retroperitoneal haematoma; lumbosacral plexus schwannoma; nerve transfer.

Introduction

Among all nervous lesions, most devastating are those affecting the lumbosacral plexus both for the patients, who face severe pain and impaired deambulation, and for the physicians, who are involved in a challenging multidisciplinary approach for diagnosis and treatment.

Lesions of the lumbosacral plexus, being anatomically protected in the retroperitoneal area and lacking rigid anatomic narrowings, are far rarer than those in the brachial district.

Giulio Casserio was the first to picture the lumbosacral plexus in his work in 1632, describing the anatomic structure of the plexus given by the confluence and mingling of the nervous fibres springing from the roots of D12-S3 [4], while the first description of lumbosacral plexus lesions only appeared in 1960 [8].

Radicular composition of the lumbosacral plexus as well as of the brachial plexus may vary in its pre or post fixation; both areas consist of anterior flexor and posterior extensor layers of fibres.

The former is composed of the anterior fibres of L4-S2 and originates in the internal popliteal sciatic, while the latter, formed by the posterior fibre of the same roots, leads to the external popliteal sciatic.

The two plans may at times be distinct in the pelvis, and the two nerves that constitute the sciatic are separate at the origin [16].

In the aetiology of plexus lesions, high-energy traumas are frequent and may lead to pelvis or sacrum fracture with consequent roots or plexus avulsion [10].

Neurodiastasis may often be associated with nerves tight and adherent to the bone plan, while simultaneous vascular lesions can result in retroperitoneal or psoas haematoma.

In firearm lesions, having perforated the abdominal wall and internal organs, bullets may hit the retroperitoneal nerve structure.

Neurogenic lesions, single or multiple as in Von Recklinghausen's disease and located in the lumbosacral plexus, may become quite extensive with moderate clinical symptomatology.

Retroperitoneal haematoma during anticoagulant therapy is an additional cause of plexus lesions [5, 7].

To diagnose such lesions an analysis of pain, sensitive disorders and motor deficits (grading 0–5), as well as an electrophysiological examination is fundamental, giving answers to questions with regard to partial or total denervations of gluteal muscles when paravertebral musculature and sphincter function are spared.

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In traumatic lesions, X-rays may reveal pelvic, spine or coxofemoral fractures, and CT myelography may help diagnose possible pseudomengoceles or interruption of roots in the cauda equina, while MR is useful in detecting retroperitoneal haematoma and fibrosis or other expansive lesions [18].

Surgery of the lumbosacral plexus is managed with four different approaches: the lateral retroperitoneal approach for the lumbar plexus; the transperitoneal approach on the midline to reach the sacral plexus; the posterior approach through laminectomy for interruptions of roots in the cauda equina; the trans-sacral approach [11].

Surgical outcome requires a protracted period of time in view of the huge distance to be covered by nerve regeneration.

Our work is meant to underline how challenging the diagnosis and treatment of lumbosacral plexus lesions are. Moreover, besides emphasising the need for a multidisciplinary approach, we would like to encourage such surgery in which positive results may be achieved.

Methods and material

Patient population

We report on our surgical experience in eight male patients, aged between 19 and 39 years. Four cases (4–7 of table) had experienced high-energy traumas in car accidents, one of these patients suffered from bilateral plexus lesion, two cases (1–2) of firearm injuries, one case of spontaneous retroperitoneal haematoma in anticoagulant therapy (case 3) and one case of schwannoma of the obturator (case 8).

Diagnosis

Clinical assessment of all patients comprised analysis of pain, sensitive disorders and motor deficit (grading 0–5). Diagnostic tools included electrophysiology, X-ray, CT myelography and MR imaging. Neurophysiology distinguished selective damage in the lumbar sacral plexus rather than at the level of the lumbosacral roots. In fact, when the plexus is compromised at either level, post-ganglionic impairment occurs and nerve conduction studies (NCS) show reduced or absent sensory action potentials (SAP); in sacral root injury, on the contrary, we are dealing with preganglionic lesion and only compound motor action potentials (cMAP) are reduced or impaired. In traumatic lesions, X-ray revealed spine, pelvic or hip fractures; CT myelography and MR imaging excluded traumatic meningoceles or interruption of roots in the cauda equina [18]. MR imaging detected retroperitoneal haematoma, fibrosis and schwannoma lesion.

Surgical techniques

The lumbar plexus was accessed through lateral retroperitoneal approach, the sacral plexus through transperitoneal approach on the midline and laminectomy was performed to reach the sacral roots.

Neurophysiologic intraoperative monitoring was performed using the PHASIS ESAOTE apparatus. Muscular activity was recorded by means of monopolar electrodes upon rectus femoris, gastrocnemius medialis and tibialis anterior (filter 20–2 k, gain 200–500 μV); bipolar stimulation was accomplished with a train of 5 square-wave pulses of 0.2 msec duration at 5 Hz; stimulus intensities did not exceed 5 mA.

In four patients neurolysis only was performed (Cases 3, 4, 6 and 7) [12], while the patient with neurogenic tumor (Case 8) required employment of a neuronavigator to limit and target the psoas section; the lesion, localized in the medial portion of the obturator, was easily excised under preservation of nerve and lumbosacral trunk.

The surgical management of injured patients (Cases 1 and 5) comprised reconstruction of the sectioned nerve trunk by sural grafting. In one patient (Case 2) ventral roots were connected by nerve graft to the sciatic nerve through dorsal approach and laminectomy.

Results

As confirmed by the literature [2, 3, 9, 10], the first result obtained in our set of patients was the drastic reduction of pain with subsequent interruption of pharmacological treatment which offered both practical and psychological advantages as patients were promptly and enthusiastically ready to start a rather lengthy rehabilitation period.

Follow-up evaluation was set at 6, 12 and 24 months following surgery.

Recovery of proximal motility was achieved between 6–12 months, whereas distal motility was regained rather

Table 1. *Characteristics, surgical treatment and outcome of 8 patients with lumbosacral plexus lesions*

No.	Age	Sex	Etiology	Deficit	Procedure	Outcome (12–24 months)
1	26	M	fire arms injuries	femoral, obturator	extraperitoneal, Graft L3, L4	walk unaided
2	32	M	fire arms injuries	sciatic	transperitoneal, graft L5-S1 roots/sciatic	improved foot function (3/5)
3	39	M	spontaneous retroperitoneal haematoma	femoral, obturator peroneal	transperitoneal neurolysis	walk; return quadriceps, 3/5 foot dorsi
4	25	M	pelvic, femoral and hip fractures, bilateral	sciatic	transperitoneal neurolysis	stand and walk with support
5	19	M	femoral and hip fractures	femoral, peroneal	transperitoneal end to end graft	walk unaided
6	36	M	injuries retroperitoneal haematoma	femoral obturator, peroneal	extraperitoneal neurolysis	walk unaided
7	23	M	retroperitoneal fibrosis	femoral, obturator	extraperitoneal neurolysis	pain relief
8	39	M	schwannoma	obturator	transperitoneal, complete exercise	walk unaided, pain relief

late, i.e. at 12–24 months follow-up. Sciatic recovery was never completely achieved.

Nevertheless, in six patients deambulation is unaided, whereas Case 4 requires a walking aid for load-bearing after replacement surgery of the right hip as a consequence of the car accident. Follow-up is limited to 6 months only for Case 7.

Case presentation

Case 3

In spring 2004, a 39-year-old man was taken to the Cardiology Unit of our institution following a heart attack. He immediately underwent angioplasty and anticoagulant therapy with good results.

A few days later, he presented a serious paresis of the lower limb with pain in L2–L4 and L5 roots together with dysesthesia and allodynia. Abdomen CT and MR (2004/05/19) (Fig. 1) showed a huge spontaneous haematoma at the right ileopsoas muscle in its medial portion extending to the pelvis with subsequent compression of the homolateral lumbosacral plexus.

An electromyographic study (2004/05/29) revealed the complete denervation of the muscles innervated by the right obturator and femoral nerves indicating neurotmesis at the lumbosacral plexus; furthermore, the exam showed a partial denervation of the muscles in the antero-external region of the leg, indicating a medium-level axonotmesis of the right sacral plexus.

Having spent a couple of months in the Rehabilitation Department without any improvement of neurologic deficiency, the patient was transferred to our Department for neurosurgical treatment of plexus decompression (2004, July).

Preoperative clinical evaluation of the right leg showed complete deficiency of the obturator and external sciatic popliteal nerve; severe deficiency of the psoas and femoral quadriceps muscles; a slight weakness of the posterior tibial muscle; anaesthesia at the internal side of the thigh; hypoesthesia in L2–L4 and L5 roots and the absence of the patellar reflex.

Prior to surgery, the patient had repeated abdomen MR that revealed dimensional reduction of the haematoma, whereas a second electromyographic study showed no relevant change.

The patient was surgically treated with a median laparotomy and subsequent complete removal of the haematoma in the right pelvis and neurolysis at the homolateral lumbosacral plexus.

The patient benefited from immediate pain relief, and clinical follow-up at 6, 12 and 18 months after surgery and physical therapy showed excellent strength improvement at the psoas, femoral quadriceps and adductor muscles; on the other hand, the peroneal nerve function did not improve at all.

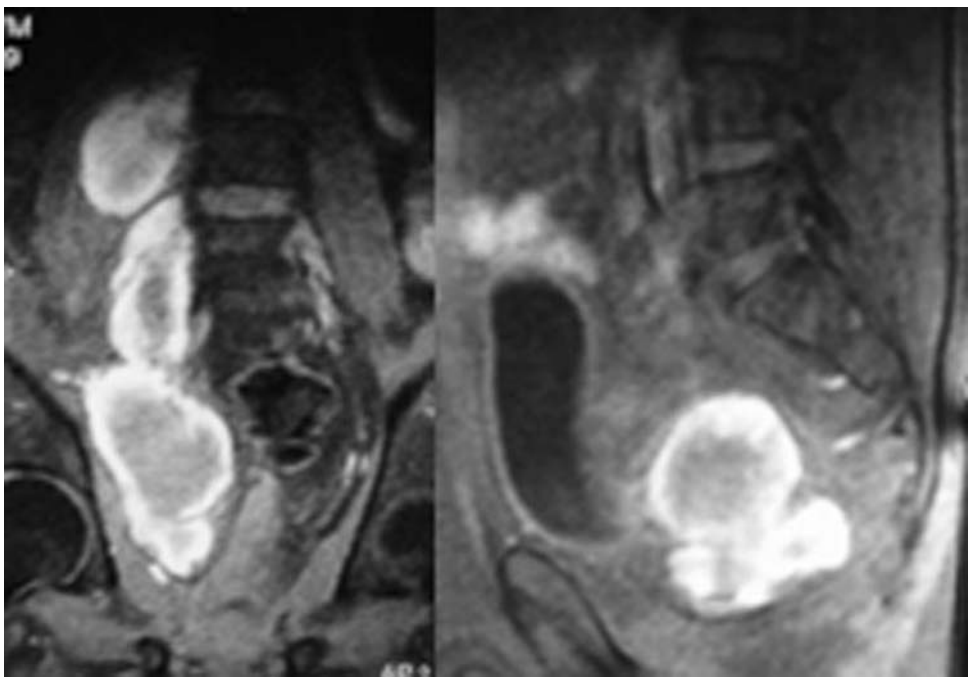

Fig. 1. Lumbosacral plexus MR, demonstrating a huge spontaneous retroperitoneal haematoma (*coronal view left*), (*sagittal view right*), long T R

Case 8

A 49-year-old male patient was brought to our attention after a 24-month period of recurring right lumbar pain. Lumbar X-ray was positive for grade I listhesis and confirmed by MR, which also revealed a suspected expansive lesion right paravertebrally. In November 2005 the patient was admitted to our hospital, and clinical examinations of the lower right limb showed positive Lasègue sign at 45°, algo-dysesthesia and areas of allodynia in L4, L5 and S1; moderate hypostenia in the dorsal flexion of foot; symmetric and effective reflexes of lower limbs. Further investigations were required, so that CT and MR (Fig. 2) examinations were planned which revealed solid bulky oviform abdominal mass of a max. longitudinal diameter of 5.2 cm, located right paravertebrally between the vertebral plan and psoas medial profile.

Electrophysiological study of the lower limbs showed a significant asymmetry in the amplitude of the super-

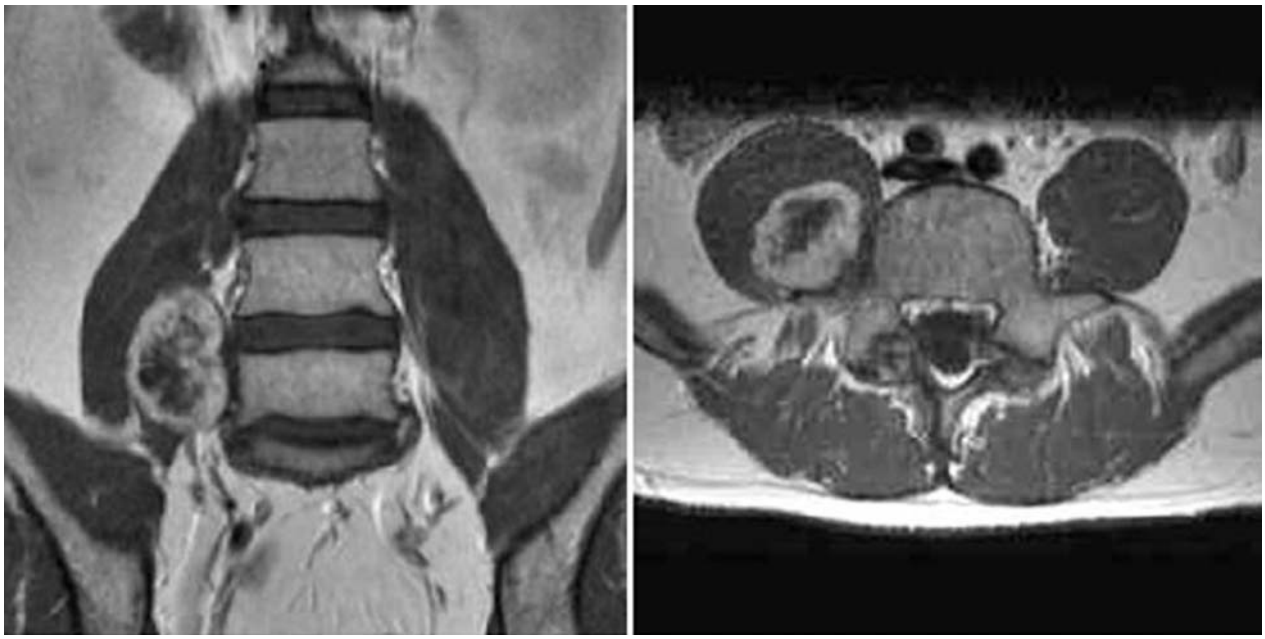

Fig. 2. Lumbosacral plexus MR demonstrating an obturator schwannoma after gadolinium (*coronal view left, axial view right*)

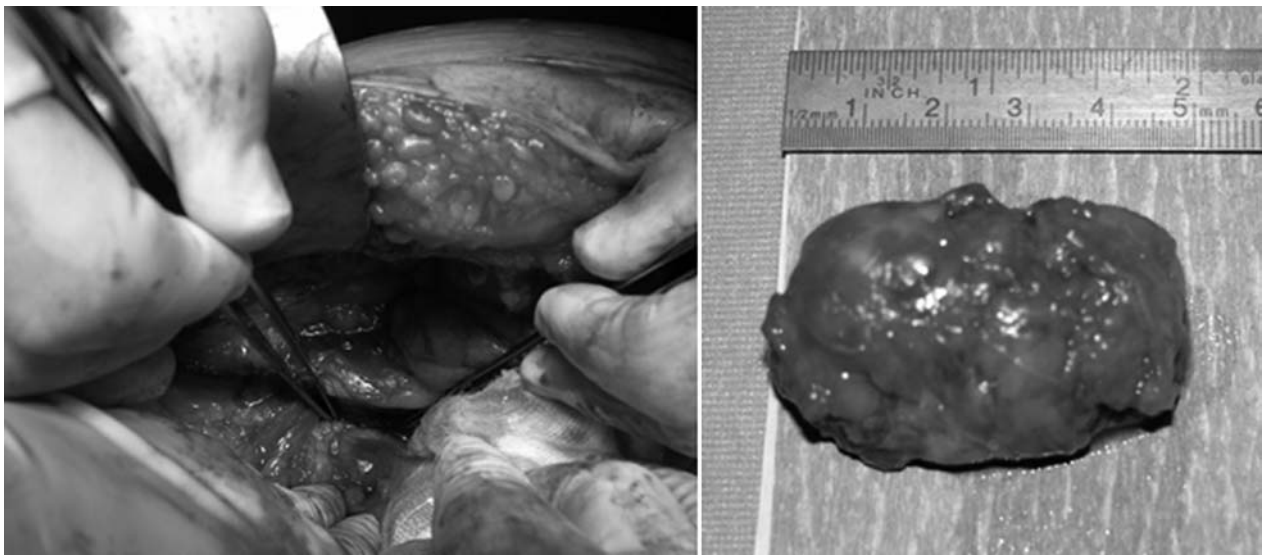

Fig. 3. Intraoperative photographs: (*left*) initial dissection and removal of the schwannoma; (*right*) complete exercise

ficial peroneal nerve (being wider on the left) and no other relevant neuro-muscular deficit. The patient was surgically managed by median laparotomy and radical excision of the mass (Fig. 3) that was confirmed by histological examination as benign schwannoma.

After an uneventful postoperative period, the patient experienced quick and prompt remission of pain and retrieved right foot dorsal flexion; however, hypoesthesia of the median internal thigh, corresponding to the obturator nerve distribution, was still present.

Evaluation of the clinical follow-up at 6 and 12 months after surgery showed complete remission of painful lower limb dysesthesia, while obturator hypoesthesia persisted.

Discussion

Literature on lumbosacral plexus lesions and their treatment [2, 3, 9, 10, 12, 13, 18] is rather scarce due to the low incidence of this peculiar trauma on the one hand and the difficulty in reaching a precise diagnosis and the complex surgical approach on the other hand. This has encouraged our team to approach post-mortem studies which proved useful for better understanding of the plexus anatomy and the adjacent structures. Also it has given us confidence and practice in surgical management.

The best technique of post-mortem dissection of the plexus implies sectioning the psoas muscle adjacent to the spine, thus detecting roots at their beginning [16].

Interesting anatomic variations emerged during our study: around 18–23% in the plexus structure of the same subject, between 8–15% for the different origin of the obturator and the presence of accessory obturator (25%), which is far higher than usually reported in the literature (8%) [1, 6, 15, 16].

For the surgical management of our cases we chose the traditional lateral extraperitoneal approach to tackle lumbar lesions, on the midline through the peritoneum for lumbosacral plexus lesions. Other authors report different surgical procedures that we are not familiar with, particularly Sedel's transileal approach or dorsal transsacral approach as described by Linarte [11], where however early mobilization is hindered and consequently patient's neuro-rehabilitation is delayed.

For the management of the schwannoma case we used a neuro-navigator, thus limiting the psoas section to expose the lesion. Although not strictly necessary in our case, this technique could prove extremely useful for minimally invasive approaches such as Rousseau's [14] excision of benign schwannoma in a young woman.

In our experience patient's outcome was successful as far as pain management and proximal musculature recovery were concerned. Unfortunately, peroneal injuries could only partially be solved.

Conclusions

Lumbosacral plexus lesions are rather rare events. Diagnosis and treatment of such lesions are demanding and imply industrious multidisciplinary cooperation. We tend to prefer surgical approaches that favour early mobilization, and, as in brachial plexus reconstruction techniques, palliative graft may be used for the treatment of roots avulsion.

In neurogenic lesions the use of a neuro-navigator may help minimize the surgical impact. In our experience, patients'outcomes, even if delayed, were very encouraging.

References

1. Artico M, Stevanato G, De Santis S, Di Paola S, Cavallotti C (1998) Morphological and functional correspondences of the lumbar and sacral plexus: techniques of anesthesia and analgesia. J Military Med Italy 5: 357–363
2. Brunelli G, Vigasio A (1986) Lumbar and sacral plexus surgery. Periph Nerve Repair Regen 4: 21–25
3. Carlstedt T, Grane P, Hallin R *et al* (1995) Return of function after spinal cord implantation of avulsed spinal nerves roots. Lancet 346: 1323–1325
4. Casserio G (1632) Tabulae Anatomicae, Bucretius
5. Chiu WS (1976) The syndrome of retroperitoneal hemorrhage and lumbar plexus neuropathy during anticoagulant therapy. South Med J 69: 595–599
6. Chotigavanich C, Sawangnatra S (1992) Anomalies of the lumbosacral nerves roots. An anatomic investigation. Clin Orthop Res 278: 46–50
7. Emery S, Ochoa J (1978) Lumbar plexus neuropathy resulting from retroperitoneal hemorrhage. Muscle Nerve 1(4): 330–334
8. Finney LA, Wulfman WA (1960) Traumatic intradural lumbar nerve root avulsion with associated traction injury to the common peroneal nerve. Am J Roentgenol Radium Ther Nucl Med 84: 952–957
9. Kline DG, Hudson AR (1995) Nerve injuries operative results for major nerve injuries, entrapments and tumors. WB Saunders: Philadelphia, pp 328–344
10. Lang EV, Borges J, Carlstedt T (2004) Surgical treatment of lumbosacral plexus injuries. J Neurosurg Spine 1: 64–71
11. Linarte R, Gilbert A (1986) Trans-sacral approach to the sacral plexus. Periph Nerve Repair Regen 4: 17–20
12. Millesi H (1981) Internal Neurolysis. In: Gorio A, Millesi H (eds) Posttraumatic peripheral nerve regeneration. Raven Press, New York, pp 197–208
13. Narakas A (1986) Discussion and conclusion of the round table sciatic nerve lesions of the G.A.M. Periph Nerve Repair Regen 4: 75–76
14. Rousseau MA, Pascal-Mousselard H, Lazennec JY, Sailant G (2005) The mini-invasive anterior extra peritoneal approach to the pelvis. Eur J Surg Oncol 8: 924–926

15. Stevanato G, Artico M (1994) Proposal for a method of conservation of preparation of the lumbosacral plexus through the use of synthetic resins. Copybook of practice anatomy. Padua 4: 43–61
16. Stevanato G, Artico M, Pastore FS, Di Paola S, Cavallotti C (1998) Surgical anatomy of lumbosacral plexus. J Military Med Italy 4: 236–239
17. Verstraete KL, Martens F, Smeets P *et al* (1989) Traumatic lumbosacral nerve root meningoceles. The value of myelography, CT and MRI in the assessment of nerve root continuity. Neuroradiology 31: 425–429
18. Zhao S, Beuerman RW, Kline DG (1997) Neurotization of motor nerves innervating the lower extremity by utilizing the lower intercostal nerves. J Reconstr Microsurg 113: 39–45

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Microsurgical management of penetrating peripheral nerve injuries: pre, intra- and postoperative analysis and results

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Summary

Background and methods. Clinical and electrophysiological motor function data were compared before and after microsurgical repair of penetrating peripheral nerve injuries. Sixty-four patients totaling 74 injured nerves (25 gunshot wounds, 49 stab wounds) were treated with external and interfascicular neurolysis and/or interfascicular nerve grafts. Microsurgery was performed 2–12 months after the injury (Group 1, 33 patients,) and 12 months–60 years after the injury (Group 2, 31 patients). The postoperative clinical and electrophysiological follow-up period ranged between 1 and 5 years.

Results. A statistically significant improvement in muscle strength occurred after the microsurgery, compared to before repair, gunshot wounds ($p < 0.001$), stab wounds ($p < 0.001$). Intraoperative and postoperative electrophysiological analysis showed statistically significant improvement. *Timing of surgery:* No statistically significant difference in muscle strength occurred between the 2 groups after the surgery, each showing statistically significant improvement, Group 1 ($p < 0.001$), Group 2 ($p < 0.001$). Patients above and below age of 40 showed an improvement in muscle strength after microsurgery, ($p < 0.001$) and ($p < 0.001$), respectively.

Conclusion. Microsurgery can progressively improve nerve function in penetrating peripheral nerve injuries and lead to significant functional improvement, even when it is delayed for more than one year after the injury.

Keywords: Penetrating peripheral nerve injury; microsurgery; timing.

Introduction

Penetrating injuries to peripheral nerves cause motor and sensory loss, as well as severe pain and disability. These injuries are often inflicted by gunshot wounds (GSW) and stab wounds (SW), many of which do not improve spontaneously over time and, therefore, become candidates for surgical repair [2–5, 9–12]. Rochkind and Alon [1, 8] suggested that even old injured peripheral nerves survive for an extended period of time, and microsurgical treatment of them results in a functional improvement of patients. The present study analyzes the pre-, intra- and postoperative clinical and electrophysiological changes in penetrating injuries of the peripheral nerve in order to show that a functional improvement is possible after surgery, even in cases where the surgery is performed more than one year after the injury.

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Material and methods

This prospective study evaluated the functional recovery of 64 patients suffering from penetrating peripheral nerve injury (from 2 months to 60 years after the injury), who underwent microsurgical repair of the injured nerve at Tel Aviv Sourasky Medical Center between October 1993 and April 1999. The patients' ages ranged between 4.5 and 86 years old, with an average of 34.6 years of age. Ten percent of the injured patients were female. The 64 patients had 74 injured nerves. In 25 (33.7%) of the injured nerves the injury was made by a gunshot wound, the remaining 49 (66.3%) were stab injuries. Fifteen (31%) of 49 stab-injured nerves were iatrogenic injuries.

The distribution of patterns of nerve injury in the 64 patients is presented in Table 1.

Preoperative and postoperative clinical evaluations

The patients were clinically evaluated using the Medical Research Council's Grading System [6]. The postoperative clinical follow-up period ranged between 1 and 5 years. The average follow up period after the operation was 19.1 months.

Preoperative and postoperative electrophysiological evaluation

Standard electromyographic test (needle EMG and nerve conduction study – NCS) was used preoperatively, and for postoperative follow-ups that were done 1–5 years after the surgery. Statistical analysis of latency,

Table 1. *Patterns of peripheral nerve injury in the 64 patients*

Nerve	No. of cases	%
Ulnar nerve	13	17.6
Median nerve	11	15
Radial nerve	4	5.4
Sciatic nerve	12	16
Tibial nerve	11	15
Peroneal nerve	19	26
Accessory nerve	4	5

compound muscle action potential (CMAP) amplitude, unit action potentials (MUAPs) recruitment or voluntary muscle activity were used to compare results before the surgery and postoperatively.

Timing of surgery

The 64 patients underwent a microsurgical repair ranging between 2 months and 60 years after the injury, with an average of 48 months and a median of 8 months. The patients were divided into two groups:

Group 1 (33 patients) was operated on between 2 and 12 months after the injury.
Group 2 (31 patients) was operated on between 12 months and 60 years after the injury.

Surgery

The procedures were performed under general anesthesia. During external and interfascicular neurolysis, compound muscle action potentials (CMAP) were recorded from corresponding muscles during stimulation of the peripheral nerve.

Surgical methods

1-Neurolysis – Using high microscopic magnification, combined external and interfascicular procedures were performed under intraoperative electrophysiological control. The degree of nerve injury is often best determined during interfascicular neurolysis [7]. When fibrosis is extreme and/or a hard neuroma in continuity was present, with minimal or no intraoperative electrophysiological responses, removal of the neuroma and interfascicular nerve grafts were done using a sural nerve. In six patients, the bioabsorbable polyglycolic acid neurotubes were used for grafts. Table 2 shows the frequency of surgical techniques.

Postoperative clinical evaluation

The comparative analysis was performed on the mean grade of all muscles tested corresponding to the injured nerve before and after surgery.

Table 2. *Frequency of surgical techniques*

Surgical technique	No. of nerves (74)	%
External neurolysis alone	12	16.2
External and interfascicular neurolysis	24	32.4
End to end suture	6	8.1
Nerve grafts	26	35.1
Neurotube grafting	6	8.1

Statistical analysis

Clinical and electrophysiological data of each patient at baseline was compared to the patient's data at follow-up examinations after the surgery. Statistical analyses were performed using univariate analysis, parametric or nonparametric, as needed.

Results

Functional motor improvement

The comparative analysis of muscle strength of the injured nerve population as a whole, prior to surgery and between 1 and 5 years after surgery, showed a statistically significant improvement in muscle strength after a microsurgical repair from mean grade 1.66 to 3.52 ($p < 0.001$).

Muscle function and etiology factor

Statistical analysis of muscle strength was compared separately in GSW and SW patients before and after the surgery and showed a statistically significant improvement in muscle strength after the microsurgical repair from mean grade 1.32 to 3.15 ($p < 0.001$) and 1.84 to 3.71 ($p < 0.001$), respectively (Fig. 1).

Muscle function and timing of surgery

The patients in *Group 1* (33 patients) who were operated on between 2 and 12 months after injury (average 4.75 months after injury) and *Group 2* (31 patients) who were operated on between 12 months and 60 years after injury (average of 10.1 years after the injury) showed a statistically significant improvement in muscle strength after a microsurgical repair, from mean grade 1.62 to 3.51 ($p < 0.001$) and 1.73 to 3.52 ($p < 0.001$), respectively. The comparative analysis of muscle functional improvement in both groups showed no statistically significant differences in the results (Fig. 2).

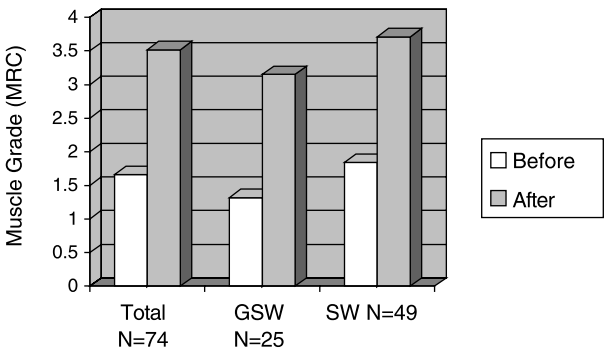

Fig. 1. Muscle strength before and after surgery, according to etiology factor ($p < 0.001$)

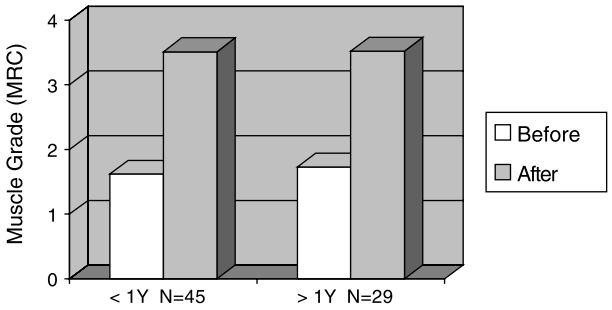

Fig. 2. Muscle strength before and after surgery according to timing of surgery ($p < 0.001$). Analysis of 74 injured nerves of 64 patients

Muscle function and age factor

Both the patients, who were under the age of 40 and those who were above the age of 40 when operated on, showed a statistically significant improvement in muscle strength after a microsurgical repair from mean grade 2.12 to 3.25 ($p < 0.001$) and 1.43 to 3.66 ($p < 0.001$), respectively. The comparative analysis of muscle functional improvement, done prior to the surgery and after surgery in both groups, showed no statistically significant difference in the results of patients under the age of 40 and those above the age of 40.

Muscle function and surgical techniques

A statistical analysis of muscle strength, which was done before and after surgery for each surgical technique, showed a statistically significant improvement in muscle strength after grafts, from mean grade 1.38 to 3.23 ($p < 0.001$); after neurolysis from 2.07 to 3.74 ($p < 0.002$), and after end-to-end suture from 0.79 to 3.74 ($p < 0.001$), respectively.

Electrophysiological improvement

Intraoperative evaluation of compound muscle action potential (CMAP)

A statistical electrophysiological analysis was done to compare latency and amplitude of CMAP at the beginning and at the end of the operation. The amplitude of intraoperative CMAP was low at the beginning of the operations and significantly increased at the end of the operations ($p = 0.009$). At the same time, latency of CMAP was reduced at the end of the operations, compared to the latency at the beginning of the operations ($p = 0.019$). These amplitude and latency changes were found in 21 patients who underwent only a neurolysis procedure.

Pre- and postoperative evaluations of CMAP

Statistical analysis of latency and amplitude of CMAP was done prior to surgery and during the last observation on 35 patients which was between 1 and 5 years after surgery. These 35 patients showed a statistically significant improvement in amplitude ($p = 0.008$), and a reduction in latency ($p < 0.001$).

Evaluation of recruitment (voluntary muscle activity) of motor unit action potentials (MUAPs)

Statistical analysis of the recruitment of MUAPs in 35 patients (11 GSW and 24 SW patients) was compared prior to surgery and during the last observation, which was between 1 and 5 years after the surgery. After surgery all 35 patients showed a significant improvement in the muscle voluntary activity ($p < 0.001$) in GSW ($p = 0.001$) and SW ($p < 0.001$), respectively.

Discussion

In this study, a statistically significant improvement of intraoperative electrophysiological activity after neurolysis was found in both groups of patients. This data supports our previous clinical and electrophysiological studies [1, 8] which suggested that old injured peripheral nerves survive for an extended period of time and decompression of the fascicles by interfascicular neurolysis improves nerve conductivity of the surviving fascicles. The pre- and postoperative comparative study of CMAP showed a statistically significant improvement in amplitude and a reduction in latency during the follow-up period. The same was true for recruitment (voluntary muscle activity) compared to the preoperative evaluation. The postoperative clinical analysis showed a statistically significant improvement in motor functional activity, not only in patients who underwent microsurgical treatment up to one year after injury, but also in patients that were operated on from one year and upwards after the injury. The statistical improvement of muscle strength was found not only in neurolysis cases but also in the patients who underwent nerve graft reconstruction procedures.

Conclusion

This data suggests that viability of nerve tissue or preservation of fascicules is longer than previously considered. Microsurgery can progressively improve nerve

function in penetrating peripheral nerve injuries, and lead to a significant functional improvement, even when it is delayed for more than one year after the injury.

References

1. Alon M, Rochkind S (2002) Pre-, intra-, and postoperative electrophysiological analysis of the recovery of old injuries of the peripheral nerve and brachial plexus after microsurgical management. J Reconstructive Microsurg 18: 77–82
2. Hurst LC, Dowd A, Sampson SP, Badalamente MA (1991) Partial lacerations of median and ulnar nerves. J Hand Surg [Am] 16: 207–210
3. Kim DH, Kline DG (1995) Surgical outcome for intra- and extrapelvic femoral nerve lesions. J Neurosurg 83: 783–790
4. Kim DH, Kline DG (1996) Management and results of peroneal nerve lesions. Neurosurgery 39: 312–319; discussion 319–320
5. Kline DG, Kim D, Midha R, Harsh C, Tiel R (1998) Management and results of sciatic nerve injuries: a 24-year experience. J Neurosurg 89: 13–23
6. Medical Research Council (1954) Peripheral nerve injuries. In: Seddon HJ (ed) Her Majesty's Stationery Office, London, pp 354–361
7. Millesi H, Rath H, Reihsner R, *et al* (1993) Microsurgical neurolysis: its anatomical and physiological basis and its classification. Microsurgery 14: 430–436
8. Rochkind S, Alon M (2000) Microsurgical management of old injuries of the peripheral nerve and brachial plexus. J Reconstructive Microsurg 16: 541–546
9. Samardzic MM, Rasulic LG, Vuckovic CD (1999) Missile injuries of the sciatic nerve. Injury 30: 15–20
10. Taha A, Taha J (1998) Results of suture of the radial, median, and ulnar nerves after missile injury below the axilla. J Trauma 45: 335–339
11. Taha A, Taha J (1998) Results of suture of the sciatic nerve after missile injury. J Trauma 45: 340–344
12. Vrebalov-Cindro V, Reic P, Ognjenovic M, Jankovic S, Andelinovic S, Karelovic D, *et al* (1999) Peripheral nerve war injuries. Mil Med 164: 351–352

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An evaluation using techniques to assess muscle and nerve regeneration of a flexible glass wrap in the repair of peripheral nerves

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Summary

In this study a flexible biodegradable wrap is compared with microsurgical epineurial suturing in the repair of cleanly divided peripheral nerves. Five groups of twelve sheep were used; one control group and four neurotmesis and repair groups. The four repair groups were;

1. Epineurial suture repair using a microscope and 9/0 polyamide; 2. Wrap secured by Tisseel glue; 3. Wrap secured by polycaprolactone glue; 4. Wrap secured by suturing.

Regeneration of the median nerve was assessed by electromyography, nerve conduction studies, wet muscle mass measurements, and morphometry.

The results suggested that nerve regeneration in the wrap + Tisseel glue group was as good as that in the epineurial repair group. The use of polycaprolactone glue is not recommended in nerve repair. Placement of the wrap was easy to learn and quick to carry out under direct vision. The wrap used in this study could prove to be a useful alternative to epineurial suturing to repair peripheral nerves and may have a particularly unique role in the developing world and battlefield.

Keywords: Muscle and nerve regeneration; peripheral nerve; release glass; peripheral nerve.

Introduction

The current standard surgical technique to repair a cleanly divided peripheral nerve is microsurgical epineurial suture. Fascicular repair may be considered in distal lesions of mixed peripheral nerves. These techniques are best carried out using an operating microscope [1] and microsurgical instruments. Fibrosis around the site of repair is thought to be caused by the sutures even if these have been confined to the epineurium [14]. Most trainees in plastic surgery in the United Kingdom receive training in microsurgery, however, microsurgical training and instruments may not be available to surgeons operating in remote areas in developing countries and operating theatres in the battlefield are often very busy making any time saving techniques useful.

The wrap used in the present study is made from *Corglaes* (Giltech Ltd, 12 North Harbour Estate, Ayr, Scotland, UK), a biodegradable, biocompatable glass, which when used in the form of a solid tube, was shown to support the regeneration of divided facial nerves in sheep [5].

Transcutaneous stimulated jitter (TSJ) is an electromyographic technique which measures the variability in the latency between the stimulation of a motor axon and the contraction of the supplied muscle fibre. TSJ has been previously shown to be a useful measurement to determine the efficacy of different types of nerve repair [9–11]. The higher the value of jitter the less mature the motor end plates of the muscle fibre tested.

Maximum nerve conduction velocity ($CV_{\max}$) is one of the most commonly used and best methods to assess the success of nerve regeneration [3, 12].

Wet muscle mass of *flexor carpi radialis* (*FCR*) was used to assess the success of muscle regeneration [2]. The FCR muscle is supplied only by the median nerve.

Morphometric measurements of *axon diameter*, *fibre diameter*, *myelin thickness* and *g-ratio* were used. As fibre diameter increases during regeneration of a neurone, there is initially a proportionately greater thickness of the myelin sheath compared to the axon diameter [16]. As regeneration continues, there is a gradual reduction in the myelin sheath thickness in proportion to the diameter of the axon itself [1]. The *g-ratio,* $\left(axondiamter/fibrediameter\right)$, has been used by many previous authors and has been found to be a good measure of the maturity of reinnervation of nerves [4, 6].

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All tests were carried out on both the operated and normal forelimbs in each sheep (the right and left sides in the control group). The ratios of the measured values in the operated side to the normal side were then used in all the statistical analyses to correct for within-groups' variation.

Methods

The sheep was anaesthetized and monitored throughout the operation. Using sterile technique, the median nerve was exposed in the upper forelimb and divided using a Meyer neurotome.

Epineurial repair

An operating microscope (Wild Heerbrugge M600), microsurgical instruments and 10/0 polyamide (Nylon, Ethilon, Ethicon Ltd, Edinburgh, UK) were used in epineurial repairs. The fascicular pattern of the proximal and distal stumps was matched as accurately as possible.

Wrap secured with glue

When using glue, the divided ends of the nerve were positioned on the top of the wrap with no gap between their ends and a small amount of glue applied to each nerve stump, the wrap folded and then glue applied down the lateral edge of the wrap as shown in Fig. 1.

Wrap secured with sutures

The wrap was applied with the sutures as shown in Fig. 2.

Nerve regeneration was assessed at seven months to allow full recovery [7]. Each sheep was anaesthetized again using and underwent electrophysiological testing.

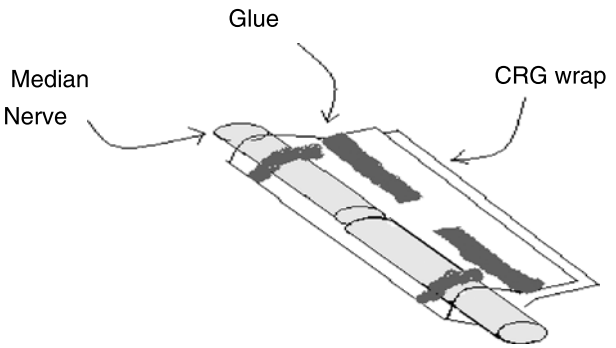

Fig. 1. Diagram of wrap placed around divided median nerve and secured with glue

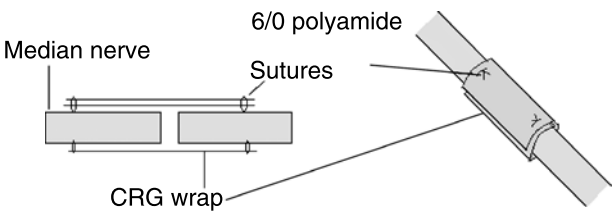

Fig. 2. Diagram of the technique used to secure the wrap with polyglactin sutures

TSJ was measured with a concentric monopolar needle using a technique described by Lenihan [9–11]. The median nerve was exposed as in the original operation and the ulnar nerve was divided. To measure maximum conduction velocity the median nerve was stimulated proximal and distal to the repair site and the compound motor action potentials produced in the FCR muscle recorded by the electromyograph (Medelec, Oxford Instruments, Manor Way, Old Woking, Surrey, GU22 9JU, England).

The mass of the muscle bellies of FCR was measured on a scientific balance (Mettler AE 163). A 1–2 cm length of nerve was excised 2 cm distal to the site of repair, stained using osmium tetroxide, fixed in Araldite and cut into 1 μm ultrathin sections and mounted on slides [8]. Sections of each nerve were chosen by a random sampling method [13] and photographed at 1000 × magnification. At least 200 neurones from each nerve were measured (*Analytical Image Station (AIS)*, Imaging research Inc., Brock University, St. Catherine's, Ontario, Canada, L2S 3A1).

One way ANOVA and *Scheffé's test* were applied respectively to determine whether any significant difference existed between the means and variances of any of the groups and if so, the groups between which the difference existed.

Results

No sheep developed a wound infection during the seven month recovery period. Macroscopically, there was no evidence of the wrap in the wrap + Tisseel or wrap + suture groups. In the wrap + polycaprolactone group however, the wrap was often still present and there was more fibrosis.

Two repairs in the epineurial suture group dehisced completely and one repair in the wrap and polycaprolactone group. The microsurgical epineurial repairs took around 25 min to complete whereas insertion of the wrap using Tisseel or polycaprolactone glue took around 3 min and placement of the wrap with 6/0 polyglactin took around 10 min.

TSJ in the wrap + polycaprolactone and wrap + polyglactin sutures was significantly higher than in the control group. This suggested that the motor end plates were not as mature in these repair groups as in the normal muscle cells but that the motor end plates had reached a normal level of maturity in the epineurial repair group and the wrap + Tisseel groups (see Fig. 3).

There was no significant difference in the results of maximum conduction velocity among the repair groups (see Fig. 3). The regenerated motorneurones in the repair groups conducted significantly more slowly than the fastest motorneurones in a normal nerve but the motorneurones in each repair group conducted at a similar maximum velocity.

There was no significant difference in the wet muscle mass of FCR among the repair groups but it was significantly smaller in all the repair groups compared with the control group ($p < 0.001$).

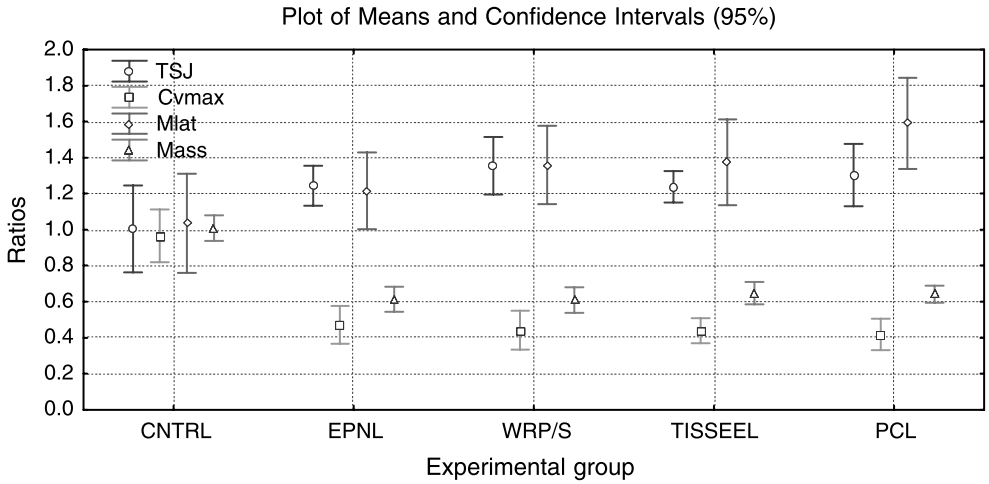

Fig. 3. Plot of means and 95% confidence intervals for variables in each group. *CNTRL* Control group; *EPNL* epineurial repair group; *WRP/S* CRG-wrap + polyglactin suture group; *TISSEEL* CRG-wrap + Tisseel group

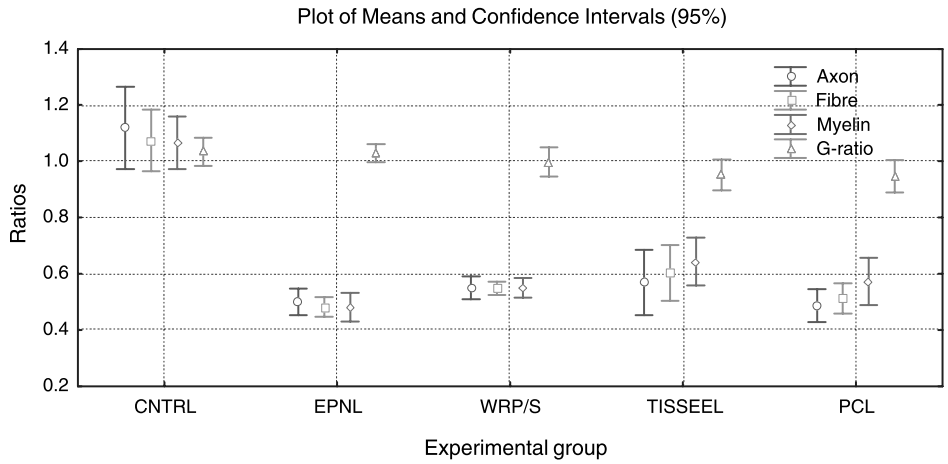

Fig. 4. Plot of means and 95% confidence intervals for morphometric variables according to experimental group

There was no significant difference between the mean axon diameter, fibre diameter, myelin thickness and g-ratio among the repair groups. The mean axon diameter, fibre diameter, myelin thickness in all the repair groups was significantly lower than in the control group. There was however no significant difference in the mean g-ratio of the repair groups compared with the control group. This suggested that myelination was complete in the repair groups. The results are shown in Fig. 4.

Discussion

The results of this study have demonstrated that a mixed peripheral nerve will regenerate to the same extent whether repaired by microsurgical epineurial suturing or wrap + Tisseel glue. We do not recommend the use of polycaprolactone glue in nerve repair.

The benefits of using the wrap in comparison with epineurial suturing are: decreased time to complete the repairs, no requirement for microsurgical instruments or skills; decreased potential for damage to the nerve fascicles by fibrosis caused by epineurial suturing [15]. The wrap maintains its structure when damp and does not collapse when folded. It has the nine ideal properties for a nerve conduit for clinical use: biocompatibility; low antegenicity[1]; minimal inflammatory stimulus; allows axon regeneration along its length; biodegradability; no potential for entrapment or compression; easily manufactured; economically available; has easy technical

[1] Although this has not been demonstrated in this study, the observation that no more fibrous tissue formation was seen at the sites of repair in the CRG-wrap + Tisseel and CRG-wrap + polyglactin groups was taken as an indication of this

handling characteristics [17]. A further benefit is that it is porous to macromolecules allowing these molecules access to the site of repair. Only basic surgical knowledge and technique is required to repair the nerve using the wrap therefore operating times and the number of primary repairs carried out in remote healthcare situations could be improved by the use of the wrap.

References

1. Aitken JT, Thomas PK (1962) Retrograde changes in fibre size following nerve section. J Anatomy 96(2): 121–129
2. Carter AJ, Kristmundsdottir F, Gilmour J, Glasby MA (1998) Changes in muscle cytoarchitecture after peripheral nerve injury and repair. A quantitative and qualitative study. J Hand Surg [Br] 23(3): 365–369
3. Dourado EPJ, Valmaseda-Castellon E, Gay-Escoda C (2004) Facial nerve repair with epineurial suture and anastomosis using fibrin adhesive: an experimental study in the rabbit. J Oral Maxillofacial Surg 62(12): 1524–1529
4. Filho OAR, Fazan VPS (2006) Streptozocin induced diabetes as a model of phrenic neuropathy in rats. J Neurosci Meth 151(2): 131–138
5. Gilchrist T, Glasby MA, Healy DM, Kelly G, Lenihan DV, McDowall KL *et al* (1998) In vitro nerve repair – in vivo. The reconstruction of peripheral nerves by entubulation with biodegradeable glass tubes-a preliminary report. Br J Plast Surg 51(3): 231–237
6. Hazari A, Johansson-Ruden G, Junemao-Bostrom K, Ljungberg C, Terenghi G, Green C *et al* (1999) A new resorbable wrap-around implant as an alternative nerve repair technique. J Hand Surg Brit Eur Vol 24B(3): 291–295
7. Lawson GM, Glasby MA (1995) A comparison of immediate and delayed nerve repair using autologous reeze-thawed muscle grafts in a large animal model. The simple injury. J Hand Surg [Br] 20(5): 663–700
8. Lenihan DV, Carter AJ, Gilchrist T, Healy DM, Miller IA, Myles LM *et al* (1998) Biodegradable controlled release glass in the repair of peripheral nerve injuries. J Hand Surg [Br] 23(5): 588–593
9. Lenihan DV, Sojitra N, Ikeda M, Carter AJ, Glasby MA (1997) Stimulated jitter measurements in the assessment of recovery after peripheral nerve repair. J Hand Surg [Br] 22(6): 772–777
10. Lenihan DV, Sojitra NM, Glasby MA (1998) Stimulated jitter measurement in the assessment of recovery after different methods of peripheral nerve repair. J Hand Surg [Br] 23(1): 12–16
11. Lenihan DV (2000) New methods for the repair and assessment of peripheral nerve injury. University of Edinburgh
12. Martins RS, Siqueira MG, Da Silva CF, Plese JPP (2005) Overall assessment of regeneration in peripheral nerve lesion repair using fibrin glue, suture, or a combination of the 2 techniques in a rat model. Which is the ideal choice? Surg Neurol 64(S1): 10–16
13. Mayhew TM, Sharma K (1984) Sampling schemes for estimating nerve fibre size. I. Methods for nerve trunks of mixed fascicularity. J Anatomy 139(1): 45–58
14. Millesi H, Meissl G, Berger A (1972) The interfascicular nerve-grafting of the median and ulnar nerves. J Bone Joint Surg Br 54A(4): 727–750
15. Millesi H (1981) Neurorrhaphy. In: Gorio A, Millesi H, Mingrino S (eds) Posttraumatic peripheral nerve regeneration; experimental basis and technique. Raven Press, New York, pp 215–228
16. Sanders FK, Whitteridge D (1946) Conduction velocity and myelin thickness in regenerating nerve fibres. J Physiol 105: 152–174
17. Wang H, Lineaweaver WC (2003) Nerve conduits for nerve reconstruction. Operative Techn Plastic Reconstructive Surg 9(2): 59–66

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Analysis of the dose-response of N-acetylcysteine in the prevention of sensory neuronal loss after peripheral nerve injury

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Summary

Background. N-Acetylcysteine (NAC) is a safe pharmaceutical agent known to protect cells from oxidative damage. Following peripheral nerve transection, NAC has been found to eliminate sensory neuronal loss. This study examines the dose-response relationship of NAC in preventing neuronal death.

Methods and Findings. The rat sciatic nerve transection model was used, and stereological quantification of sensory neuron survival carried out at two weeks post-axotomy. NAC was administered systemically as an intraperitoneal injection to five groups of rats at a range of doses (1–300 mg/kg/day). Significant neuronal loss was observed in the 1 mg/kg/day dosage group (18.5% loss, $p = 0.067$ vs. sham treatment). A degree of neuroprotection occurred with 10 mg/kg/day (9.1% loss, $p < 0.005$ vs. control), whilst there was no significant loss with either 150 or 300 mg/kg/day.

Conclusions. The prevention of sensory neuronal loss with NAC is dose dependent and effective over a wide therapeutic range. This analysis confirms the efficacy of systemic administration and provides a dose framework with which NAC has clinical potential to improve outcome after peripheral nerve trauma.

Keywords: N-Acetylcysteine; nerve injury; axotomy; neuroprotection; optical fractionator; dorsal root ganglia.

Introduction

The loss of up to 50% of the sensory neuronal population demonstrated in experimental models of nerve injury [12, 16] is a likely major factor explaining poor sensory functional outcome after peripheral nerve trauma [18], which is particularly evident when there is a delay before repair [7]. Quality of sensation regained depends on innervation density attained upon completion of regeneration [18], and the survival of sufficient numbers of sensory neurons remains a prerequisite for adequate reinnervation [4, 8].

N-Acetylcysteine (NAC) is a safe pharmacological treatment which maintains intracellular glutathione levels to protect hepatocytes from oxidative damage [1]. Initial studies demonstrated that administration of NAC after nerve transection improves the cellular morphology of axotomised sensory neurons, significantly reduces the rate of cell death, and virtually eliminates neuronal loss [5]. The aim of this present work is to determine the lowest dose of NAC that is optimally effective, using stereological counts of sensory neurons in the dorsal root ganglia (DRG).

Materials and methods

All work was performed in accordance with the terms of the Animals (Scientific Procedures) Act 1986. The experimental design is similar to previously published studies employing an established rat sciatic nerve transection model of nerve injury [5]. Under halothane anaesthesia adult rats underwent unilateral sciatic nerve division, and proximal and distal nerve stumps were ligated and secured into silicone caps to prevent spontaneous regeneration. Treatment with N-acetylcysteine at doses of 300, 150, 10 and 1 mg/kg ($n = 5$/group) commenced immediately postoperatively and was administered as a daily 1 ml intraperitoneal injection. A 'sham treatment' group ($n = 5$) received an equal volume of saline (0.9% NaCl). The weight of the animals was assessed every three days and the dose adjusted accordingly.

Two weeks following axotomy the L4 and L5 dorsal root ganglia were harvested bilaterally and fixed in 4% paraformaldehyde. Each entire ganglion was cut into 30 μm serial cryosections which were stained with 1 μg/ml solutions of Hoechst (Bisbenzimide H33342, BioChemika) and propidium iodide (BioChemika), to allow fluorescence microscopic examination of nuclear and cytoplasmic morphology, respectively.

The large number of primary sensory neurons in each ganglion precludes the absolute determination of their total number. Therefore the optical fractionator was employed to efficiently obtain an estimate of the

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Table 1. *Effect of two-week NAC treatment on neuron survival after axotomy*

Treatment group	Neuron counts				Percentage neuronal loss (L4 + L5)		Statisitical significance	
	Axotomised ganglia (L4 + L5)		Control ganglia (L4 + L5)				Axotomy vs. control	Dosage group vs. sham
	Mean	SD	Mean	SD	Mean	SD	*p* value	*p* value
Sham	33281	1532	39470	4139	15.10	7.68	0.016*	–
1 mg/kg	32764	5089	40442	2042	18.51	15.30	0.059	0.668
10 mg/kg	37011	1110	40605	1700	8.78	3.08	0.004**	0.126
150 mg/kg	37217	2142	37268	6459	−1.96	15.94	0.983	0.063
300 mg/kg	35031	3230	37662	1929	7.00	7.05	0.087	0.121

Neuron counts are expressed as the mean and SD of the combined L4 plus L5 counts for dorsal root ganglia in each dosage group. Neuronal loss is calculated as the reduction in neuronal count of axotomised ganglia compared to their contralateral controls, expressed as a percentage and SD for each group, $^{*}p < 0.05$, $^{**}p < 0.01$.

number of surviving L4 and L5 DRG neurons as previously described [5, 6]. The mean neuron count for each group is expressed as the combined figure for L4 plus L5 DRG, both for the axotomised and the contralateral non-axotomised control sides. Neuronal loss was calculated by subtracting the neuron count in the axotomised L4 plus L5 ganglia from that in the contralateral control ganglia for each animal, and expressed as a percentage of the number of neurons in the control ganglion pair. From these figures the mean percentage neuronal loss for each dosage group was calculated.

Statistical analysis was performed using the GraphPad Prism® software package. Pair-wise comparisons were performed using Student's *t*-test and comparison between dosage groups by ANOVA one-way analysis of variance, or Kruskal-Wallis test where data was non-normally distributed.

Results

No adverse reactions or change in animal behaviour were observed as a result of NAC treatment. There was no significant difference in the rate of weight gain between sham and treatment groups. Table 1 reports the mean number of neurons present within the axotomised and contralateral control L4 plus L5 ganglia in each group at 2 weeks post-axotomy. The number of neurons present within control ganglia was consistent in all groups (one-way ANOVA $p = 0.5025$). Axotomised ganglia in the sham treatment group contained significantly fewer neurons than their contralateral control ganglia (mean difference, 6188 neurons, $p < 0.05$), representing a 15.0% loss of neurons. Neuron loss was similar in the 1 mg/kg/day dosage group (mean loss, 7677 neurons [18.5%], $p = 0.67$ vs. sham treatment), but a degree of neuroprotection was found with a dose of 10 mg/kg/day (mean loss, 3595 neurons [9.1%], $p < 0.005$ vs. contralateral ganglia). Neuronal counts after axotomy in both the 150 mg/kg group (mean loss, 51 neurons) and 300 mg/kg groups (mean loss, 2632 neurons) were not significantly different from their contralateral controls. In summary, a progressive increase in neuroprotection was evident, with a dose of 150 mg/kg/day NAC providing complete neuroprotection.

Discussion

The results of this study demonstrate a dose-dependent effect of NAC on the prevention of sensory neuronal loss after peripheral nerve injury. The two-week time point used in this study coincides with the peak rate of neuronal death, when the majority of neurons that are destined to die have already been lost, or reached an unsalvageable point in the cell death cycle [12]. This time point was selected as the neuroprotective effect has previously been demonstrated to persist throughout a more extended timeframe during which neurons otherwise continue to die [5].

The neuroprotective effect improves with an increasing dose, a partial response being evident at a dose of 10 mg/kg. In initial studies almost complete preservation of the neuronal population was demonstrated with 30 mg/kg NAC (1% neuron loss) [5] and this effect plateaus, with no significant difference between the responses seen at 150 and 300 mg/kg/day. The complete elimination of neuronal loss observed at 150 mg/kg/day was not merely an artefact of the way in which loss is calculated, since there was no significant difference in the non-axotomised contralateral ganglion counts between groups, and this consistency confirms that NAC treatment did not affect the non-axotomised ganglia. The minor loss calculated in the highest dose group lies within the variability of the counting technique and random sampling, since it is reported for other models of peripheral nerve injury that NAC did not lose efficacy at systemic doses up to 750 mg/kg/day, and also with intrathecal administration at even higher concentrations [20].

NAC undergoes considerable first-pass metabolism [2], hence the importance of parenteral administration in this study. Once in circulation NAC is quickly delivered to neurons due to the absence of a blood-nerve barrier with-

in the neuron-containing region of the DRG. Although the plasma half-life of NAC is reportedly 5.6 h for intravenous administration in human adults [15], it is intracellular levels that are most relevant and once daily intraperitoneal dosing has been previously found to be efficacious in terms of neuroprotection in rat models. In clinical practice this would translate to intravenous administration with the aim of achieving peak tissue levels as soon as possible after injury. The time it takes for a patient to present to hospital is unlikely to be of detriment to outcome, since neuronal loss only begins to develop a number of days after the injury [12]. Acetyl-L-carnitine has previously been observed to maintain complete sensory neuroprotection if therapy is initiated 24 h after axotomy [19], whilst a one-week delay in NAC treatment after ventral root avulsion is reported to have no impact on the degree of motor neuron loss [20].

Neuronal cell death is an active process, and the principal trigger in peripheral nerve injury is the loss of target derived neurotrophic support [4, 17]. The precise neuroprotective mechanism of NAC remains unclear, but is thought likely to involve redox regulation of cell signalling, or an antioxidant effect, most probably mediated by its role as a glutathione substrate [5]. Whilst other pharmaceutical agents and exogenous neurotrophic factors have previously shown promising experimental results with regard to neuronal rescue [17], none has yet achieved clinical application due to complex interactions [13, 14], side effects and practical difficulties with administration [3, 10, 11]. In contrast, NAC has proven to be a clinically safe and effective pharmaceutical, eliciting few side effects in humans other than minor hypersensitivity reactions at an incidence estimated to be between 0.2–3% for intravenous dosage [9]. Although immediate nerve repair partially reduces neuronal loss by reconnecting neurons with distal nerve stump neurotrophic factors [12], this is a very incomplete protective effect, and is only clinically relevant for primary repair. So the potential benefit of NAC administration lies not just in keeping neurons alive until the time of surgery, but also in maximising survival even after repair.

References

1. Corcoran GB, Wong BK (1986) Role of glutathione in prevention of acetaminophen-induced hepatotoxicity by N acetyl-L-cysteine in vivo: studies with N-acetyl-D-cysteine in mice. J Pharmacol Exp Ther 238(1): 54–61
2. Cotgreave IA, Berggren M, Jones TW, Dawson J, Moldeus P (1987) Gastrointestinal metabolism of N-acetylcysteine in the rat, including an assay for sulfite in biological systems. Biopharm Drug Dispos 8(4): 377–386
3. Dyck PJ, Peroutka S, Rask C, Burton E, Baker MK, Lehman KA *et al* (1997) Intradermal recombinant human nerve growth factor induces pressure allodynia and lowered heat-pain threshold in humans. Neurology 48(2): 501–505
4. Fu SY, Gordon T (1997) The cellular and molecular basis of peripheral nerve regeneration. Mol Neurobiol 14(1–2): 67–116
5. Hart AM, Terenghi G, Kellerth JO, Wiberg M (2004) Sensory neuroprotection, mitochondrial preservation, and therapeutic potential of N-acetyl-cysteine after nerve injury. Neuroscience 125(1): 91–101
6. Hart AM, Terenghi G (2004) Frozen-section fluorescence microscopy and stereology in the quantification of neuronal death within dorsal root ganglia. J Mol Histol 35(6): 565–580
7. Kallio PK, Vastamaki M, Solonen KA (1993) The results of secondary microsurgical repair of radial nerve in 33 patients. J Hand Surg [Br] 18(3): 320–322
8. Lundborg G (2000) A 25-year perspective of peripheral nerve surgery: evolving neuroscientific concepts and clinical significance. J Hand Surg [Am] 25(3): 391–414
9. Mant TG, Tempowski JH, Volans GN, Talbot JC (1984) Adverse reactions to acetylcysteine and effects of overdose. Br Med J (Clin Res Ed) 289(6439): 217–219
10. Martin D, Merkel E, Tucker KK, McManaman JL, Albert D, Relton J *et al* (1996) Cachectic effect of ciliary neurotrophic factor on innervated skeletal muscle. Am J Physiol 271(5 Pt 2): R1422–R1428
11. McArthur JC, Yiannoutsos C, Simpson DM, Adornato BT, Singer EJ, Hollander H *et al* (2000) A phase II trial of nerve growth factor for sensory neuropathy associated with HIV infection. AIDS Clinical Trials Group Team 291. Neurology 54(5): 1080–1088
12. McKay HA, Brannstrom T, Wiberg M, Terenghi G (2002) Primary sensory neurons and satellite cells after peripheral axotomy in the adult rat: timecourse of cell death and elimination. Exp Brain Res 142(3): 308–318
13. Novikova LN, Novikov LN, Kellerth JO (2000) BDNF abolishes the survival effect of NT-3 in axotomized clarke neurons of adult rats. J Comp Neurol 428(4): 671–680
14. Novikova LN, Novikov LN, Kellerth JO (2000) Survival effects of BDNF and NT-3 on axotomized rubrospinal neurons depend on the temporal pattern of neurotrophin administration. Eur J Neurosci 12(2): 776–780
15. Prescott LF, Donovan JW, Jarvie DR, Proudfoot AT (1989) The disposition and kinetics of intravenous N-acetylcysteine in patients with paracetamol overdosage. Eur J Clin Pharmacol 37(5): 501–506
16. Tandrup T, Woolf CJ, Coggeshall RE (2000) Delayed loss of small dorsal root ganglion cells after transection of the rat sciatic nerve. J Comp Neurol 422(2): 172–180
17. Terenghi G (1999) Peripheral nerve regeneration and neurotrophic factors. J Anat 194 (Pt 1): 1–14
18. Wiberg M, Hazari A, Ljungberg C, Pettersson K, Backman C, Nordh E *et al* (2003) Sensory recovery after hand reimplantation: a clinical, morphological, and neurophysiological study in humans. Scand J Plast Reconstr Surg Hand Surg 37(3): 163–173
19. Wilson DH, Hart A, Brannstrom T, Wiberg M, Terenghi G (2007) Delayed acetyl-L-carnitine administration and its effect on sensory neuronal rescue after peripheral nerve injury. J Plast Reconstr Aesthet Surg 60: 114–118
20. Zhang C-G, Welin D, Novikov L, Kellerth J-O, Wiberg M, Hart A (2005) Motorneuron protection by N-acetyl-cysteine after ventral root avulsion and ventral rhizotomy. Br J Plast Surg 58: 765–773

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Did the partial contralateral C7-transfer fulfil our expectations? Results after 5 year experience

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Summary

Objective. Within the last decade contralateral C7-transfer has become a new source of axon donor in complete brachial plexus lesions.

Methods. Ten adult patients with a complete posttraumatic brachial plexus lesion and a follow-up of more than 5 years are analyzed. As shown by GU we are using a two stage procedure with exploration and extraplexuel neurotization of the suprascapular nerve using 1/2 spinal acessory nerve. Depending on the intraoperative findings, the musculocutaneous nerve is neurotized by the phrenic nerve at the time of primary operation or secondarily neurotized by the contralateral C7 root. If the musculocutaneous nerve could be neurotized by the phrenic nerve, C7-transfer is used to reinnervate the median nerve. If ever possible, the vascularized ulnar nerve graft or if not availabe two sural nerves are used. Neurotization of the musculocutaneous nerve was carried out in 6, and of the median nerve in 4 patients. There are 6 patients in the MC group and 4 patients in the Median group. Criterias for evaluation used are: donor site (morbidity, classification), time for recovery, time for autonomization, and functional result. Successful elbow flexion is achieved if muscle power > M3, successful median nerve motor function is achieved if a primitive power grip pattern is achieved.

Results. All patients were complaining of temporary paresthesia in the dorsal part of P3 of the thumb, index and middle finger. There was complete sensory at the 3-month postoperative examination. There was no evident clinical motor loss at the donor extremity.

A successful elbow flexion, i.e. muscle power > M3 was achieved in all 6 patients after 9–15 months. 4 of 6 patients are able to use this function individually. In the other two patients a start command must be given voluntarily from the contralateral side (contraction of the contralateral latissimus dorsi muscle).

A functional primitive grip pattern could be achieved in 1 out of 4 patients after 18 months. In three patients, although there is movement, this mouvement must be judged "academic" at the present state.

Conclusions. The C7-transfer proved to be a safe transfer if at the time of operation no fascicles innervating wrist and finger extension are taken. Provided adequate biceps muscle organ function, active elbow flexion can be reconstructed in most of the patients. However, for median nerve reinnervation motor results are moderate up to now.

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Keywords: C7 Transfer; neurotization; donor nerve; brachial plexus lesion.

Introduction

Within the last decade contralateral C7-transfer has become a new source of axon donor in complete brachial plexus lesions.

Patients and methods

Ten adult patients with a complete posttraumatic brachial plexus lesion and a follow-up of more than 5 years are analyzed. As shown by GU we are using a two stage procedure with exploration and extraplexuel neurotization of the suprascapular nerve using 1/2 spinal acessory nerve. Depending on the intraoperative findings, the musculocutaneous nerve is neurotized by the phrenic nerve at the time of primary operation, or secondarily neurotized by the contralateral C7 root. If the musculocutaneous nerve could be neurotized by the phrenic nerve, C7-transfer is

Table 1. *Results donor site morbidity*

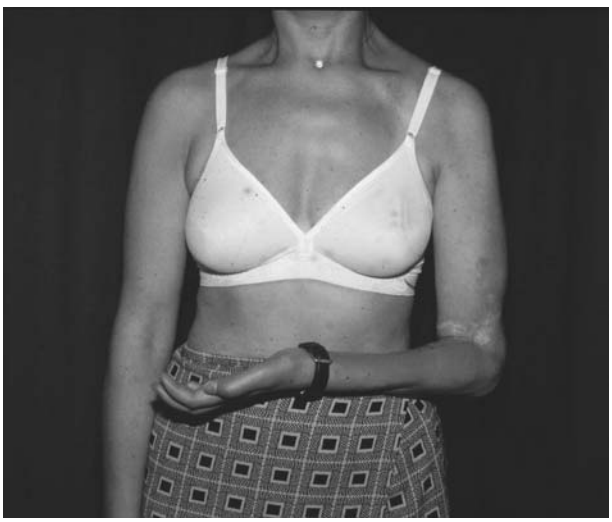
Uneventful wound healing	10/10
Paresthesia P3 DI,II,III	10/10 (6–52 weeks)
Motor deficits on clinical examination	0/10
EMG denervation signs	(10/10)

Table 2. *Functional results after C7 transfer for elbow flexion*

Epidemiology:	
$N = 6$ (5 m, 1f)	
Delay: 4–7 m	
Nerve graft: vasc. ulnar nerve 6/6	
Recovery:	
HT-progession: 6/6	
1st contraction: 10–16 months	
Recovery plateau: 18–24 months	
Functional result:	5/6
(EF >90° + 1,5 kg at wrist)	
Autonomization:	3/6

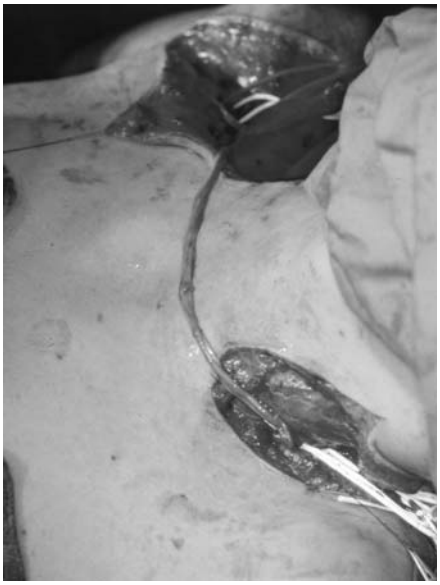
used to reinnervate the median nerve. If ever possible, the vascularized ulnar nerve graft or – if not availabe – two sural nerves are used. Neurotization of the musculocutaneous nerve was carried out in 6, and of the median nerve in 4 patients. There were 6 patients in the MC group and 4 patients in the Median group. Criteria for evaluation used are: donor site (morbidity, classification), time of recovery, time of autonomization, and functional result. Successful elbow flexion is achieved if muscle power is $>$M3, successful median nerve motor function is achieved if a primitive power grip pattern is achieved.

a

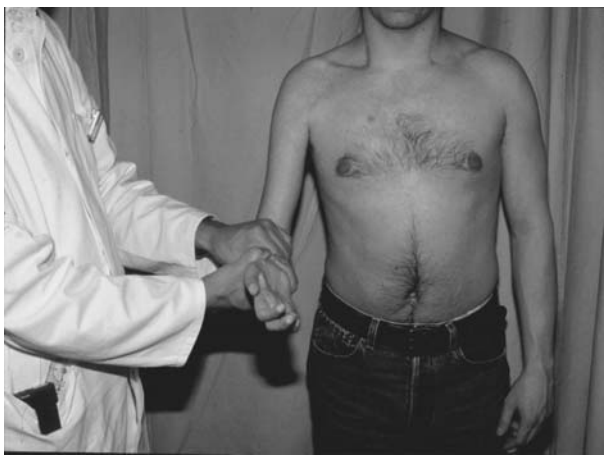


b

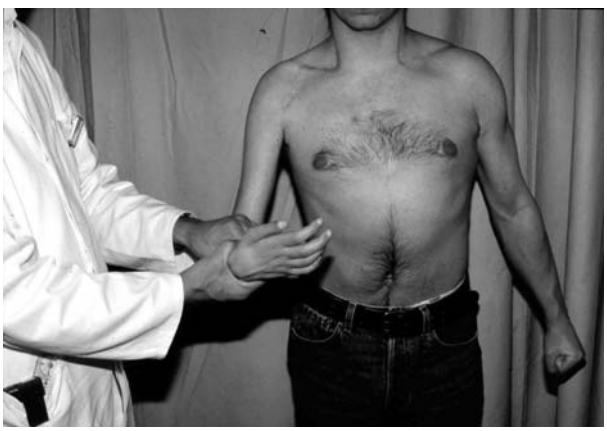
Fig. 1. Functional results 18 months after partial contralateral C7 transfer to reinnervate the musculocutaneous nerve. (a) Clinical aspect after 10 months: to contract the elbow flexors it is necessary to voluntarily start the movement at the contralateral side. (b) Clinical aspect after 24 months: elbow flexion can be done without contralateral start command (positive autonomization)



a



b



c

Fig. 2. Functional results 24 months after partial contralateral C7 transfer to reinnervate: (a) intraoperative aspect: nerve graft is placed subcutaneously; (b) postoperative aspect: wrist extension; (c) postoperative aspect: wrist flexion

Results

All patients were complaining of temporary paresthesia in the dorsal part of P3 of the thumb, index and middle finger. There was complete sensory at the 3-month post-operative examination. There was no evident clinical motor loss at the donor extremity (Table 1).

A successful elbow flexion, i.e. muscle power $>$M3 was achieved in all 6 patients after 9–15 months. 4 of 6 patients were able to use this function individually. In the other two patients a start command must be given voluntarily from the contralateral side (contraction of the contralateral latissimus dorsi muscle) (Table 2, Fig. 1a,b).

A functional primitive grip pattern could be achieved in 1 out of 4 patients after 18 months. In three patients, although there is movement, this movement must be judged "academic" at the present state (Table 3, Fig. 2a–c).

Conclusions

The C7-transfer proved to be a save transfer if at the time of operation no fascicles innervating wrist and finger extension are taken. Provided there is adequate biceps muscle organ function, active elbow flexion can be reconstructed in most of the patients. However, for median nerve reinnervation motor results are moderate up to now.

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Bridging defects: autologous nerve grafts

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Summary

Autologous nerve grafting has been performed since 1876 without success. Since the 1960s, the interfascicular nerve grafting technique has provided a reliable method to bridge very long nerve defects with predictable results. Personal experience of over 40 years has demonstrated that donor site morbidity is minimal if certain precautions are observed. Donor site morbidity does not justify the use of alternative techniques. However, in vast defects like brachial plexus lesions, the number of available autografts is insufficient. We do need alternative techniques like tubulisation, however, not to replace autologous nerve grafts but to supplement them.

Keywords: Nerve regeneration; nerve graft; autograft; brachial plexus.

Introduction

In spite of the fact that free nerve grafting was attempted for the first time already in 1876 by Albert [1], nerve grafting was considered a salvage procedure with rather poor chances of success.

Why did nerve grafts not yield satisfactory results?

Regenerating axons have to cross two lines of coaptation at the proximal end of the graft and at the distal end of the graft to reach the distal stump. This consideration alone is a logical argument that results after grafting must be inferior to an end-to-end neurorhaphy. Nerve trunks as free grafts do not survive very well and utilization of cutaneous nerves [2] with a small diameter cannot really bridge defects of a nerve trunk. The introduction of so called cable grafts [8] (several segments of thin cutaneous nerves glued or sutured together to form a cable of the same size as the nerve to be repaired) could not solve the problem. The free grafts packed together to a cable did not survive free grafting well because part of their surface was in contact with another free graft and not available for contact with the recipient site. The suggestion by Strange [9, 10] to improve the survival of trunk grafts by transposing a trunk graft as a pedicled flap did not become popular. Another nerve trunk had to be sacrificed, e.g., ulnar for median nerve. The advantage of maintaining the blood supply was neutralized by the fact that – as in all trunk grafts – nobody could predict where an axon that entered the graft proximally would leave the graft at the distal end thus making an ''aimed connection'' impossible. In addition, surgeons were convinced that the length of graft would have a negative influence on the final result. Therefore, the two stumps of the transected nerve were approximated as much as possible thus combining the disadvantages of a coaptation under tension with the disadvantage of grafting with two sites of coaptation.

The new approach to free nerve grafting

Results of free nerve grafting improved significantly in the late sixties of the last century when a new biologic approach was introduced [4–7]. This development was based on the following considerations:

- The result is dependent on the **length of a given defect** of nerve tissue, but is **not dependent on the distance** between the two stumps. Therefore, the length of graft is not important.
- **Tension** at the site of coaptation should be avoided not only at the time of surgery by flexing the adjacent joints but also during mobilization.

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- If one uses **long nerve grafts** even in long defects, coaptation without tension is possible to improve the quality of coaptation.
- The individual grafts should **be placed individually** and not as a cable to improve survival.
- By **interfascicular dissection** the two stumps are dissected into individual fascicles or pre-existent fascicle groups to be coapted individually with one segment of a cutaneous graft.
- By comparing the fascicular pattern an attempt is made to define corresponding fascicles or fascicle groups to connect them by one graft segment to achieve an ''aimed connection''.

Donor site morbidity

Excision of a cutaneous nerve to be used as a graft causes formation of a neuroma at the proximal site of transection. This neuroma may give rise to pain, and a pain syndrome may develop.

According to my experience of over 40 years of nerve grafting, painful neuroma after harvesting a cutaneous nerve graft occurs only if transection is located in the subcutaneous tissue. The site of transection should consequently be located deep in the subfascial space. This means that if we need a 6 cm long graft and we want to harvest the sural nerve, we have to excise the whole sural nerve up to the level below the knee joint and dispose of the rest. It is evident that for a short defect of a not important nerve like digital nerve of the ring finger, sacrification of the whole sural or any other of the donor nerves is not justified. It is this consideration and not the donor site morbidity which raises the wish to have some alternative to an autograft.

Quantity of donor nerves

There are many donor nerves available. The number is certainly sufficient to bridge the vast majority of nerve defects we meet in clinical practice. But in a long defect of the sciatic nerve or in many brachial plexus cases we are short of donor nerves. This is a second reason why alternatives to autologous nerve grafts are wanted. This is not to substitute autologous grafts but to augment the number of grafting material.

The ideal nerve graft

- The ideal nerve graft should be available in large quantities.
- It should consist of a stroma with structure and the mechanical properties of endoneurium.
- It should contain capillaries and a few fibroblasts.
- It should contain a large quantity of Schwann cells.
- To avoid any immunologic reaction, the Schwann cells should be autologous cells from the patient as well as the capillaries and the fibroblasts.
- Since we know that there is a difference between motor and sensory Schwann cells [3] they should be selected accordingly.
- Preferably the Schwann cells should originate from the nerve to be repaired.

Summary

I am still convinced that autologous nerve grafts are the gold standard to bridge defects of peripheral nerves. For the reasons outlined above I support any attempt to develop alternatives, not to substitute but to supplement autologous grafts. The final goal of research should be the ''ideal graft'' as a vision.

References

1. Albert E (1885) Einige Operationen am Nerven. Wien Med Press 26: 1285
2. Foerster O (1916) Vortrag: Außerordentliche Tagung der deutschen orthopädischen Gesellschaft Berlin, 2.-9. Feb. 1916. Münch Med WSCHR 63: 283
3. Hoke A, Redett R, Hameed H, Jari R, Zhou C, Li ZB, Griffin JW, Brushart TM (2006) Schwann cells express motor and sensory phenotypes that regulate axon regeneration. J Neurosci 26(38): 9646–9655
4. Millesi H, Ganglberger J, Berger A (1966) Erfahrungen mit der Mikrochirurgie peripherer Nerven. Chir Plastica 3: 47
5. Millesi H, Berger A, Meissl G (1972) Experimentelle Untersuchungen zur Heilung durchtrennter peripherer Nerven. Chir Plastica 1: 174–206
6. Millesi H, Meissl G, Berger A (1972) The interfascicular nerve-grafting of the median and ulnar nerves. J Bone Joint Surg Am 54(4): 727–750
7. Millesi H, Meissl G, Berger A (1976) Further experience with interfascicular grafting of the median, ulnar, and radial nerves. J Bone Joint Surg Am 58(2): 209–218
8. Seddon HJ (1947) The use of autologous grafts for the repair of large gaps in peripheral nerves. Brit J Surg 35: 151
9. Strange FG StC (1947) An operation for nerve pedicle grafting. Preliminary communication. Brit J Surg 34: 423
10. Strange FG StC (1950) Case report on pedicled nerve-graft. Br J Surg 37(147): 331–333

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The allogenic nerve graft

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Introduction

A segmental nerve defect is still best be treated by autologous nerve graft. However, besides its donor side morbidity, extensive nerve defects of peripheral nerves or the brachial plexus often cannot be completely treated due to missing quantity of autologous nerve graft material. Thus two solution are possible: first, incomplete reconstruction – the abandoned nerve trunk is used as additional source of autologous graft material (ulnar nerve in complete brachial plexus palsy), or second, the application of new reconstruction techniques, such as nerve distraction [1], tubes [7] or nerve allografts [3, 9, 14]. Nerve distraction is still in the laboratory phase [1, 11]. Nerve tubes should not be chosen in cases of mixed nerve and/or defect larger than 10 mm [2]. Thus the nerve allograft still seems to be the best substitute.

Immunological rejection and how to cope with it

The major problem of adult nerve allograft lies within the immunologic rejection leading to poor functional results. To avoid the rejection phenomenon, either the reagibility of the host and/or the antigenicity of the allograft could theoretically be reduced.

Systemic immunosuppression

To reduce the reagibility of the host, systemic immunosuppression is needed. With adequate immunosuppression the functional results of nerve allograft are equal to those of autologous nerve grafts [5] (Fig. 1a–c).

Although the nerve graft will largely be replaced by autologous tissue during regeneration, immunosuppres-

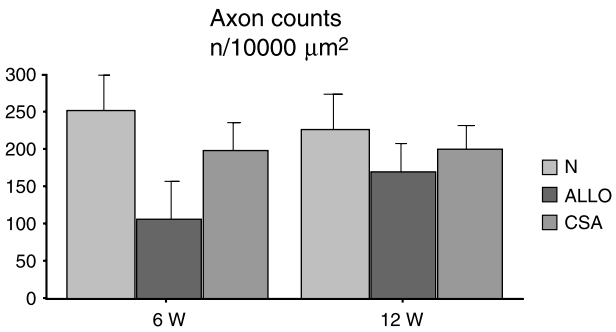

Fig. 1. Axon counts after nerve transplantation: (*a*) autologous nerve graft (“gold standard”), (*b*) allogenic nerve graft without immunosuppression, (*c*) allogenic nerve grafting with adequate immunosuppression

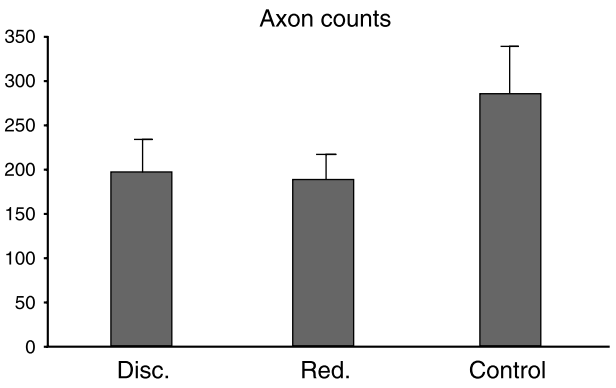

Fig. 2. Results of allogenic nerve grafting with temporary immunosuppressive treatment

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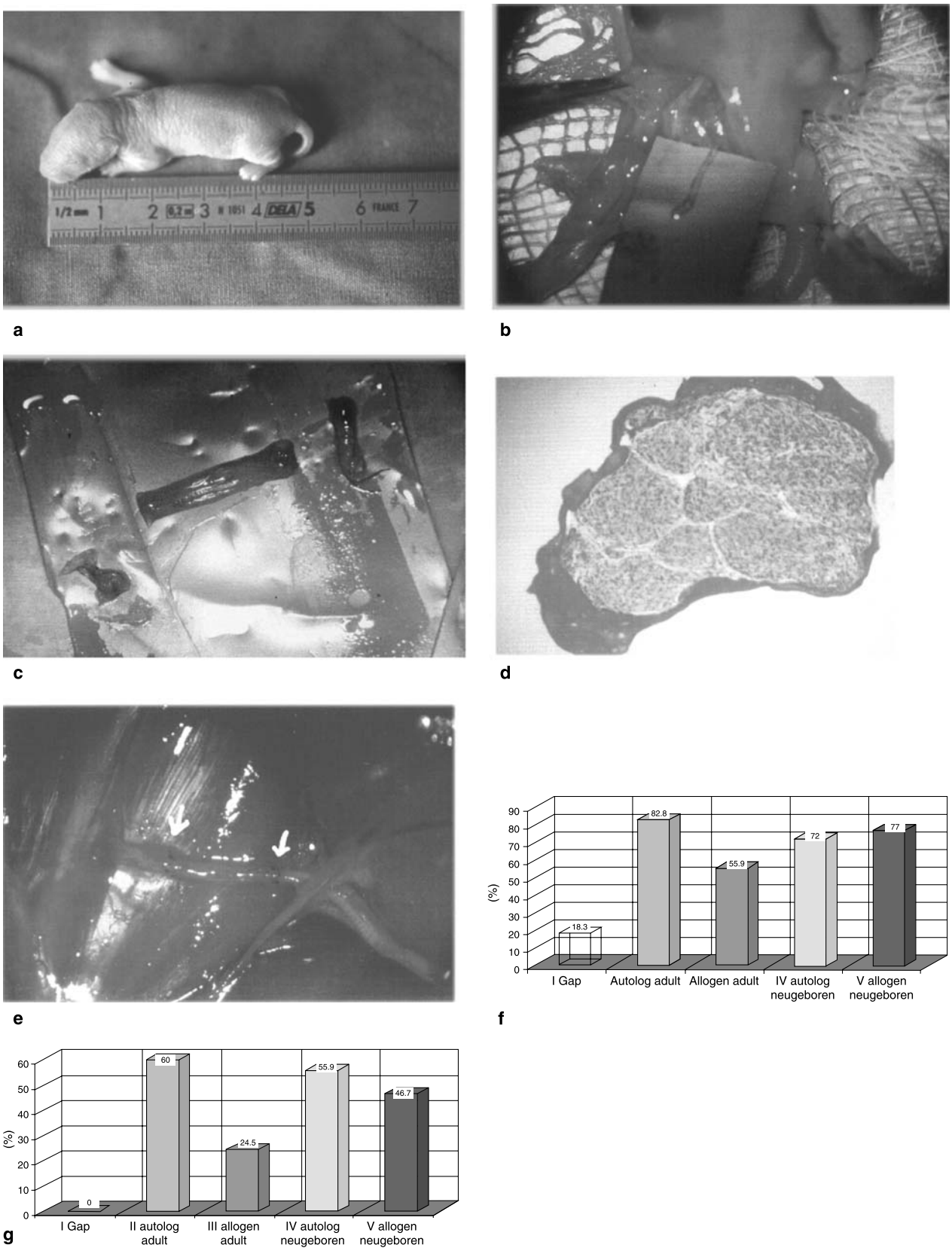

Fig. 3. The composed nerve allogenic nerve graft from the newborn: (a) clinical aspect of a newborn rat, (b) harvesting the sciatic nerve under the microscope, (c) assembly of several sciatic nerves to create a composed nerve allograft from the newborn, (d) cross-section of the composed nerve allograft from the newborn, (e) clinical aspect after transplantation for reconstruction of segmental nerve defect of the peroneal nerve in an adult rat, (f) results of axon counts at the distal nerve stump 6 months after transplantation, (g) results of (dry) muscle weight 6 months after transplantation

sive treatment must not be under a certain dosage or even stopped, and has to be given lifelong (Fig. 2) [6]. However, the risk of known (skin tumors) and still unknown side-effects of immunosuppressive medication does not justify its application for a non life-threatening situation like nerve repair procedure.

Reduction of antigenicity of the graft

Reduction of antigenicity of the allograft can theoretically be achieved by graft pretreatment, tissue grouping, or the use of low-antigenic graft material.

Pretreatment of nerve allografts with high dose irradiation or lyophilization markedly diminished the nerve allograft response. All other methods of treatment that have been used either experimentally or clinically (freezing, predegeneration, low dose irradiation [12] – 200 or 10.000 rads, or a combination of predegeneration, freezing, and irradiation) did not significantly decrease antigenicity of the nerve allograft, and the allograft appeared no less antigenic than a fresh nerve allograft [8]. However, all those techniques more or less destroy the Schwann cells. The importance of maintaining viable Schwann cells in grafts has been emphasized by studies of peripheral nerve transplantation, which have shown that the antigenicity of grafts can be decreased by methods that kill Schwann cells. But graft lacking viable Schwann cells usually do not provide significant neurologic recovery, on careful analysis of the results [8, 9, 13].

Tissue grouping [15] is routinely used in transplantation medicine but the probability to find a largely identical tissue is very low except for twins.

Within the last ten years several studies on different foetal or newborn neural tissue used as allograft have reported restricted or missing graft rejection [4, 10]. The immunologic system in the foetus is not completely developed, and this could reduce graft immunogenicity. In contrast to grafts from adults, the foetal neural tissue is not less immunologically reactive but becomes vascularized in solid grafts, and apparently more migratory in suspension grafts [10]. Theoretically this reduced graft rejection could lead to better functional results. The functional and immunological behaviour of peripheral nerve tissue harvested from the newborn rat and transplanted into an adult rat was studied in an isogenic and allogenic experimental model [4]. The following results have been found: 1) It is technically possible to use the fragile peripheral nerve tissue of a newborn to create a nerve graft for reconstruction of peripheral nerve defects in adults. 2) The composed nerve allograft does lead to comparable functional results of autologous nerve graft, and 3) The composed allogenic nerve graft differs in its immunological rejection from adult allogenic nerve graft. This concept is charming. However, there are two shortcomings. Legal and ethic problems to have access to this material, which are by the way the same problems as encountered in stem cell engineering (Fig. 3a–g).

References

1. Anjou de B, Allieu Y, Cenac P, Sanz J (1989) Expansion nerveuse. Ann Chir Main 8 336–337
2. Chiu DTW, Strauch B (1990) A prospective clinical evaluation of autogenous vein grafts used as a nerve conduit for distal sensory nerve defects of 3 cm or less. Plast Reconstr 86: 928–934
3. Comtet J-J (1988I) Is there a future for nerve homograft? In: Brunelli GA (ed) Textbook of microsurgery. Masson, Milano, pp 629–632
4. Hierner R (1999) Das allogene modifizierte Kabeltransplantat vom Neugeborenen. Medizinische Hochschule Hannover, Habilitationsschrift
5. Lassner F, Schaller E, Steinhoff G, Wonigeit K, Walter GF, Berger A (1989) Cellular mechanisms of rejection and regeneration in peripheral nerve allografts. Transplantation 48: 386–392
6. Lassner F, Becker MH-J, Führer S, Walter GF, Berger A (1996) Die zeitlich begrenzte Immunsuppression am allogenen Nerventransplantat der Ratte. Handchir Mikrochir Plast Chir 28: 176–180
7. Lundborg G (1988) Nerve injury and repair. Churchill Livingstone, Edinburgh
8. Mackinnon SE, Hudson AR, Falk RE, Kline D, Hunter D (1984) Peripheral nerve allograft: an assessment of regeneration across pretreated nerve allografts. Neurosurg 15: 690–693
9. Mackinnon SE, Hudson AR, Bain JB, Falk RE, Hunter DA (1987) The peripheral nerve allograft: an assessment of regeneration in the immunosuppressed host (invited Discussion by Yaremchuk M.J.) Plast Reconstr Surg 79: 436–445
10. Mahowald MB, Silver J, Ratcheson RA (1988) The ethical options in transplanting fetal tissue. Hastings Center Report 9–15
11. Milner RH (1989) The effect of tissue expansion on peripheral nerves. Br J Plast Surg 42: 414–421
12. Pollard JD, Fitzpatrick L (1973) A comparison of the effects of irradiation and immunosupressive agents on regeneration through peripheral nerve allografts: an ultrastructural study. Acta Neuropath (Berl) 23: 166–180
13. Ruwe PA, Trumble TE (1990) A functional evaluation of cryopreserved peripheral nerve autografts. J Reconstr Microsurg 6: 239–244
14. Schaller E, Lassner F, Becker M, Walter GF, Berger A (1991) Regeneration of autologous nerve grafts in a rat genetic model: preliminary report. J Reconstr Microsurg 7: 9–12
15. Singh R, Medrelse K, Stefanko S (1984) Role of tissue typing on preserved nerve allografts. Exp Neurology 83: 659–663

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Alternative techniques for peripheral nerve repair: conduits and end-to-side neurorrhaphy

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Summary

Nowadays new techniques may help the surgeon in difficult cases of nerve tissue loss: when a gap is produced in a mixed nerve, the use of conduits can be an alternative to nerve grafts, which still represent the ''gold standard'' for this kind of lesions. We have applied biologic conduits (muscle inside a vein) in more than 40 cases since 1993 with 85% of good functional results for both sensory and mixed nerves up to 5 cm. The advantages of this technique are: 1) all graft material is easily withdrawn in the lesion area and thus is not necessary to perform any new incision; 2) the possibility of reconstructing nerve gaps up to 5 cm avoids secondary damage created by the withdrawal of healthy nerves; 3) the possibility for spontaneous orientation of regenerating nerve fibers is offered as fibers are allowed to search for their final target (chemiotropism).

Furthermore, when the tissue loss is important or the proximal nerve stump is not available, so jeopardizing the possibility of recovery with traditional reconstruction, the use of end-to-side neurorrhaphy has been described to solve the problem. However the use of end-to-side neurorrhaphy in the clinical setting for motor recovery remains controversial. In our experience we had satisfying results only in 20% of cases and thus motor reconstruction in the absence of an available proximal nerve may be best handled by nerve to nerve transfers. By contrast we had good results in sensory nerve reconstruction (especially digital nerves) by end-to-side coaptation.

Keywords: Nerve repair; conduits; neurorraphy.

Introduction

Nowadays, surgical resolution of a disability resulting from a peripheral nerve lesion is no longer an impossible task for the surgeon even if diagnosis and treatment still require a thorough knowledge of the physiopathology of the nervous system together with the most recent sophisticated microsurgical techniques. Basic knowledge as to the pathophysiological events that take part in a nerve trunk and its neurons after transection injury (degeneration and regeneration) is essential in order to choose the correct surgical treatment, its timing and the rehabilitation program.

Since the observations of Waller [46] in 1850 describing the changes of the distal segment of a transected nerve in the frog, there has been extensive research and numerous studies. However, only in the seventies did this lead to the fundamental works of Millesi on nerve repair by means of interfascicular nerve grafting [31]. Two decades have now passed and we are able to understand the rules of nerve regeneration better, also thanks to the studies by Rita Levi Montalcini, Lundborg and other researchers on nerve growth factors, chemotropism and many other fields of interest [15, 19, 43]. This led us to develop new techniques which may help the surgeon in difficult cases together with traditional reconstructive methods.

These new methods can be useful especially in case of loss of nerve tissue: when a gap is produced in a mixed nerve the use of conduits can be an alternative to nerve grafts, which still represent the ''gold standard'' for this kind of lesions. Furthermore, when the tissue loss is important or the proximal nerve stump is not available, so jeopardizing the possibility of recovery with traditional reconstruction, the use of end-to-side neurorrhaphy has been described to solve the problem.

We aim to present the rationale and the technical details of these kinds of alternative techniques discussing their indications and limits.

Conduits

The withdrawal of a nerve for an autograft creates damage in a sound area (skin scar, sensory loss in the donor

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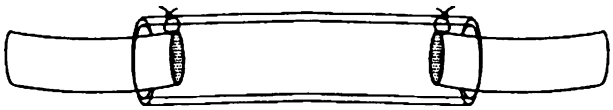

Fig. 1. Tubes allow spontaneous orientation of regenerating axons

area, risk of neuroma formation); moreover, at times these autografts are not long enough to repair an extensive nerve gap. The use of tubes (synthetic or biologic such as veins) may guide the axonal regeneration without sacrificing sound nerves (Fig. 1). We shall examine the advantages and problems of this kind of reconstruction while describing the influence of biomolecular factors on nerve regeneration.

Orientation of fascicles when using grafts conditions the final result. Misdirection could lead the regenerating axons to a wrong final target. For this reason several authors continue to study the mechanisms of axonal orientation during regeneration. At the site of nerve injury sprouts start to grow distally and several biomolecular factors are involved to support the outgrowth and direction of axonal growth.

Simplifying, these biomolecular factors could be subdivided into three major groups: neurotrophic factors, neurotropic factors and Neurite Promoting Factors (NPF) [17, 18].

Neurotrophic factors are endogenous soluble proteins influencing survival, development, morphological plasticity of nerve cells (''neurotrophism''). These factors are synthesized in neurons, muscle, glands and are classified on the basis of their receptors: *Neurotrophins* (NGF, BDNF, NT-3, NT-4/5), *Neuropoietic Cytokines* (CNTF, IL-6), *Fibroblast Growth Factors* (aFGF, bFGF, FGF-5, FGF-6), *Insuline Gene Family* (ITF-I, IGF-II, insulin) and *Others* (LIF, EGF, TGFα, TGFβ, CDNF). The prototype for a trophic factor, the Nerve Growth Factor (NGF), binds to its receptors, is internalized in vesicles and then transported, by retrograde axonal transport, to the cell body, where it exerts its action.

Neurotropic factors influence the axonal growth direction by exerting an attraction at a distance (''neurotropism''). These factors, delivered by the distal nerve segment, create a concentration gradient. It is not strictly correct to separate ''trophic'' and ''tropic factors'' completely, and it has been suggested to use the terms ''trophic'' and ''tropic influence'': factors secreted by non-neuronal cells in a distal nerve segment after an injury which normally have a trophic influence that may act like tropic factors, thereby exerting an attraction at a distance, influencing also the axonal growth direction.

Neurite promoting factors (*NPF*) are substances that axons like to grow on, promoting the growth cone formation. *Laminin* and *fibronectin* are examples of substances included in the extracellular matrix wile *N-CAM* and *L1* are examples of cell surface molecules providing adequate adhesions for the advancing sprouts.

The better understanding of these biological factors involved in the nerve regeneration process guided researchers in their efforts to improve nerve repair. Indeed, much has been done to overcome problems connected with the correct orientation of fascicles not only in direct sutures but also as to nerve repair in the case of loss of nerve substance. These two problems have both been faced by means of the so-called *tubulization techniques* or *conduit repair.*

The tubulization rationale represents a biological approach to a nerve injury, in which the role of the surgeon is limited and special emphasis is given to the role of intrinsic healing capacities of the nerve tissue itself.

To solve the problem of *misdirection* of the regenerating fibres leading to inappropriate distal reinnervation Lundborg suggested to encase both ends of a transected nerve in a silicon tube, leaving a short gap in between (3–4 mm), allowing the accumulation of these biological factors inside the tube. The early results from a prospective, randomised, clinical study showed that tube repair gives at least as good prerequisites for recovery of nerve function as conventional repair technique [9].

Many biological and synthetic materials have been tested to bridge a peripheral loss of substance: arteries, veins, mesothelial chambers, predegenerated or fresh skeletal muscle, empty artificial tubes, resorbable or not, tubes filled with growth factors and/or Schwann cells. Unfortunately, all of these ''tubes'' are useful for short distances only. In particular, vein or other empty tubes collapse in gaps over 1–2 cm and axon loss may occur in muscle grafts. Therefore, the major limitation of tubulization grafting techniques is the fact that they can be used only for short distances (1–2 cm).

Biologic conduits

Since 1993, we have carried out some experimental and clinical trials on the use of ''tubes'' made from a *vein filled with fresh skeletal muscle* (Fig. 2a–c). This *biological tubulization* combines two elements that have been individually previously shown to have limitations in their application for nerve repair. The vein guides regeneration and the muscle prevents vein collapse.

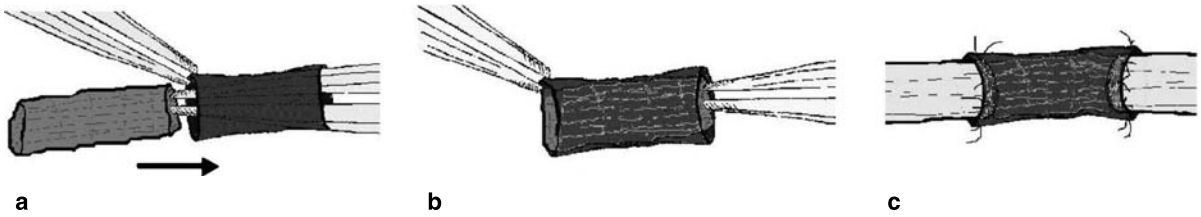

Fig. 2. Combined muscle-in-vein conduit; (a, b) the muscle is pulled inside the vein by a small forceps previously inserted into the vein itself; (c) nerve stumps are inserted 2–3 mm into the vein

Moreover, the muscle provides an adequate ''adhesion'' for the advancing sprouts by means of neurite promoting factors present in its basal lamina (laminin and fibronectin) mimicking the Schwann cell adhesion role. Many studies have been carried out on the possibility to use the muscle basal lamina as scaffold for nerve regeneration [13]. Moreover, extracts of fresh skeletal muscle have been shown to increase neurite outgrowth [38]. We demonstrated that vein conduits, filled with fresh skeletal muscle, provide morphological results in rats similar to traditional nerve grafts, in cases with a substance loss of up to 3 cm [5]. In our experimental works these biological conduits show good nerve regeneration and regrowing axons are organized in minifascicles of various dimension. The observed persistence of some muscle fibres inside the vein grafts at the end of the regeneration process does not appear to prevent a good morphological pattern of nerve regeneration in the distal stump. Distal nerve stumps are rich in both myelinated and unmyelinated nerve fibers. The morphological appearance of regenerated nerve fibres, as well as quantitative data on their number and size is similar to those previously reported for direct nerve repair in the rat. The role of Schwann cells in the regeneration process deserves a particular mention. In the lesioned nerve they are essential in axonal regeneration because they synthesise surface cell adhesion molecules, elaborate basement membrane rich in laminin and fibronectin and produce neurotrophic factors forming an environment that is particularly conducive to axonal regrowth. Migratory Schwann cells colonize the muscle-in-vein graft early [10] and reproduce inside it a situation that mimes what happens in the transected injured nerve. Even at very early postoperative stages, we found in these conduits an environment rich in neurotrophic factors that are suitable to promote nerve fibre regeneration. The production of these factors seems to be sustained by these early migrating Schwann cells [34]. We demonstrated that the Schwann cells not only migrate inside these conduits but they also proliferate inside them [11]. Then, the need for Schwann cells seems to be the critical point for long-distance tubulizations. Anyway, the good environment given by the muscle inside the vein may explain the good functional results given by these conduits in front of those reported with other kinds of artificial or biological conduits. Moreover, it was demonstrated that using the muscle-in-vein combined graft regenerating axons are able to correctly grow and orientate under the guide of the neurotropic lure exerted by the distal nerve stump [42].

The surgical technique for muscle-in-vein conduit harvesting is easy: the vein and the muscle may be found in the same region of the nerve lesion avoiding new incisions. A vein of the same length of the nerve gap and of the diameter of the lesioned nerve is harvested: it is then filled with a piece of muscle with its fibres longitudinally oriented. The muscle is pulled inside the vein by a small forceps previously inserted into the vein itself (Fig. 2).

Then, this biologic conduit is positioned in the nerve gap and the two nerve stumps are introduced for few millimetres inside the vein and sutured to the vein wall.

We have applied this technique in more than 40 cases since 1993 with good functional results for both sensory and mixed nerves (Fig. 3) up to 5–6 cm [6]. We reviewed our cases according to international criteria (evaluation by British Medical Research Council Criteria and classification of the results by Sakellarides system) and we had good results not only for sensory nerves repairs (as described even with other kind of tubes or conduits) but also for mixed or pure motor nerves: we reported up to 85% of good results with a minimum follow-up of 14 months.

The advantages of this surgical technique are several: 1) all graft material (vein and muscle) is easily withdrawn in the lesion area and therefore it is not necessary to perform any new incision in other sites; 2) the possibility of reconstructing nerve gaps up to 6 cm is given avoiding secondary damage created by the withdrawal of healthy nerves as autografts; 3) the possibility for spontaneous orientation of regenerating nerve fibres inside the tube is offered as fibres are allowed to search for their final target in response to chemical signals coming from the distal nerve segment (chemiotropism).

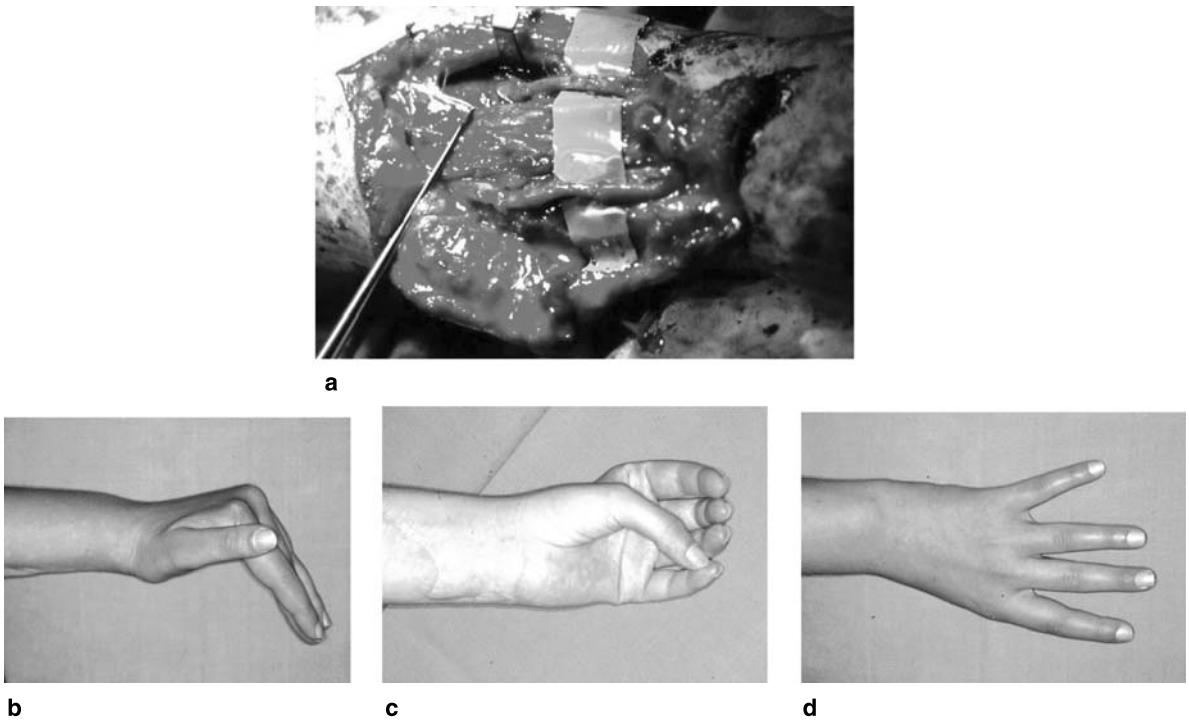

Fig. 3. Muscle-in-vein combined tube on median and ulnar nerve (4 cm lesion); (a) intraoperative; (b–d) clinical results 6 months later

The support of the basal lamina, and chemiotrophic and chemiotropic substances originating from the distal stump, do indeed reach the regenerating axons and correctly guide them to their final target tissue. This may well explain our good clinical results, whilst traditional nerve grafting techniques sometimes forces the orientation of regenerating axons.

Synthetic conduits

Regarding synthetic tubulation the first studies on rats were carried on by Seckel in 1984, using polimeric synthetic bioabsorbable tubes.

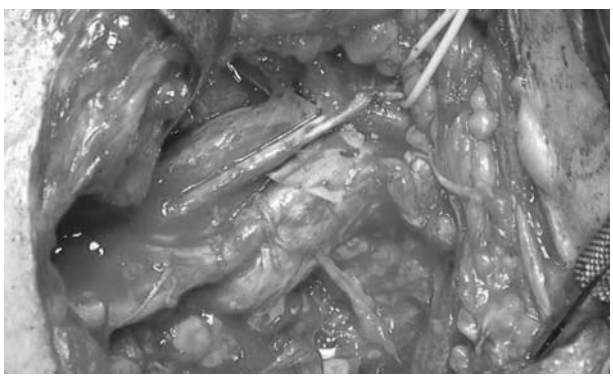

Fig. 4. End-to-side neurorraphy (grafts to the musculocutaneous nerve from the phrenic nerve)

In 1988 Dellon and Mackinnon report their experimental results in baboons, using bioabsorbable polyglycolic acid nerve conduit.

In 1990 the same authors report their results in 15 patients with digital nerve lesions.

In 1991 Lundborg reports his results in treating with a silicone tube an ulnar nerve lesion and in 1994 with the same technique 2 median nerve lesions. Lundborg doesn't use the silicone tube in nerve lesions with a loss of substance, but in cases in which a normal suture could be used. He leaves a gap to demonstrate his theory: when leaving a chamber, inside it all neurobiological factors will facilitate axonal regeneration and orientation.

We have some experience in nerve reconstruction with bioabsorbable polyglycolic acid tubes (neurotube® Neuroregen L.L.C.-acido poliglicolico). The neurotube is a woven polyglycolic acid tube and presents technical and biological features that makes it an optimal synthetic tube.

Its permeable: this permits the passage of nutritional factors and oxygen, basical for nerve regeneration and most of all for production of the neurotrophic and neurotopic factors.

Its bioabsorbable: the tube is completely absorbed by hydrolysis over a 6 months period. In animal studies Dellon and Mackinnon demonstrate in 1988 the complete absorbation and its substitution with a pseudo-nerve tissue after a one year period from implantation [26].

Table 1. *Case series June 1998–December 2002*

Age	Nerve grafted	Level	Gap (cm)	Interval from injury to grafting (months)	Follow-up (months)	Recovery	Results	
							Weber	Q-DASH
36 CA m	digital	P1	1.5	14	74	S3+	VG [7]	5.83
67 CG f	digital	hand	2	4	71	S3+	E [5]	8.33
53 CD m	digital	hand	4	primary	56	S3+	E [4]	9.16
47 BA m	digital	P1	2	primary	40	S3+	VG [10]	7.5
15 AI f	digital	P1	1.5	primary	42	S3+	VG [8]	8.33
40 DM f	digital	P1	2	primary	35	S4	VG [5]	4.6
23 MF m	digital	P1	1	1	46	S3+	VG [7]	4.6
20 LA m	digital	P1	2	5	26	S3+	VG [7]	2.5
27 RA m	digital	P1	3	1	24	S3+	VG [8]	2.5
44 CG f	digital	P1	1	7	20	S3	G [12]	17.5
42 LP f	digital	P1	1	2	17	S3	G [9]	1.67
42 DC m*	digital	P1	3	18	17	S2	P [15] crush inj	20.83
42 DC m*	digital	hand	2	18	17	S2	P [15] crush inj	20.83
42 DC m*	digital	hand	4	18	17	S2+	G [4] crush inj	20.83
30 ID m	digital	P1	1.5	primary	16	S3+	VG [8]	4.16
66 ZG m	digital	hand	1	16	14	S4	E [4]	2.5
63 SL f	digital	P1	2.5	primary	14	S3+	VG [13]	16.67
36 VD m	digital	P1	2	primary	12	S3	G [12]	10
30 CM m	digital	P1	1.5	primary	6	S3+	VG [11]	8.33

* Same patient.

The external corrugation of the tube prevents its collapse under normal physiological soft tissue pressure and this permits its use in anatomical sites where external pressure of tissue is quite high. This happens at the digital level, where other tubulation techniques are difficult to use for this reason (digital sites). The neurotube is a synthetic tube ready to use, also in emergency. There are precise indications: it can be used in digital and palmar nerve lesions with gap less than 3 cm [25]. There are also negative aspects in using neurotube: high costs and possibility of extrusion.

In 1990 Mackinnon and Dellon [28] reports 1 patient who presented extrusion of the tube after a tenolysis during the consequent early mobilisation program. Other 3 cases are reported by Weber *et al.* [47] in patients who presented skin necrosis and infection.

From June 1998 to December 2002, a total of 17 patients were treated in our department for digital and palmar nerve injury with a nerve gap from 0.5 to 3 cm. In all of them we used a polyglycolic acid conduit to repair the nerve gap. One of the patients had a double injury in 2 different fingers of the same hand. We used 3 tubes for the 2 fingers, so the total cases are 19. Eight patients were treated immediately in emergency, while the others after a period of 1 to 16 months.

We evaluated our patients by means of Highet modified Mackinnon-Dellon scale and by Dellon MTP testing. We also classified our patients according to Sakellarides parameters in three groups: very good (S3 + S4), good (S2 + S3), poor (S0 S1 S1 + S2). We also considered patients satisfaction according to pain disappearance and patients personal evaluation with Quick-DASH questionnaire (Table 1).

The follow-up was between 6 and 74 months.

Thirteen patients had an excellent or very good recovery, whilst 3 of them had good results.

In only one patient we had a poor result. The patient had a crush injury and we used 2 tubes for the index and one tube for the middle finger. He also presented open fractures of the phalanxes and tendon injury (very complex lesion). Anyway he is satisfied for the resolution of pain.

We had no cases of extrusion of the polyglycolic acid tube.

Part of this series was compared with biologic conduit repairs showing similar results [36].

End-to side neurorraphy

Sometimes, trauma may create an *avulsion injury* of the peripheral nerve by means of traction – elongation forces: the nerve may be avulsed at its origin from the spinal chord or when it comes into contact with the final target (muscle or sensory receptors). These lesions cannot be repaired by means of sutures or grafts because there are not two nerve stumps to be faced.

In case of *root avulsion* Carlstedt recently proposed the reimplantation of the roots into the spinal chord [8]. For the moment this is an experimental technique: it has

been utilized by Carlstedt only in selected cases and the evaluation of the results is still in progress. Several authors have described the use of sound nerves to be transposed on the distal injured nerves (*nerve transfers or neurotizations*). In these type of lesions various nerves may be used for these ''neurotizations'': intercostal nerves (generally to reconstruct the musculocutaneous nerve) [32], the XI cranial nerve or the cervical plexus [1, 7], contralateral C7 root [12], some funiculi of the ulnar nerve for the musculocutaneous nerve [33]. We personally reported a neurotization technique that is not used for avulsion injuries but rather to improve the results of very proximal ulnar nerve lesions [4]. We use two terminal branches of the median nerve (the thenar sensory branch and the motor branch for the pronator quadratus muscle) to neurotize the ulnar nerve at the wrist thus obtaining a distal, topographical reconstruction with faster recovery.

Recently, a new technique of nerve repair has been described to solve avulsion injuries or when the proximal cut nerve stump is not available for traditional end-to-end repair: the ''end-to-side'' suture. The coaptation of a distal severed nerve stump laterally to a sound neighboring nerve gave good nerve regeneration in several experimental works [44], and some clinical reports [22] seem to suggest a promising role for this technique in special selected cases.

The renewed interest in termino-lateral (end-to-side) neurorrhaphy (TLN) since its reintroduction by Viterbo *et al.* [45] in 1991, has resulted in many studies aimed at elucidating the potentiality and limits of this surgical approach to nerve repair. One of the main points in favor of TLN, in comparison to other nerve repair methods, is represented by the possibility to recover the function of a damaged nerve without loosing the function of a donor nerve. However, despite the body of experimental data supporting the effectiveness of nerve regeneration in TLN, few studies have reported the clinical employment of this surgical technique so far, and therefore scientific data on its clinical efficacy are poor and contrasting. One of the possible explanations of the caution of surgeons is that, so far, data in favor of the recovery of the voluntary control of the motor function of a nerve restored by TLN are very poor. It has been demonstrated that TLN leads to partial recovery of the function of the repaired nerve [16, 24, 30, 41, 49, 50]. Kallianen *et al.* [14] have even find out that there is no statistical significant difference in the mechanical function of skeletal muscles reinnervated by TLN versus end-to-end neurorrhaphy. A still unanswered question is: can functional reinnervation obtained by TLN become independent from the functional activity of the donor nerve? In other words, will the two functions controlled by the same donor nerve eventually be under a separate voluntary control or will they remain reciprocally bound? Furthermore, the side effects of TLN on the donor nerve have not been extensively studied yet.

Another area of controversy has been the source of regenerating axons to the distal nerve after TLN. The possibilities are: (1) invasion from donor nerve axons that were damaged during nerve preparation, or (2) from collateral (nodal) sprouting from the TNL site. In fact, some authors [37] distinguish between end-to-side coaptations in which the surgeon deliberately or involuntary transects donor nerve axons (the majority of cases according to Rovak) from coaptations in which the surgeon attempts to leave the donor nerve axons intact. True collateral sprouting has been confirmed in elegant double-labelling studies [3, 23, 50]. Collateral sprouting to the recipient nerve occurs from the nodes of Ranvier [2]. Normal, uninjured sensory axons spontaneously sprout de novo, but motor axons may need to be injured to sprout [39, 40]. Matsumoto *et al.* [29] demonstrated that invasion of the Schwann cells from the distal segment into the epineurium of the donor nerve is critical in initiating collateral sprouting from intact axons.

An important theorical and technical point is the necessity to create an epineurial window when performing an end-to-side repair. Epineurium may constitute a barrier to a good axonal sprouting, but leaving it intact may avoid involuntary lesions to the sound donor nerve. Experimental data have shown that TLN leads to reinnervation of the peripheral territories belonging to the severed nerve, also without an epineural window [21, 24, 48]. Whilst, Rovak's review states that axon damage occurs with any degree of invasiveness, and TLN efficacy mirrors the spectrum of invasiveness; therefore the amount of damage inflicted upon the donor nerve at the time of coaptation is probably the most important factor in determining the success of a TLN repair.

To really understand the clinical effectiveness of end-to-side repairs we also did an experimental research on rats [35] aiming at demonstrating the residual damage in the donor nerve and the real functional recovery by means of functional tests. In this study, the severed rat median nerve was sutured in an end-to-side fashion to the intact ulnar nerve. The progression of recovery of the flexion of the fingers was assessed by means of the grasping test. Seven months after surgery, the animals were sacrificed and morphological and morphometrical

analysis was performed on the regenerated median nerve and on the donor ulnar nerve. Results of the functional assessment showed that voluntary motor control of the muscles innervated by the median nerve was partially and progressively recovered by termino-lateral neurorrhaphy with a mean strength in the flexion of the fingers that reached about 20% of the normal before sacrifice. Morphological and morphometrical analysis showed that nerve fiber regeneration occurred in all repaired median nerves. Signs of nerve fiber atrophy were detected in the ulnar nerve distally to the point of suture suggesting the possible occurrence of a secondary damage to the donor nerve after termino-lateral neurorrhaphy that should be taken into consideration in the clinical perspective.

We recently presented also our clinical experience: we used this technique in selected cases of brachial plexus lesions [9] alone or together with other traditional repairs: 80% were considered fair or poor results. Then, the majority of poor results let us think that end-to-side repairs may lead to some clinical recovery but it is often unpredictable. In our hands this could be due to incorrect indications (TLN performed on mixed nerves) or to technical mistakes.

We also performed 6 TLN repairs for sensory nerves lesions (mainly digital nerves lesions repaired by suture of the damaged distal nerve stump to the other sound collateral digital nerve) with a good percentage of sensory recovery (5 cases): S3+.

Then, the use of end-to-side neurorrhaphy in the clinical setting for motor recovery remains controversial. Motor reconstruction in the absence of available proximal nerve is best handled by nerve-to-nerve transfers (neurotizations). We use end-to-side neurorrhaphy in sensory nerve reconstructions (especially digital nerves) in cases in which the distal nerve ends would remain without a source of proximal neurons. For motor repairs we suggest to limit this technique to well selected cases in which no other reconstruction is possible.

References

1. Allieu Y, Privat JM, Bonnel F (1984) Paralysis of the brachial plexus. Neurotization by the spinal accessory nerve. Clin Plastic Surg 11: 133–137
2. Al-Qattan M, Al-Thunayan A (1998) Variables affecting axonal regeneration following end-to-side neurorrhaphy. Br J Plast Surg 51: 238–242
3. Andreopoulos E, Skoulis TG, Luizzi F *et al* (1998) Double-labelling technique to trace axonal sprouting after end-to-side neurorrhaphy [abstract]. J Reconst Microsurg 14: 591–599
4. Battiston B, Lanzetta M (1999) Reconstruction of high ulnar nerve lesions by distal double median to ulnar nerve transfer. J Hand Surg [Am] 24: 1185–1189
5. Battiston B, Tos P, Cushway T, Geuna S (2000) Nerve repair by means of vein filled with muscle grafts. I. Clinical results. Microsurgery 20: 32–36
6. Battiston B, Tos P, Geuna S *et al* (2000) Nerve repair by means of vein filled with muscle grafts. II. Morphological analysis of regeneration. Microsurgery 20: 37–41
7. Brunelli G, Monini L (1984) Neurotization of avulsed roots of brachial plexus by means of anterior nerves of cervical plexus. Clin Plastic Surg 11: 149–153
8. Carlstedt T (1995) Spinal nerve root injuries in brachial plexus lesions: basic science and clinical application of new surgical strategies. Microsurgery 16: 13–16
9. Felici N, Del Bene M, Battiston B, Amadei F (2003) Functional results of end-to-side nerve anastomosis in 39 consecutive patients, Abstracts Volume Second Congress of the World Society for Reconstructive Microsurgery, Heidelberg, p 42
10. Fornaro M, Tos P, Geuna S, Giacobini-Robecchi MG, Battiston B (2001) Confocal imaging of Schwann-cell migration along muscle-vein combined grafts used to bridge nerve defects in the rat. Microsurgery 21: 153–155
11. Geuna S, Raimondo S, Nicolino S, Boux E, Fornaro M, Tos P, Battiston B, Perroteau I (2003) Schwann-cell proliferation in muscle-vein combined conduits for bridging rat sciatic nerve defects. J Reconstr Microsurg 19: 119–123
12. Gu YD, Chen DS, Zhang GM *et al* (1998) Long-term functional results of contralateral C7 transfer. J Reconstr Microsurg 14: 57–59
13. Ide C (1984) Nerve regeneration through the basal lamina scaffold of the skeletal muscle. Neurosci Res 1: 379–391
14. Kalliainen LK, Cederna PS, Kuzon WM (1999) Mechanical function of muscle reinnervated by end-to-side neurorrhaphy. Plast Reconstr Surg 103: 1919–1927
15. Levi-Montalcini R, Hamburger V (1951) Selective growth stimulating effects of mouse sarcoma on sensory and sympathetic nervous system of the chick embryo. J Exp Zool 116: 321–362
16. Liu K, Chen LE, Seaber AV, Goldner RV, Urbaniak JR (1999) Motor functional and morphological findings following end-to-side neurorrhaphy in the rat model. J Orthop Res 17: 293–300
17. Lundborg G (ed) (1988) Nerve injury and repair. Churchill Livingstone, Edinburgh
18. Lundborg G, Dahlin L, Danielsen N, Zhao Q (1994) Trophism, tropism and specificity in nerve regeneration. J Reconstr Microsurg 5: 345–354
19. Lundborg G, Longo FM, Varon S (1982) Nerve regeneration model and trophic factors in vivo. Brain Res 232: 157–161
20. Lundborg G, Rosen B, Dahlin L *et al* (1997) Tubular versus conventional repair of median and ulnar nerves in human forearm: early results from a prospective, randomized, clinical study. J Hand Surg [Am] 22: 99–106
21. Lundborg G, Zhao Q, Kanje M, Danielsen N (1994) Can sensory and motor collateral sprouting be induced from intact peripheral nerve by end-to-side anastomosis? J Hand Surg 19B: 277–282
22. Luo Y, Wang T, Fang H (1997) Preliminary investigation of treatment of ulnar nerve defect by end-to-side neurorrhaphy. Chung Kuo Hsiu Fu Chung Chien Wai Ko Tsa Chih 11: 338–339
23. Lutz B, Chuang D, Hsu J *et al* (1998) End-to-side neurorrhaphy: functional and double labelling study in rat upper limb. J Reconstr Microsurg 14: 590–602
24. Lutz BS, Chuang DC, Ma SF, Wei FC (2000) Selection of donor nerves – an important factor in end-to-side neurorrhaphy. Br J Plast Surg 53: 149–154
25. Mackinnon S, Dellon AL (1990) A study of nerve regeneration across synthetic (maxon) and biologic (collagen) nerve conduits for nerve gaps up to 5 cm in the primate. J Reconstr Microsurgery 6: 117–121

26. Mackinnon S, Dellon AL (eds) (1988) Nerve repair and nerve grafting. Surgery of the peripheral nerve. Thieme Medical Publishers, New York
27. MacKinnon SE (1996) Nerve allotransplantation following severe tibial nerve injury. Case report. J Neurosurg 84: 671–676
28. Mackinnon SE, Dellon AL (1990) Clinical nerve reconstruction with a bioabsorbable polyglycolic acid tube. Plast Reconstr Surg 85: 419–424
29. Matsumoto M, Hirata H, Nishiyama M *et al* (1999) Schwann cells can induce collateral sprouting from intact axons: experimental study of end-to-side neurorrhaphy using a Y-chamber model. J Reconstr Microsurg 15/4: 281–286
30. McCallister WV, Tang P, Trumble TE (1999) Is end-to-side neurorrhaphy effective? A study of axonal sprouting stimulated from intact nerves. J Reconstr Microsurg 15: 597–603
31. Millesi H (1981) Interfascicular nerve grafting. Orthopaedic Clin North Am 12: 287–301
32. Narakas A (1977) The surgical management of brachial plexus injuries. In: Daniel RK, Terzis JK (eds) Reconstructive surgery. Little, Brown, Boston
33. Oberlin C (1994) Nerve transfer to biceps muscle using a part of ulnar nerve for C5-C6 avulsion of the brachial plexus. J Hand Surg [Am] 19: 232–237
34. Pagnotta A, Tos P, Fornaro M, Gigante A, Geuna S, Battiston B (2002) Neurotrophins and their receptors in early axonal regeneration along muscle-vein-combined grafts Microsurgery 22: 300–303
35. Papalia I, Geuna S, Tos PL, Boux E, Battiston B, Stagno d'Alcontres F (2003) Morphological and functional study of rat median nerve repair by means of termino-lateral neurorrhaphy on the ulnar nerve. J Reconstr Microsurg 19(4): 257–264
36. Risitano G, Battiston B, Coppolino S, Tos P (2001) Risultati clinici sull'utilizzo della tubulizzazione biologica e sintetica nella ricostruzione dei nervi digitali della mano. Riv Chir Mano 38: 28–35
37. Rovak JM, Cederna PS, Kuzon WM Jr (2001) Terminolateral neurorrhaphy: a review of the literature. J Reconstr Microsurg 7: 615–624
38. Smith RG, Appel SH (1983) Extracts of skeletal muscle increase neurite outgrowth and cholinergic activity of fetal rat spinal motor neurons. Science 219: 1079–1081
39. Tarasidis G, Watanabe O, Mackinnon S *et al* (1997) End-to-side neurorrhaphy resulting in limited sensory axonal regeneration in a rat model. Ann Otol Rhinol Laryngol 106: 506–512
40. Tarasidis G, Watanabe O, Mackinnon S *et al* (1998) End-to-side neurorrhaphy: a long term study of neural regeneration in a rat model. Otolaryngol Head Neck Surg 119: 337–344
41. Tarasidis G, Watanabe O, Mackinnon S, Strasberg SR, Haughey BH, Hunter DA (1988) End-to-side neurorrhaphy: a long term study of neural regeneration in a rat model. Otolaringol Head Neck Surg 119: 337–341
42. Tos P, Battiston B, Geuna S *et al* (2000) Tissue specificity in rat peripheral nerve regeneration through combined skeletal muscle and vein conduit grafts. Microsurgery 20: 65–71
43. Varon S, Adler R (1981) Tropic and specifying factors directed to neuronal cells. Adv Cell Neurobiol 2: 115–163
44. Viterbo F, Trindade JC, Hoshino K, Mazzoni Neto A (1994) End-to-side neurorrhaphy with removal of the epineurial sheath: an experimental study in rats. Plast Reconstr Surg 94: 1038–1047
45. Viterbo F, Trinidade JC, Hoshino K, Mazzoni A (1992) Latero-terminal neurorrhaphy without removal of the epineural sheat: experimental study in rats. Sao Paulo Med J 110: 267–275
46. Waller A (1850) Experiments on the section of glossopharyngeal and hypoglossal nerves of the frog, and observations of the alterations produced thereby in the structure of their primitive fibers. Philos Trans R Soc London (Biol) 140: 423–429
47. Weber RA, Breidenbach WC, Brown RE, Jabaley ME, Mass DP (2000) A randomized prospective study of polyglycolic acid conduits for digital nerve reconstruction in humans. Plast Reconstr Surg 106: 1036–1045
48. Yamauchi T, Yajima Y, Tamai S *et al* (2001) Neurohistochemical analysis of regeneration in rat peripheral nerve after end-to-side neurorrhaphy. J Orthop Sci 6: 82–87
49. Yüksel F, Karacaolu E, Güler M (1999) Nerve regeneration through side-to-side neurorrhaphy sites in a rat model: a new concept in peripheral nerve surgery. Plast Reconstr Surg 104: 2092–2099
50. Zhang Z, Soucacos P, Bo J, Beris AE (1999) Evaluation of collateral sprouting after end-to-side coaptation using a fluorescent double labelling technique. Microsurgery 19: 281–286

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The beneficial effect of genetically engineered Schwann cells with enhanced motility in peripheral nerve regeneration: review

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Summary

Background. The importance of Schwann cells in promoting nerve regeneration across a conduit has been extensively reported in the literature, and Schwann cell motility has been acknowledged as a prerequisite for myelination of the peripheral nervous system during regeneration after injury.

Methods. Review of recent literature and retrospective analysis of our studies with genetically modified Schwann Cells with increased motility in order to identify the underlying mechanism of action and outline the future trends in peripheral nerve repair.

Findings. Schwann cell transduction with the pREV-retrovirus, for expression of Sialyl-Transferase-X, resulting in conferring Polysialyl-residues (PSA) on NCAM, increases their motility in-vitro and ensures nerve regeneration through silicone tubes after end-to-side neurorraphy in the rat sciatic nerve model, thus significantly promoting fiber maturation and functional outcome. An artificial nerve graft consisting of a type I collagen tube lined with the genetically modified Schwann cells with increased motility, used to bridge a defect in end-to-end fashion in the rat sciatic nerve model, was shown to promote nerve regeneration to a level equal to that of a nerve autograft.

Conclusions. The use of genetically engineered Schwann cells with enhanced motility for grafting endoneural tubes promotes axonal regeneration, by virtue of the interaction of the transplanted cells with regenerating axonal growth cones as well as via the recruitment of endogenous Schwann cells. It is envisaged that mixed populations of Schwann cells, expressing PSA and one or more trophic factors, might further enhance the regenerating and remyelinating potential of the lesioned nerves.

Keywords: Peripheral nerve regeneration; Schwann cells motility; polysialylated neural cell adhesion molecule, end-to-side nerve grafting, end-to-end nerve grafting.

Introduction: the problem

Peripheral nerve injuries are associated with considerable disability, due to loss of motor and sensory function, and represent one of the most challenging microsurgical problems. The prognosis becomes even worse in the case of a nerve defect that prohibits end-to-end neurorraphy. Interposition of a nerve graft minimizes the hazardous tension [25] but forces regenerating axons to cross two coaptation sites, with subsequent loss of fibers at each suture line. Due to the limited availability of nerve autografts and the associated donor site morbidity, new treatment modalities have been sought. Nerve tubes [4, 14, 23, 38]; and end-to-side neurorraphy [21, 27, 28, 36, 37, 40, 41]; are potentially of enormous therapeutic value. Tubes of different materials, either autologous [14, 38]; or synthetic [4, 23]; have yielded promising results in bridging short nerve gaps. However in every animal model investigated, the 3 cm nerve gap appears to be the critical distance above which a hollow conduit will not support nerve regeneration [5]. The fact that the usual clinical situation involves nerve gap greater than 3 cm, led investigators to pre-load nerve tubes with exogenous cells [12, 31]; or growth factors [17, 11]; in order to manipulate and improve the microenvironment through which axons regenerate.

End-to-side neurorraphy also presents the potential to reduce the re-innervation distance. Despite promising results from experimental studies [21, 27, 28, 36, 37, 40, 41]; further investigation is required to understand the underlying mechanism and to identify factors promoting axonal regeneration after terminolateral neurorraphy, in order to achieve constant and reproducible results prior to clinical use.

There is no doubt that axonal regeneration after nerve repair is far from perfect by all currently used methods. Consequently, our research for the development of new

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therapeutic strategies has to profit at many levels from the advances in cellular and molecular neurobiology of the peripheral nerve system, that may have important clinical impact.

Schwann cell: the biology

The behavior of Schwann cells (SCs) has been acknowledged as the key in Wallerian degeneration and subsequent regeneration for over a century [30]. After peripheral nerve injury, SCs migrate and form bridges between the severed ends, facilitating growth cone navigation, axonal re-ensheathment and elongation [39]. Schwann cells produce basal lamina components, such as laminin and collagen type IV, and secrete a variety of growth factors like nerve growth factor, fibroblast growth factor 1 and 2, insulin like growth factor 1 and 2, brain-derived growth factor, ciliary neurotrophic factor, neuregulin and neurotrophin-3. Interestingly, experimental studies demonstrated that regrowing axons do not elongate through acellular nerve grafts or if SC migration is impeded. In contrast, if the graft contains SCs, this has a positive effect both on the functional recovery and the graft's ability to bridge larger defects [13].

Schwann cell motility is considered as a prerequisite for myelination of the peripheral nervous system during regeneration after injury [24]. Several authors have focused on identifying factors capable of eliciting Schwann-cell migration. Growth factors such as β-neuregulin [24, 22], insulin-like growth factor [3] and neural cell adhesion molecule (NCAM) [33] have been identified to promote SC migration in vitro. NCAM belongs to the immunoglobulin (Ig) superfamily, displays homophilic and heterophilic binding activities and is highly expressed on the surface of axons and SCs during nervous system development and after peripheral nerve injury [32]. Its properties are strongly influenced by polysialylation, and the carbohydrate polysialic acid (PSA) attached uniquely to NCAM, through a developmentally regulated process, modulates neural cell interactions [16], promotes neurite outgrowth [6] and has been correlated with cell migration during remyelination [2, 29].

Schwann cells enhanced motility: in vitro studies

Cultured SCs were transduced with the pREV retrovirus [20] encoding for Sialyl-Transferase-X (STX), the enzyme that converts NCAM to the polysialylated form PSA-NCAM [2] as described previously [18]. The transduction efficiency was verified with immunohistochemistry for PSA. Schwann cell phenotype was assessed with a-GFAP and a-S-100 immunohistochemistry, and was found not to been affected by the transduction procedure. The migrational behavior of SC was assessed with a gap-bridging assay, that efficiently permits the assessment of effector molecules on SC migration (Fig. 1) [24]. The in vitro motility assay showed that the motility of STX-transduced SCs' was significantly higher compared to control cells throughout the observation period.

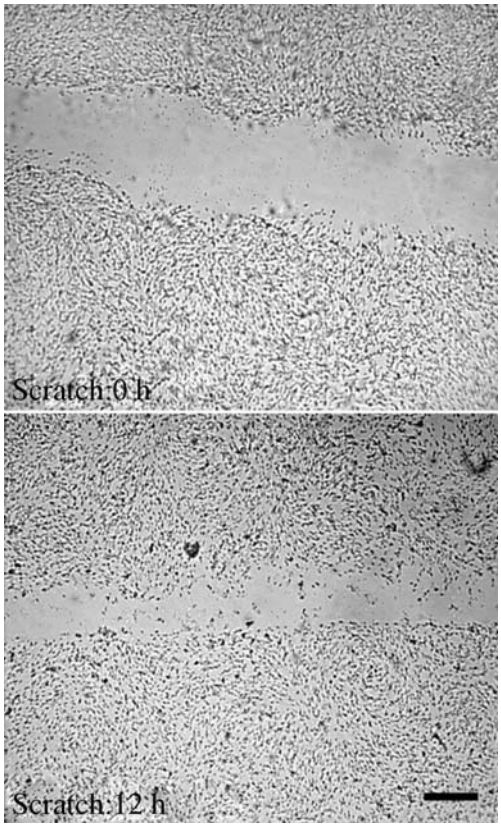

Fig. 1. STX-transduced Schwann cell migration within the scratch area. Cells were grown to confluency and a cell-free area, 1 mm in width, was generated. Micrographs represent digitized phase-contrast images obtained at zero time and 12 h after scratch formation. Note the reduction in the gap width after 12 h. Scale bar = 80 μm

Schwann cells were then used to line silicone tubes. In order to make the cells traceable, a solution of DiI in DMSO was added to a final concentration of 25 μg/ml DiI in medium and the cells were incubated for 15 min. The DiI-containing medium was washed off and the cells were detached from the dish, centrifuged and resuspended in a concentration of 12×10^5/ml in medium.

Using a Hamilton syringe, the suspension was injected into the lumen of the silicon tubes that had been pre-treated with 5 μg/ml PPL and had been left to dry. The cell suspension was left for 2 h in the incubator with no extra medium, so as to allow the cells to attach

to the tube wall. Then, medium was added to the petri dish to completely cover the tubes. For documentation of the presence of living SCs, silicone tubes were photographed using the inverted microscope and then used for transplantation. Some tubes were not transplanted but kept for up to 1 month in vitro to assess survival (Fig. 2).

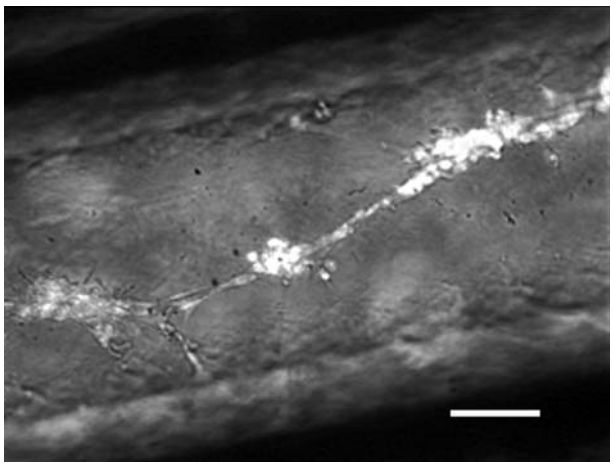

Fig. 2. Fluorescently stained Schwann cells lining the lumen of a silicone conduit, kept in vitro for 1 month after seeding. Scale bar = 40 μm

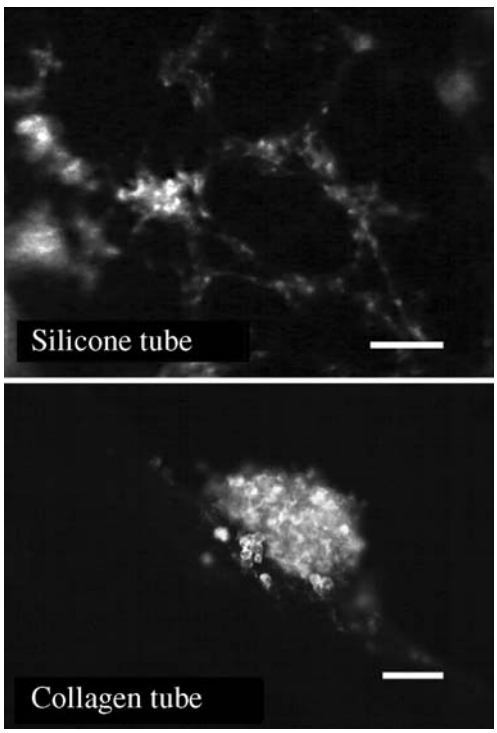

Fig. 3. Schwann cells in longtitudinal view of unsectioned silicone tube (A) and in cross section of cryosectioned collagen tube (B), that had been kept for 2 days in vitro. Immunostaining for Schwann cell marker GFAP reveals the presence of immunoreactive living Schwann cells. Scale bars = 40 μm

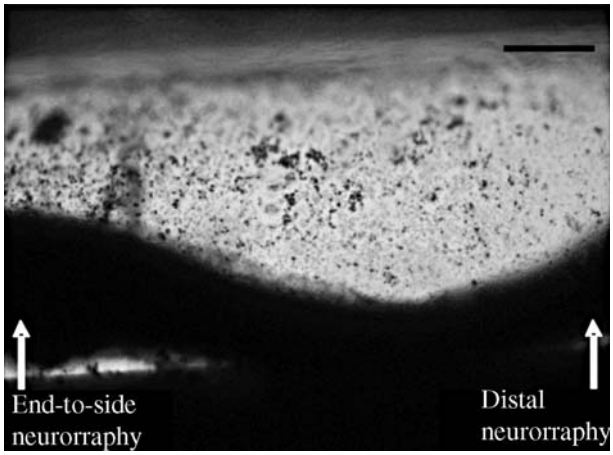

Fig. 4. Light microscopy of the silicone tubes in situ, revealed that the newly formed nerve-like structure was surrounded by a plethora of viable, stained and unstained control Schwann cells (black dots). This means that the implanted cells have survived (as the AP marker is not expressed by endogenous cells), and that endogenous cells have also been recruited in the graft. Expression of STX on Schwann cell membranes is postulated to enhance the recruitment of endogenous SCs. Scale bar = 50 μm

A similar procedure was followed to line type I collagen tubes, but in this case SCs were traced by using an Alkaline Phosphatase (AP) expressing retrovirus instead of DiI [20]. AP-tracing was only applied to control cells (Fig 4). The survival, biochemical phenotype and morphology of the SCs is not affected by their transduction by either the AP or the STX retrovirus [18], so the presence of AP-transduced cells in the tubes can be used as an index of survival for both AP- and STX- expressing SCs. Immunostaining cryosectioned collagen tubes that had been kept for 2 days in vitro for SC markers GFAP and S-100 revealed the presence of immunoreactive cells, thus demonstrating the viability of the cellular population that had been grafted (Fig. 3).

Schwann cell enhanced motility: in vivo studies

End-to-side nerve grafting

In our earliest studies we investigated nerve regeneration after end-to-side neurorraphy in the rat sciatic nerve model. A nerve graft or silicone tube was sutured terminolateral to the perineurium of the intact tibial nerve and its distal end was sutured to the distal peroneal stump [7]. A 100% regeneration rate through nerve grafts was achieved, compared to 40% through the hollow silicone tubes. Al-Qattan and Al-Thynyan [1], in the same animal model, sutured a nerve graft or silicone tube terminolateral to the perineurium of an intact nerve but its

distal end was not attached to a distal nerve stump. The authors demonstrated 50% regeneration rate through the nerve grafts, but they failed to demonstrate nerve regeneration through the silicone tubes. The findings of the two comparable studies clearly indicate the necessity of a distal nerve stump. The microenvironment of a regrowing axon is mainly composed of its ensheathing SC and the distal nerve stump is a persistent source of SCs. The cells emerging from the distal stump have the ability to migrate more than a few mm into the surrounding connective tissue [34]. The presence of a short inter-stump gap (10 mm) in this experimental model [7] seems to facilitate SC migration from the distal stump towards the growth cone, thus supporting regeneration after end-to-side neurorraphy through hollow silicone tubes in 40% of cases.

In an effort to improve these results, silicone tubes were lined with cultured Schwann cells [7]. The presence of SCs ensured significantly higher regeneration rate in silicone tubes (87%), as compared to 40% in hollow silicone tubes in the previous study, and comparable to that of nerve grafts (100%). Moreover, the presence of SCs in the lumen of the silicone tubes improved fiber maturation, as indicated by the increased fiber diameter and myelin thickness. Despite the presence of SCs into the lumen of the silicone tubes, nerve regeneration was not ensured in all cases. Thus, it was very important to identify additional parameters, which would facilitate and promote nerve regeneration after terminolateral neurorraphy.

The fact that SC motility is a prerequisite for myelination during peripheral nerve regeneration [24] stimulated our interest to make use of the in-vitro tested SCs with increased motility. Silicone tubes were lined with STX-transduced SC and were used for end-to-side nerve grafting, in the same animal model. Regeneration was achieved in all cases, indicating that this reconstruction method, as well as nerve grafting, ensures nerve regeneration after end-to-side neurorraphy [8]. The presence of STX-transduced SC into the lumen of the silicone tubes improved regenerated fiber maturation, as indicated by the significantly higher fiber diameter and myelin thickness, compared to all other reconstructive methods used. Most importantly, walking track analysis demonstrated that the functional outcome was significantly better [8].

End-to-end nerve grafting

These initial promising results prompted us to use STX-transduced SC in end-to-end nerve grafting, this time in a bio-degradable artificial tube. Semipermeable collagen matrix has been proved to permit nutrient exchange and accessibility of neurotrophic factors to the axonal growth zone during regeneration [19, 15]. The clinical application of type I collagen tubes in bridging short nerve gaps, motivated our decision to use collagen type I tubes as the matrix to line the cultured SCs. Our in-vitro studies demonstrated that SCs remained attached and viable to the wall of the collage tubes indicating the appropriateness of this artificial nerve graft as a potential nerve guiding matrix. Therefore we designed a new artificial nerve graft consisting of a type I collagen tube lined with STX-transduced SC with increased motility [9], and we investigated its efficacy in bridging nerve gaps in end-to-end fashion in our established rat sciatic nerve model [10, 11].

The results of the in-vivo study confirmed the biocompatibility and long-term stability of type I collagen tubes, and most importantly demonstrated that the biodegradation of the tubes was slowly enough to maintain a stable support structure for extended regeneration processes.

The different reconstructive methods (nerve graft, acellular collagen tube, collagen tube lined with SCs and collagen tube lined with STX-transduced SCs) did not significantly influence the number of regenerated fibers, among the different experimental groups, but resulted in significant differences in fiber diameter and myelin thickness. The presence of SCs (non-transduced) in the lumen of the collagen tubes improved both fiber diameter and myelin thickness compared to acellular collagen tubes. The presence of STX-transduced SCs further improved fiber diameter and myelin thickness, and achieved results comparable to that of nerve autografts. Motor function, as measured by the walking pattern test, was superior in animals treated with collagen tubes lined with STX-transduced SCs and nerve grafts compared to acellular collagen tubes and tubes lined with non-transduced SCs. This finding was verified by the faster recovery of the compound muscle action potential in the group treated with STX-transduced SCs, which reached the normal value at 8 weeks postoperatively.

The better histological-morphometric results seen in the collagen tubes lined with STX-transduced SCs were matched by significantly better functional outcome over acellular collagen tubes and SCs lined collagen tubes. Most importantly, collagen tubes lined with genetically modified SCs with increased motility achieved qualitatively equivalent nerve regeneration with the gold standard of nerve autografts.

Underlying mechanism of action of genetically modified Schwann cells in peripheral nerve regeneration

The superior results seen with STX-transduced SC are most likely attributable to their migration behavior. Three lines of evidence point to this conclusion: first, there is strong in vitro evidence of the SC viability into the lumen of both silicon and collagen tubes. Second, the phenotype of the transduced cells does not seem to be influenced in any other way, as assessed by morphology and a-GFAP and a-S100 immunocytochemistry. Third, there is bibliographical evidence that SC motility is a prerequisite for myelination of the peripheral nervous system during regeneration after injury [1].

Understanding the mechanism underlying peripheral nerve regeneration is the basis for adequate design of novel therapeutic strategies for the treatment of injured peripheral nerves. Wallerian degeneration occurs in the distal nerve segment (secondary degeneration) and a few nodes of the proximal nerve end (primary degeneration). Axonal sprouting begins within 96 hours and proliferating SCs support and myelinate the injured axons. Experiments with autologous venous grafts used to bridge 10 mm gap in the sciatic nerve model showed that axonal advancement into the conduit consistently lags behind SC migration, scaffold formation, and bridging of the nerve gap [35].

Taking the above into account, the overall improvement of nerve regeneration by STX-transduced SC transplantation can be attributed to two events. First, transplanted STX-transduced SCs constitute a favorable, more permissive environment for advancing axons [39]. Second, transplanted SCs recruit endogenous SCs as well. In Fig. 4 the newly formed nerve-like structure in the lumen of the silicone conduit is surrounded by stained and unstained cells. This means that the implanted cells have survived (as the AP marker is not expressed by endogenous cells), and that endogenous cells have also been recruited in the graft, thus supporting the regeneration process. The enhanced motility of STX-transduced cells is likely to cause them to have increased interactions with native cells and thus accelerate the recruitment process.

The future: how to improve the results

The collagen type I tube lined with SCs with enhanced motility is a promising artificial nerve graft [9], but it requires further investigation in a primate animal model in order to bridge longer nerve gaps, before proceeding to clinical application.

Moreover, we currently investigate the feasibility of transplantating STX-tranduced SCs to enhance motor end-plate reinnervation after direct nerve-to-muscle neurotization, with encouraging results.

Professor Millesi recently raised the question ‘‘How to improve the results of peripheral nerve surgery?’’ [26]. There is strong evidence that all efforts should be made towards the engagement of microneural surgery with molecular research. It is envisaged that mixed populations of SCs, expressing PSA and one or more trophic factors, might further enhance the regenerating and remyelinating potential of lesioned nerves.

References

1. Al-Qattan MM, Al-Thynan A (1998) Variables affecting axonal regeneration following end-to-side neurorraphy. Br J Plast Surg 51: 238–242
2. Angata K, Fukuda M (2003) Polysialytransferase major players in polysialic acid synthesis on the neural cell adhesion molecule. Biochimie 85(1–2): 195–206
3. Cheng HL, Steinway ML, Russell JW, Feldman EL (2000) GTPases and phosphatidylinositol 3-kinase are critical for insulin-like growth factor-I-mediated Schwann cell motility. J Biol Chem 275(35): 27197–27204
4. Cuadros CL, Granatir CE (1987) Nerve regeneration through a synthetic microporus tube (Expanded Polytetrafluorothylene): experimental study in the sciatic nerve of the rat. Microsurgery 8: 41–46
5. Doolabh VB, Hertl MC, Mackinnon SE (1996) The role of conduits in nerve repair: a review. Rev Neurosci 7: 47–84
6. Franceschini I, Angata K, Ong E, Hong E, Doherty P, Fukuda M (2001) Polysialytransferase ST8SiaII (STX) polysialylates all of the major isoforms of NCAM and facilitates neurite outgrowth. Glycobiology 11(3): 231–239
7. Gravvanis AI, Karvelas M, Lykoudis E, Lavdas A, Papalois A, Patralexis C, Matsas R, Stamatopoulos C, Ioannovich J (2003) The use of silicone tubes in end-to-side nerve grafting. An experimental study. Eur J Plast Surg 26: 111–115
8. Gravvanis AI, E, Lavdas A, Papalois A, Franceschini I, Tsoutsos D, Dubois-Dalcq M, Matsas R, Ioannovich J (2005) The effect of Genetically-modified Schwann cells with increased motility in end-to-side nerve grafting. Microsurgery 25: 423–432
9. Gravvanis, A. Lavdas, A. Papalois, D. Tsoutsos, P. Panayotou, D. Chuang, M. Dubois-Dalcq, R. Matsas (2005) Collagen tube lined with genetically modified Schwann cells with increased motility: a new promising bioartificial nerve graft. Eur Surg 37(4): 204–212
10. Gravvanis AI, Lykoudis EG, Tagaris GA, Patralexis CG, Papalois AE, Panayotou PN, Stamatopoulos CN, Ioannovich JD (2002) Microsurgical repair of nerve lesions with nerve grafts: the effect of nerve growth factor 7S. Eur J Plast Surg 25: 187–192
11. Gravvanis AI, Tsoutsos, DA, Tagaris GA, Papalois A, Patralexis C, Iconomou T, Panayotou P, Ioannovich J (2004) The beneficial effect of nerve growth factor 7S on peripheral nerve regeneration through inside-out vein grafts: an experimental study. Microsurgery 24(5): 408–415
12. Hadlock TA, Sundback CA, Hunter DA, Vacanti JP, Cheney ML (2001) A new artificial nerve graft containing rolled Schwann cell monolayers. Microsurgery 21: 96–101
13. Hall SM (1986) Regeneration in cellular and acellular autografts in the peripheral nervous system. Neuropathol Appl Neurobiol 12: 27–46

14. Itoh S, Shinomiya K, Samejima H, Ohta T, Ishizuki M, Ichinose S (1996) Experimental study of nerve regeneration through the basement membrane tubes of the nerve, muscle, and artery. Microsurgery 17: 525–534
15. Keilhoff G, Stang F, Wolf G, Fansa H (2003) Bio-compatibility of type I/III collagen matrix for peripheral nerve reconstruction. Biomaterials 24(16): 2779–2787
16. Kiss JZ, Rougon G (1997) Cell biology of polysialic acid. Curr Opin Neurobiol 7(5): 640–646
17. Laquerriere A, Peulve P, Jin O, Tiollier J, Tardy M, Vaudry H, Hemet J, Tadie M (1994) Effect of basic fibroblast growth factor and α-melanocytic stimulating hormone on nerve regeneration through a collagen channel. Microsurgery 15: 203–210
18. Lavdas AA, Franceschini I, Dubois-Dalcq M, Matsas R (2006) Schwann cells genetically engineered to express PSA show enhanced migratory potential without impairment of their myelinating ability. Glia (in press)
19. Li ST, Archibald SJ, Krarup C, Madison R (1992) Peripheral nerve repair with collagen conduits. Clin Mat 9: 195–200
20. Lopez-Lastra, M, Gabus C, Darlix JL (1997) Characterization of an internal ribosomal entry segment within the 5′ leader of avian reticuloendotheliosis virus type A RNA and development of novel MLV-REV-based retroviralvectors. Hum Gene Ther 8(16): 1855–1865
21. Lundborg G, Zhao Q, Kanje M, Danielsen N, Kerns JM (1994) Can sensory and motor collateral sprouting be induced from intact peripheral nerve by end-to-side anastomosis? J Hand Surg 19B: 277–282
22. Mahanthappa NK, Anton ES, Matthew WD (1996) Glial growth factor 2, a soluble neuregulin, directly increases Schwann cell motility and indirectly promotes neurite outgrowth. J Neurosci 16(15): 4673–4683
23. Meek MF, Dijkstra JR, Den Dunnen WFA, Ijkema-Paassen J, Schakenraad JM, Gramsbergen A, Robinson PH (1999) Functional assessment of sciatic nerve reconstruction: Biodegradable Poly (DlLA-ε-CL) nerve guides versus autologus nerve grafts. Microsurgery 19: 381–388
24. Meintanis S, Jessen KR, Mirsky R, Matsas R (2001) The neuron-glia signal β-neuregulin promotes Schwann cell motility via the MARK Pathway. Glia 39(1): 39–51
25. Millesi H (1980) Nerve grafts: indications, techniques and prognosis. In: Omer G, Spinner M (eds) Management of peripheral nerve problems. Saunders, Philadelphia, pp 410–430
26. Millesi H (2005) Editorial. Eur Surg 37(4): 185–186
27. Noah EM, Williams A, Jorgenson C, Skoulis TG, Terzis JK (1997) End-to-side neurorraphy: a histologic and morphometric study of axonal sprouting into an end-to-side nerve graft. J Reconstr Microsurg 13(2): 99–106
28. Okajina S, Terzis JK (2000) Ultrastructure of early axonal regeneration in end-to-side neurorraphy model. J Reconstr Microsurg 16(4): 313–323
29. Oumesmar BN, Vignais L, Duhamel-Clerin E, Avellana-Adalid V, Rougon G, Baron-Van Evercooren A (1995) Expression of the highly polysialylated neural cell adhesion molecule during postnatal myelination and following chemical demyelination of the adult spinal mouse spinal cord. Eur J Neurosci 7: 480–491
30. Ramon y Cajal, S (1928) Degeneration and regeneration of the nervous system. University Press, Oxford, London, Vol 1
31. Shen ZL, Berger A, Hierner R, Allmeling C, Ungewickell E, Walter GF (2001) A Schwann cell-seeded intrinsic framework and its satisfactory biocompatibility for a bioartificial nerve graft. Microsurgery 21: 6–11
32. Storms SD, Rostihauser U (1998) A role for polysialic acid in neural cell adhesion molecule heterophilic binding to proteoglycans. J Biol Chem 273: 27124–21129
33. Thomaidou D, Coquillat D, Meintanis S, Noda M, Rougon G, Matsas R (2001) Soluble forms of NCAM and F3 neuronal cell adhesion molecules promote Schwann cell migration: identification of protein tyrosine phosphatases zeta/beta as the putative F3 receptors on Schwann cells. J Neurochem 78(4): 767–778
34. Torigoe K, Tanaka H-F, Takahashi A, Awaya A, Hashimoto K (1996) Basic behavior of migratory Schwann cells in peripheral nerve regeneration. Exp Neurol 137: 301–308
35. Tseng CY, Hu G, Ambron RT, Chiu DTW (2003) Histologic analysis of Schwann cell migration and peripheral nerve regeneration in the autogenous venous nerve conduit (AVNC). J Reconstr Microsurg 19(5): 331–339
36. Viterbo F, Trinidade JC, Hoshimo K, Mazzoni Neto A (1992) Latero-terminal neurorraphy without removal of the epineurial sheath: experimental study in rats. Sao Paulo Med J 110(6): 267–275
37. Viterbo F, Trinidade JC, Hoshimo K, Mazzoni Neto A (1994) End-to-side neurorraphy with removal of the epineurial sheath: an experimental study in rats. Plast Reconstr Surg (7): 1038–1047
38. Wang KK, Costas PD, Bryan DJ, Jones DS, Seckel BR (1993) Inside-out vein graft promotes nerve regeneration in rats. Microsurgery 14: 608–618
39. Williams LR, Longo FM, Powell HC, Lundborg G, Varon S (1983) Spatial-temporal progress of peripheral nerve regeneration within a silicone chamber: parameters for a bioassay. J Comp Neurol 218: 460–470
40. Zhang Z, Soucacos PN, Bo J, Beris AE, Malizos KN, Ioachim E, Agnantis NJ (2001) Reinnervation after end-to-side nerve coaptation in a rat model. Am J Orthop 30(5): 400–406
41. Zhao JZ, Chen ZW, Chen TY (1997) Nerve regeneration after terminolateral neurorraphy: experimental study in rats. J Reconstr Microsurg 13: 31–37

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Schwann cells, acutely dissociated from a predegenerated nerve trunk, can be applied into a matrix used to bridge nerve defects in rats

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Summary

Background. The gold standard to reconstruct a nerve defect is a conventional autologous nerve graft. There may be a lack of such grafts in severe nerve injuries. Alternatives to autologous nerve grafts are needed.

Methods. We have developed a technique where mainly Schwann cells are acutely dissociated from the ends of the severed nerve trunk after nerve injury. The technique does not require long-term cell culture procedures. The obtained cells, which can be dissociated within a few hours, are applied to a silicone tube or a tendon autograft used to bridge a nerve defect.

Findings. Dissociated cells from the ends of the severed nerve ends consist of more than 85% of Schwann cells. The remaining cells are ED1 stained macrophages. The cells survive transfer to a silicone tube or a tendon autograft which bridge the nerve defect. Axons do grow through such a graft filled with dissociated cells.

Conclusion. Our novel model to obtain mainly Schwann cells by dissociation of the cells from the severed nerve ends after injury and add them to a matrix, thereby creating an artificial nerve graft, may be a new technique with potential clinical application in nerve reconstruction.

Keywords: Schwann cells; nerve graft; macrophages.

Introduction

When a peripheral nerve trunk is severely injured creating a defect, the gold standard of reconstruction is nerve grafting, preferably using an autologous nerve graft. In some situations there may be a lack of graft material. Furthermore, harvesting a nerve graft may create some sequele at the donor site. Therefore there is a need to develop alternatives to conventional nerve grafts. A large number of different structures have been used to experimentally bridge nerve defects, such as various designs of tubes and basal lamina preparation obtained by freeze-thawing or extraction of muscles and nerves. The structures can support regeneration but the extent of regeneration is far from sufficient. Regeneration can be improved if cultured Schwann cells are added to such matrices. However, the technique to obtain Schwann cells by culture is time consuming. These Schwann cells are usually of isogenic origin since allogenic cells are not an optimal alternative. It would be an advantage to obtain the Schwann cells that have proliferated in the stumps of the severed nerve trunk after the nerve injury and add to a suitable matrix, especially if the cells can be prepared during the nerve reconstruction procedure. For that reason we have developed a method where particularly Schwann cells from the nerve ends of the severed nerve trunk are acutely dissociated and added to two different tube structures, a silicone tube or a tendon autograft, to bridge nerve defects. In this minireview we will describe the procedure and results based on published papers [1, 6].

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The procedure of dissociation of cells

In rats the sciatic nerve on one side was transected without any attempt to repair the nerve injury. One week later the animals were reanaesthetised and a 2–3 mm long piece of each end (proximal and distal end) of the severed nerve trunk was removed. The nerve pieces were placed in 0.25% collagenase A and D in the culture medium RPMI-1640 and incubated for two hours in 37°. The treated nerve pieces were dissolved through repeated pipetting and transferred to a tube containing 10 or 15% bovine albumin in culture medium. The mixture was centrifuged for 10 min, the supernatant was removed

and the cells were washed in phosphate-buffered saline. The cell pellets were resuspended in plasma or culture medium.

The obtained dissociated cells consist particularly of Schwann cells achieving 81–88% of the recovered cells. The remaining cell types are mainly ED1 positive macrophages. The number of living cells that could be obtained from the proximal and distal nerve stumps reach up to 3×10^5 living cells which is somewhat lower than are presented when implanted cultured Schwann cells are used (range from 4×10^5 to 2.4×10^6, [3, 5]). However, one should consider that in the latter procedure cells may die from mild rejection even if they are isogeneic.

Creation of a matrix containing acutely dissociated cells

In our two previous studies [1, 6], we have used a silicone tube or an autologous tendon membrane, obtained from the tail tendons of the rat, to create a matrix that could harbour the dissociated cells (Fig. 1).

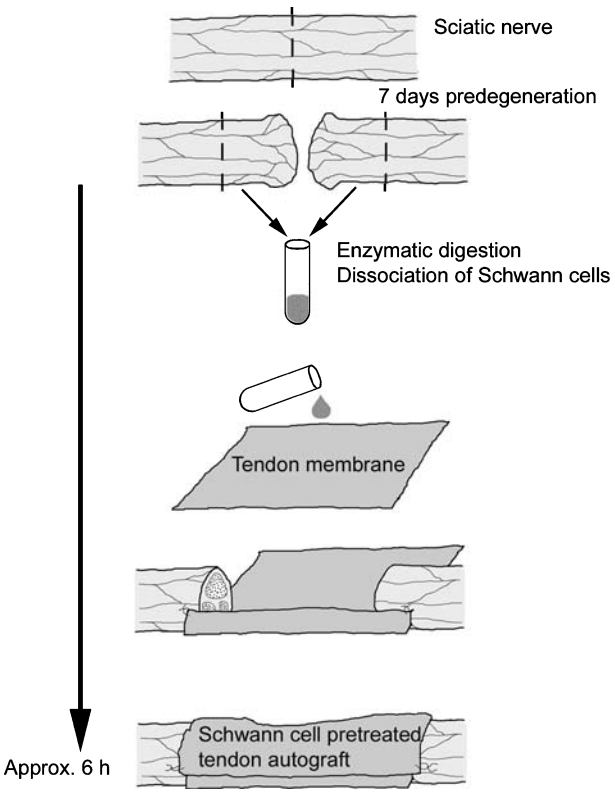

Fig. 1. The procedure in which cells are acutely dissociated by enzymatic digestion from the proximal and distal nerve stumps and applied to the tendon membrane used to bridge nerve defect. Reproduced by kind permission from Scand J Plast Reconstr Surg Hand Surg [1]

Nerve regeneration

In short term experiments (up to 10 days) neurofilament positive axons could be seen in the 10 mm long tendon autografts that were used to bridge a nerve defect. The axonal outgrowth was significantly longer after a week, but not at later time intervals, in tendon autografts that were filled with acutely dissociated cells. However, the regeneration rate was not different in tendon autografts filled with dissociated cells than in empty tendon autografts which could be based on the fact that Schwann cells had migrated from the proximal and distal nerve segments forming a continuous Schwann cell cable at later time points. This indicates that acutely dissociated cells may be of importance during a short time interval in limited rat sciatic nerve defects. However, this new principle may be more successful in long nerve grafts.

In more long-term experiments (one month) [6], we analysed nerve regeneration in silicone tubes with and without acutely dissociated cells. In such experiments, a new nerve-like tissue structure was formed between the proximal and distal nerve stumps as previously seen in animals and humans [2, 4]. We particularly investigated the effects of adding acutely dissociated cells to the silicone tube and evaluated results four weeks after the

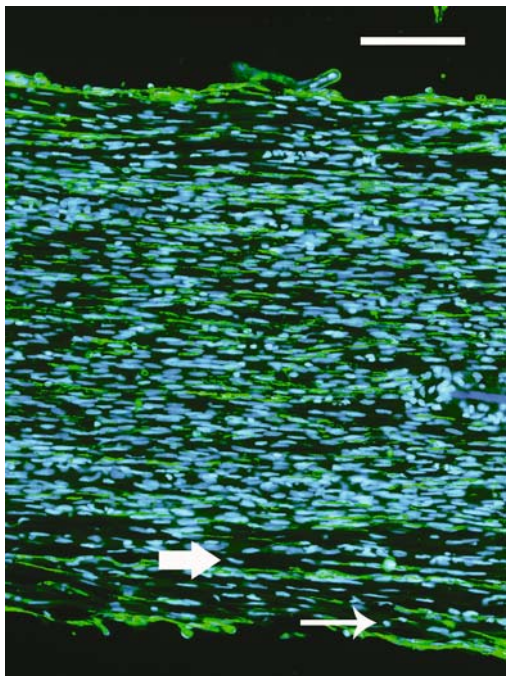

Fig. 2. Longitudinal section of the content of a silicone tube four weeks after bridging severed rat sciatic nerves with neurofilament positive axons (green) and Schwann cells (large oval nucleus) and inflammatory cells (small round nucleus) are seen (large and thin arrows, respectively). Reproduced by kind permission from Scand J Plast Reconstr Surg Hand Surg [6]

implantation procedure. After one day the cells were evenly distributed throughout the formed cable in the silicone tube. Regenerating neurofilament positive nerve fibres were organised into regenerating units with a well-developed perineurium surrounding the nerve structure at four weeks, but the width of the structure was not larger if dissociated cells were added to the tube. However, the area of Schwann nuclei were significantly larger in tubes where dissociated cells were added (Fig. 2).

The axons that regenerated through a silicone tube with dissociated cells were more evenly distributed in the distal nerve segment compared to axons that had grown through an empty silicone tube. Furthermore, the cross-sectional area of nerve fibres (detected by neurofilament reactivity in the distal nerve segment) was significantly higher at four weeks when dissociated cells had been added to the silicone tube.

Conclusion

The present model, where cells, consisting mainly of Schwann cells, are acutely dissociated from the severed nerve ends and added to a matrix, can be used to bridge nerve defects [1, 6]. Previous conventional methods to apply Schwann cells to matrices include cultured Schwann cells which may take up to six weeks to obtain. The present method is much more rapid (h) and has potential to be used clinically in the future. However, more developmental work is needed to for example improve the dissociation process and technique of cell application to the matrix, evaluate the optimal time of predegeneration and to evaluate the procedure in different models to find out which are the most favourable nerve defects that can be bridged.

References

1. Brandt J, Nilsson A, Kanje M, Lundborg G, Dahlin LB (2005) Acutely-dissociated Schwann cells used in tendon autografts for bridging nerve defects in rats: a new principle for tissue engineering in nerve reconstruction. Scand J Plast Reconstr Surg Hand Surg 39: 321–325
2. Dahlin LB, Anagnostaki L, Lundborg G (2001) Tissue response to silicone tubes used to repair human median and ulnar nerves. Scand J Plast Reconstr Surg Hand Surg 35: 29–34
3. Kim DH, Connolly SE, Kline DG, Voorhies RM, Smith A, Powell M, Yoes T, Daniloff JK (1994) Labeled Schwann cell transplants versus sural nerve grafts in nerve repair. J Neurosurg 80: 254–260
4. Lundborg G, Dahlin LB, Danielsen N, Gelberman RH, Longo FM, Powell HC, Varon S (1982) Nerve regeneration in silicone chambers: influence of gap length and of distal stump components. Exp Neurol 76: 361–375
5. Mosahebi A, Fuller P, Wiberg M, Terenghi G (2002) Effect of allogeneic Schwann cell transplantation on peripheral nerve regeneration. Exp Neurol 173: 213–223
6. Nilsson A, Dahlin LB, Lundborg G, Kanje M (2005) Graft repair of a peripheral nerve without the sacrifice of a healthy donor nerve by the use of acutely dissociated autologous Schwann cells. Scand J Plast Reconstr Surg Hand Surg 39: 1–6

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Comparative neuro tissue engineering using different nerve guide implants

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Summary

At the moment autologous nerve grafting remains the only reasonable technique for reconstruction of peripheral nerve defects. Unfortunately, this technique has a lot of complications and disadvantages. These problems are related to the autologous nerve that is harvested for this procedure. Donor site morbidity with loss of sensitivity, painful neuroma formation and of course the restricted availability of autologous nerves stimulates the idea for alternative techniques on that field. In this paper we describe our experience with different graft materials for reconstruction of a 2 cm nerve gap in a median nerve model in rats.

After implantation of various materials (biological/synthetic) the main experiments were conducted with a synthetic, biodegradable nerve conduit seeded with autologous Schwann cells. With this material we were able to reconstruct successfully a 2 cm gap in the rat median nerve. Regeneration with this material was found to be equally to an autologous nerve graft.

Keywords: Epsilon-caprolactone; nerve repair; nerve conduit; rat median nerve; Schwann cells.

Introduction

The procedure of autologous nerve grafting remains the golden standard for reconstruction of peripheral nerve defects [6]. An autologous nerve, most commonly the sural nerve, is harvested and transplanted into a gap between two nerve stumps. The main problem with this technique is found in the harvesting area were numbness remains and painful neuroma formation is possible. Furthermore, grafting material is available only to a limited extent. Thus, especially in the case of very extensive nerve injuries (brachial plexus) a complete reconstruction seems to be impossible [7].

Therefore, and due to restricted regeneration rates achieved by this technique, in the past various materials were suggested to replace autologous nerve grafting. These different materials can be divided in two groups: biological and synthetic materials. Moreover, synthetic conduits may be differentiated into resorbable and non-resorbable [4].

Among biological materials especially veins have been proposed as favourable transplant since this material demonstrated successful regeneration across short gaps with only limited donor-site morbidity and sufficient availability [3]. To circumvent the problem of veins to collapse, skeletal muscles with a higher mechanical stability and an axial structure (muscular laminin) as guiding substratum for regenerating axons were proposed as an alternative approach [2].

Among synthetic nerve guides the first material introduced were non-resorbable silicon tubes described by Lundborg. After successful reconstruction, these foreign bodies induced compression syndromes making a second surgical intervention necessary to remove the tubes [5]. Therefore, in the following years material specialists focused their work on developing resorbable nerve conduits with defined degradation profiles and characteristics. Efforts aimed to adjust the time point of implant disintegration to allow sufficient time for axonal regeneration and furthermore to choose polymer components that limit the amount of adverse degradation products (lactate, oxygen radicals, etc.).

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We found these characteristics in a trimethylencarbonat-ε-caprolacton polymer (TMC/CL) [8]. Our first in vivo experiments revealed that the degradation time of the selected nerve guide implants were around six months. To foster axonal regeneration, we decided to co-implant autologous Schwann cells (SC) inside the tubes in order to profit from nerve growth factors and other neurotrophic factors that were physiologically synthesized by these cells [10]. The viability of the cells inside the nerve guide tubes was proven in a former in vitro study and was secured by the micro porous structure of the polymer wall [8]. In this work we describe our experience with different materials for tubulization of peripheral nerve defects as an alternative to autologous nerve grafting. Moreover, we present results from a study with a trimethylencarbonat-ε-caprolacton polymer guiding tube in conjunction with syngenic Schwann cells for reconstruction of a 2 cm gap in a rat median nerve model [9].

Materials and methods

Animal model

For our experiments we used female inbred Lewis rats. The rats weighed 200–220 g. The median nerve model (see below) was used because it provides an easy way to monitor neuronal regeneration by a quantitative functional test, i.e. grasping. All experiments were conducted following strictly the German and European guidelines for animal research. Animals were observed for nine months.

Conduits

First, autologous veins were harvested from the upper extremity of the animals and interposed into a musculocutaneous nerve in order to train the surgical expertise for the handling of the material ($n = 4$) (Fig. 1).

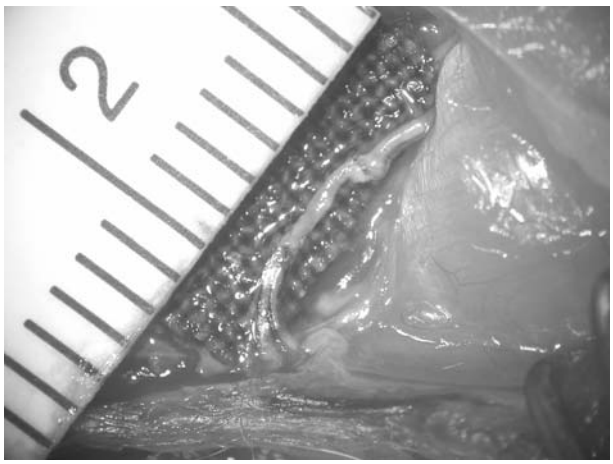

Fig. 1. Vein implant. Depicted is a fresh implanted vein in a gap across the musculocutaneous nerve in a rat. Note the collapse of the venous wall

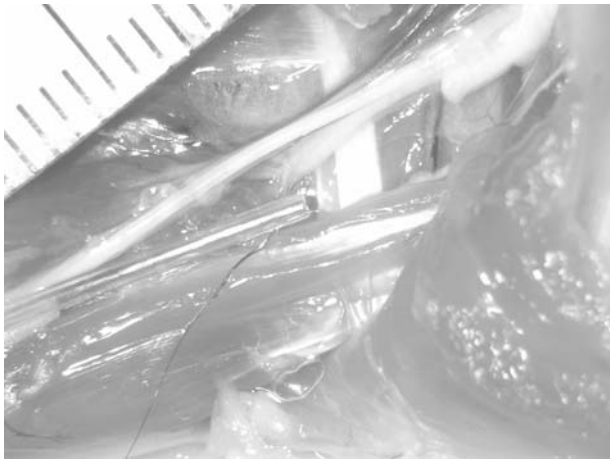

Fig. 2. Silicone tube implant. Silicon tube that is introduced across a 2 cm defect in a median nerve. The needle is damaged due to the stiffness of the material

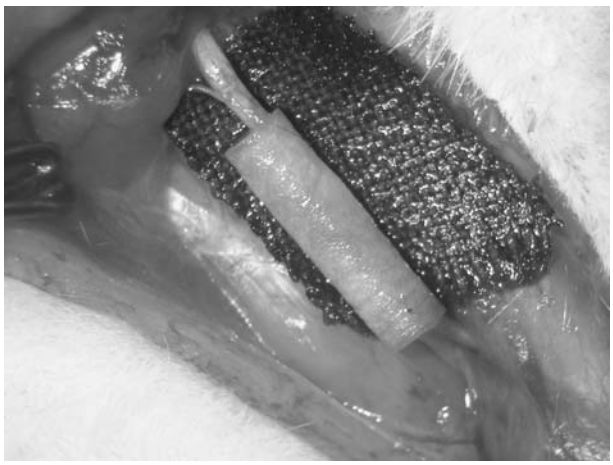

Fig. 3. Collage tube implant. Implanted collagen tube in a rat median nerve. Note the size-mismatch between the lumen and the nerve

In a second series, non-resorbable silicone tubes were introduced into 2 cm defects in a severed median nerve ($n = 2$) (Fig. 2). Finally, before starting with the main experiments four additional animals received a resorbable collagen tube (Neuragen^{TM}, Integra Neuroscience; USA) (Fig. 3) [1]. The majority of animals received a TMC/CL polymer to guide regenerating axons across a 2 cm defect in the rat median nerve. We designed our protocol as follows:
Group 1: no operation ($n = 22$); group 2: autologous nerve graft ($n = 22$); group 3: empty TMC/CL conduit ($n = 16$); group 4: TMC/CL CL conduit and SC ($n = 22$).

Functional test

For functional evaluation the grasping test described by Bertelli and Mira was used. It is based on the inability of rats to flex their digits after median nerve transection. After successful reconstruction of the nerve the rats gain back the ability to flex the forepaw. The force developed by this manoeuvre can be measured using an electronic balance and a wire grid while pulling the animals gently at their tails.

Target muscle regeneration

For further information about the state of regeneration, the flexor digitorum sublimis muscle was additionally harvested and weighed. This muscle reflects median nerve regeneration since it is specifically innervated by that nerve.

Histology

For a general overview specimens were stained with Nissl stain. Specific staining with antibodies was performed with a Schwann cell marker (S-100) and a pan axonal marker (PAM). Finally, electron microscopy was used to assess the integrity of neural tissue.

Eletrophysiology

The nerve conduction velocity was analyzed using a NIM-Pulse^{TM} electrophysiological device from Medtronic, USA. The measurement was carried out before animals were sacrificed in a supine position in a standardized stimulation paradigm (same stimulation site).

Results

Venous grafts demonstrated pronounced luminal instability. Another disadvantage was the need for a second surgical step to harvest the vein before nerve reconstruction. Due to adverse implant characteristics, animals subjected to this procedure were monitored only up to four weeks. Silicon tubes used for implantation, demonstrated another problem that had to be handled by the surgeon, i.e. stiffness and rigidity. That means especially the microsurgical application of the material may be hampered by a damage of the needle that was observed in all cases (Fig. 2). Collagen tubes revealed another problem. All the tubes provided by Integra had an internal diameter of 2 mm that was by far too wide for the rat median nerve (Fig. 3). Consequently we proceeded with the experiments with the TMC/CL tubes which at an early state had their own problems as well. This was a too rapid degradation that led to broken prostheses after only a few weeks in vivo (Fig. 4). This problem was solved by the reduction of the radiation time of that material in order to prolong degradation. A certain degree of radiation which also breaks chemical bonds of the polymer, is needed for sterilization of implants.

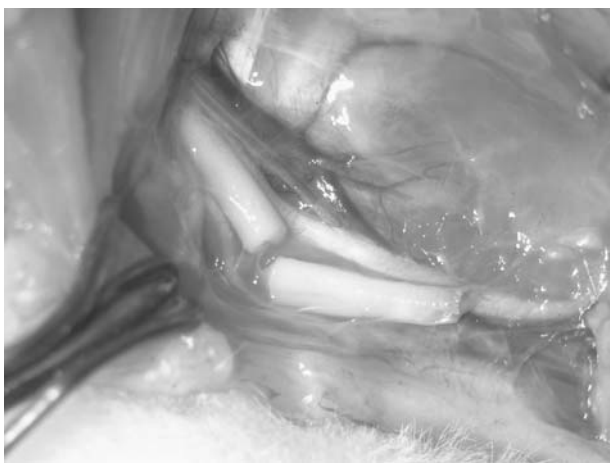

Fig. 4. Broken TMC/CL tube four weeks after implantation

After nine postoperative months, the functional regeneration in group 2 and 4 demonstrated a complete regeneration compared to the non-operated animals. Group 3 animals (TMC-CL tube without SC) showed no functional regeneration during the complete observation period. Furthermore, the measurement of the muscle weight revealed comparable data among groups 2 and 4 (gr. 2: 71.86 ± 2.03, gr. 4: 67.12 ± 1.25 [% of control]) while animals supplied with an empty tube demonstrated a progressive atrophy (gr. 3: 13.96 ± 1.29 [% of control]). These observations were supported by the histological analyses that confirmed the presence of intact axons with surrounding myelin sheaths at the distal coaptation point of the specimens gathered from groups 2 and 4. In animals from group 3 no axons were found. Finally, the measurement of the nerve conduction velocity displayed no statistical significant differences among the groups 1, 2 and 4, while animals supplied with an empty tube did not display any recordable potentials.

Discussion and conclusion

In an in vivo study of various nerve conduits for restoration of a peripheral nerve gap of 2 cm in a rat median nerve model, we found a trimethylencarbonat-ε-caprolacton polymer to provide ideal characteristics for nerve regeneration. The regeneration induced by this material in conjunction with cellular elements, namely Schwann cells was comparable to that obtained with an autologous graft. In contrast, an empty tube was not sufficient to induce regeneration across this distance as judged from functional (grasping test), electrophysiological, and histological parameters and from the weight of the flexor digitorum sublimis muscle innervated by the median nerve.

Other materials were chosen as well for application across a nerve gap in rats. These materials demonstrated various disadvantages. Venous grafts showed to be instable across longer defects while an inappropriate stiffness was found with silicon tubes that made it impossible for implantation across joints leading into immobilization and ankylosis of the joints. The material provided by Integra had an unacceptable large diameter for application in that model. The smallest diameter was about 2 mm raising the question whether this is enough for a small finger nerve in man. However, the advantage of

these collagen tubes is the resorbable character of the material while exact resorption time remains to be evaluated [1].

Reviewing our results, it seems that cellular elements, especially those producing neurotrophic factors are most helpful to drive a successful regeneration across large nerve gaps. Further improvement of the nerve conduits is still needed. Though in group 4 animals (nerve guide tube with SC) functional regeneration was found resulted, the onset and the velocity regeneration was still behind of that observed in the autologous group.

References

1. Archibald SJ, Shefner J, Krarup C, Madison RD (1995) Monkey median nerve repaired by nerve graft or collagen nerve guide tube. J Neurosci 15: 4109–4123
2. Battiston B, Tos P, Cushway TR, Geuna S (2000) Nerve repair by means of vein filled with muscle grafts I. Clinical results. Microsurgery 20: 32–36
3. Chiu DT (1999) Autogenous venous nerve conduits. A review. Hand Clin 15: 667–671
4. IJkema-Paassen J, Jansen K, Gramsbergen A, Meek MF (2004) Transection of peripheral nerves, bridging strategies and effect evaluation. Biomaterials 25: 1583–1592
5. Lundborg G, Gelberman RH, Longo FM, Powell HC, Varon S (1982) In vivo regeneration of cut nerves encased in silicone tubes: growth across a six-millimetre gap. J Neuropathol Exp Neurol 41: 412–422
6. Millesi H (2000) Techniques for nerve grafting. Hand Clin 16: 73–91
7. Schaller E, Berger A (1987) Peripheral nerve allograft. Plast Reconstr Surg 80(6): 870–871
8. Schlosshauer B, Muller E, Schroder B, Planck H, Muller HW (2003) Rat Schwann cells in bioresorbable nerve guides to promote and accelerate axonal regeneration. Brain Res 963: 321–326
9. Sinis N, Schaller HE, Schulte-Eversum C, Schlosshauer B, Doser M, Dietz K, Roesner H, Müller HW, Haerle M (2005) Nerve regeneration across a 2 cm gap in the rat median nerve using a resorbable nerve conduit filled with Schwann cells. J Neurosurg 103: 1067–1076
10. Strauch B, Rodriguez DM, Diaz J, Yu HL, Kaplan G, Weinstein DE (2001) Autologous Schwann cells drive regeneration through a 6-cm autogenous venous nerve conduit. J Reconstr Microsurg 17: 589–595

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The "bioartificial living nerve graft"

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Summary

Introduction. Nerve tubes seeded with cultured Schwann cells have become a promising alternative to nerve autografts. However, the functional results of these bioartificial cellular grafts remain to be improved. To imitate the three-dimensional structure of peripheral nerves, we designed a Schwann cell-seeded intrinsic framework within a semipermeable biodegradable collagen nerve tube (Integra®).

Material and methods. In 90 rats a 25 mm gap was created at the sciatic nerve of the right lower limb. In group I, the gap was treated using the "bioartificial nerve graft". In group II, the tube filled with non-seeded filaments was implanted in order to evaluate the influence of the Schwann cells on regeneration. In group III, the gap was bridged using an autologous nerve graft. For evaluation clinical testing, gait analysis, electrophysiological conduction testing, tibialis anterior muscle weight recording and axon counts from the distal nerve stump were used.

Results. There was a significant difference between the "bioartificial nerve graft" (group I) and the non-seeded bioartificial nerve graft (group II) indicating the importance of the living Schwann cells. Comparing the results of the "bioartificial nerve graft" (group I) with the autologous nerve grafts (group III), there was a significant difference in all the examinations indicating a still slower regeneration in the artificial graft.

Conclusions. We conclude that the unique three-dimensional net allowed the settlement of Schwann cells onto the biodegradable filaments, which can be used as "artificial Bünger bands". With further refinements of the "artificial Bünger bands" and Schwann cell cultures there should be improved functional and histological results in the "bioartificial nerve graft" group.

Keywords: Tissue engineering; bioartificial nerve graft; nerve substitute; peripheral nerve.

Introduction

Nerve tubes seeded with cultured Schwann cells have become a promising alternative to nerve autografts. However, the functional results of these bioartificial cellular grafts remain to be improved. To imitate the three-dimensional structure of peripheral nerves, we designed to fabricate a Schwann cell-seeded intrinsic framework within a semipermeable biodegradable collagen nerve tube (Integra®).

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Material and methods

In 90 rats a 25 mm gap was created at the sciatic nerve of the right lower limb. In group I the gap was treated using the "bioartificial nerve graft" (Fig. 1a–f). In group II, the tube filled with non-seeded filaments was implanted, in order to evaluate the influence of the Schwann cells on regeneration. In group III, the gap was bridged using an autologous nerve graft. For evaluation we were using clinical testing, gait analysis, electrophysiological conduction testing, tibialis anterior muscle weight recording and axon counts from the distal nerve stump.

Results

There was a significant difference between the "bioartificial nerve graft" (group I) and the non-seeded bioartificial nerve graft (group II) indicating the importance of the living Schwann cells. Comparing the results of the "bioartificial nerve graft" (group I) with the autologous nerve grafts (group III), there was a significant difference in all the examinations indicating a still slower regeneration in the artificial graft (Fig. 2).

Conclusions

We conclude that the unique three-dimensional net allowed the settlement of Schwann cells onto

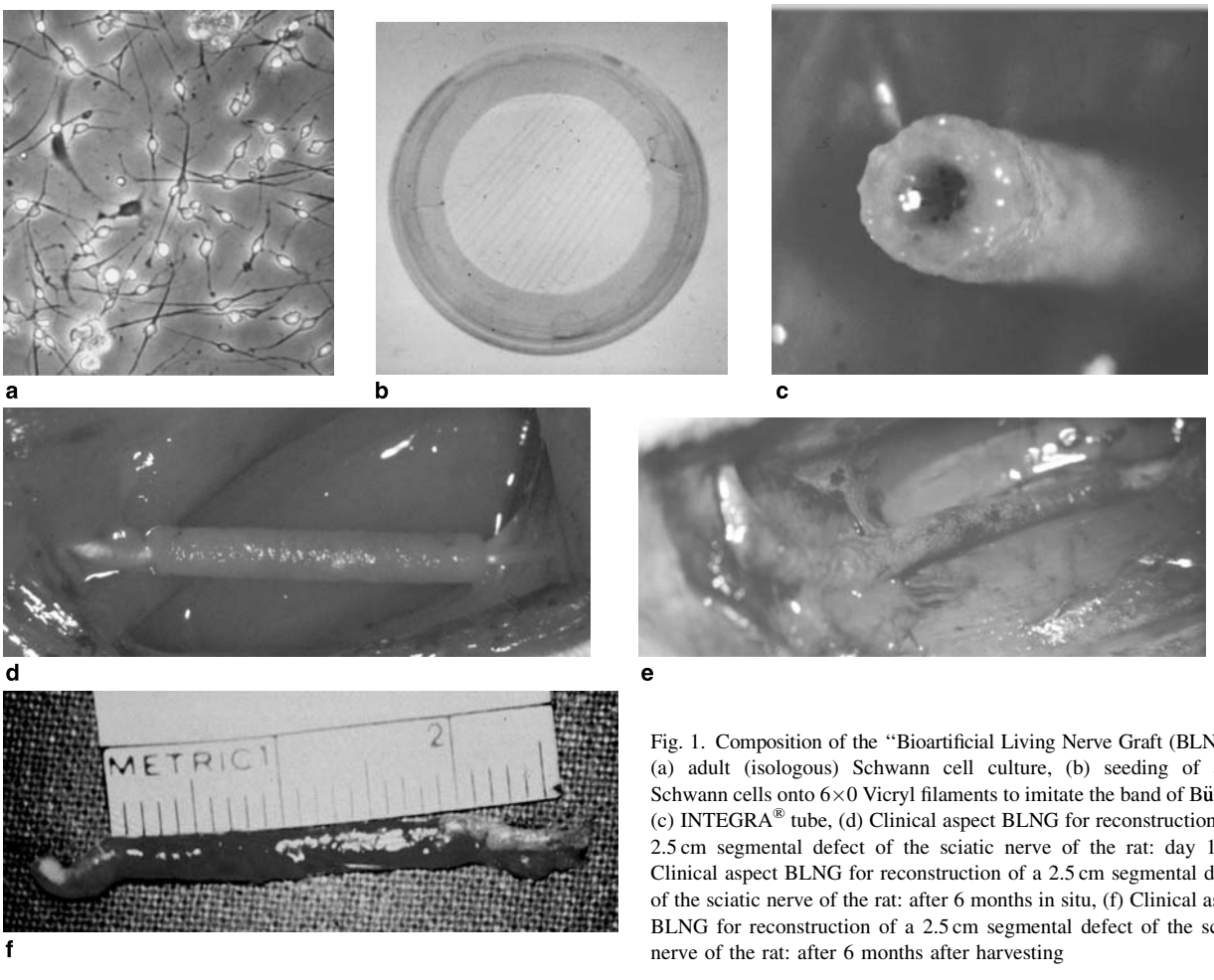

Fig. 1. Composition of the "Bioartificial Living Nerve Graft (BLNG)". (a) adult (isologous) Schwann cell culture, (b) seeding of adult Schwann cells onto 6×0 Vicryl filaments to imitate the band of Bünger, (c) INTEGRA® tube, (d) Clinical aspect BLNG for reconstruction of a 2.5 cm segmental defect of the sciatic nerve of the rat: day 1, (e) Clinical aspect BLNG for reconstruction of a 2.5 cm segmental defect of the sciatic nerve of the rat: after 6 months in situ, (f) Clinical aspect BLNG for reconstruction of a 2.5 cm segmental defect of the sciatic nerve of the rat: after 6 months after harvesting

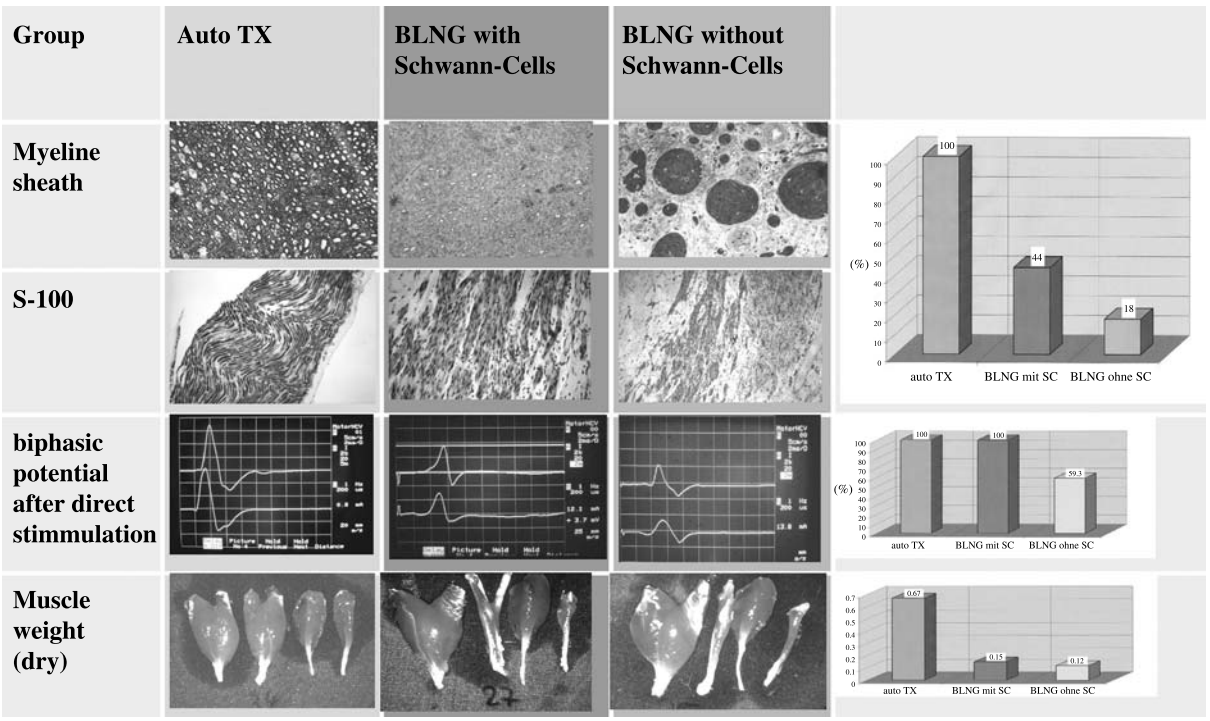

Fig. 2. Results

the biodegradable filaments, which can be used as ‘‘artificial Bünger bands’’. With further refinements of the ‘‘artificial Bünger bands’’ and Schwann cell cultures there should be improved functional and histological results in the ‘‘bioartificial nerve graft’’ group.

References

1. Lohmeyer J (2003) Überbrückung peripherer Nervendefekte unter Verwendung eines bioartifiziellen Nerventransplantates im Tiermodell. Medizinische Hochschule Hannover, Dissertation
2. Shen ZL, Berger A, Hierner R, Walter GF (2001) A Schwann cell-seeded intrinsic framework and its satisfactory biocompatibility for a bioartificial nerve graft. Microsurgery 21: 6–11

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Nerve regeneration using tubular scaffolds from biodegradable polyurethane

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Summary

Introduction. In severe nerve lesion, nerve defects and in brachial plexus reconstruction, autologous nerve grafting is the golden standard. Although, nerve grafting technique is the best available approach a major disadvantages exists: there is a limited source of autologous nerve grafts.

This study presents data on the use of tubular scaffolds with uniaxial pore orientation from experimental biodegradable polyurethanes coated with fibrin sealant to regenerate a 8 mm resected segment of rat sciatic nerve.

Methods. Tubular scaffolds: prepared by extrusion of the polymer solution in DMF into water coagulation bath. The polymer used for the preparation of tubular scaffolds was a biodegradable polyurethane based on hexamethylene diisocyanate, poly(ε-caprolactone) and dianhydro-D-sorbitol.

Experimental model. Eighteen Sprague Dawley rats underwent mid-thigh sciatic nerve transection and were randomly assigned to two experimental groups with immediate repair: (1) tubular scaffold, (2) 180° rotated sciatic nerve segment (control). Serial functional measurements (toe spread test, placing tests) were performed weekly from 3rd to 12th week after nerve repair. On week 12, electrophysiological assessment was performed. Sciatic nerve and scaffold/nerve grafts were harvested for histomorphometric analysis. Collagenic connective tissue, Schwann cells and axons were evaluated in the proximal nerve stump, the scaffold/nerve graft and the distal nerve stump.

The implants have uniaxially-oriented pore structure with a pore size in the range of 2 µm (the pore wall) and 75 × 700 µm (elongated pores in the implant lumen). The skin of the tubular implants was nonporous.

Animals which underwent repair with tubular scaffolds of biodegradable polyurethanes coated with diluted fibrin sealant had no significant functional differences compared with the nerve graft group.

Control group resulted in a trend-wise better electrophysiological recovery but did not show statistically significant differences.

There was a higher level of collagenic connective tissue within the scaffold and within the distal nerve stump. Schwann cells migrated into the polyurethane scaffold. There was no statistical difference to the nerve graft group although Schwann cell counts were lower especially within the middle of the polyurethane scaffold. Axon counts showed a trend-wise decrease within the scaffold.

Conclusion. These results suggest that biodegradable polyurethane tubular scaffolds coated with diluted fibrin sealant support peripheral nerve regeneration in a standard gap model in the rat up to 3 months. Three months after surgery no sign of degradation could be seen.

Keywords: Nerve regeneration; tubular scaffolds; biodegradable; allogenic nerve graft.

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Introduction

Five basic possibilities are described by Millesi to manage a nerve defect [6].

1) Restoration of continuity by end-to-end coaptation. 2) Restoration of continuity by adding tissue. 3) By passing a defect by transfer of synergistic nerve fibers of equal destination as suggested by Millesi and Schmidhammer [3]. 4) By passing a defect by nerve fiber transfer from another nerve if the proximal stump is lacking. 5) Nerve-muscle neurotization if the distal stump is lacking.

The golden standard for bridging nerve defects and in brachial plexus reconstruction is autologous nerve grafting [1, 2]. However, there is a very limited source of autologous nerve grafts. Thus, in the last decades with the rapid advances in biomaterial technology, a number of materials were tested for a nerve guiding function. Different materials, such as polymers, silicone and collagen were used for nerve tubes and nerve scaffolds [1].

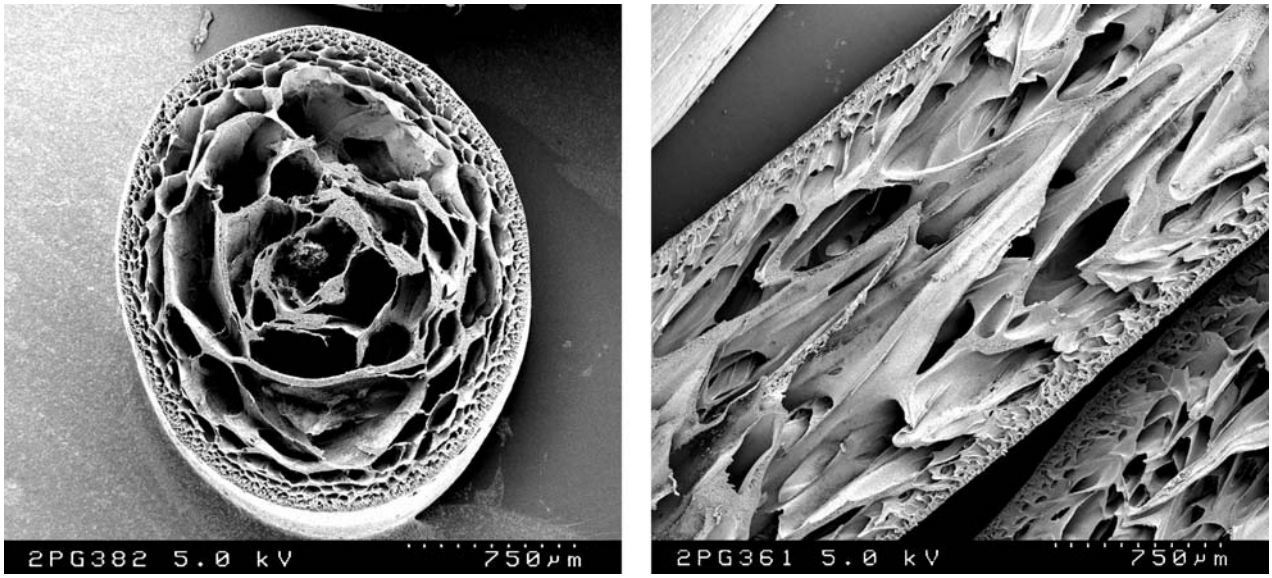

Fig. 1. Scanning electron micrographs of polyurethane tubular scaffolds with uniaxial pore orientation. (A) Cross-section perpendicular to the tube longer axis; (B) Cross-section parallel to the tube longer axis

One major demand to those nerve guiding materials is biodegradability within a short time. Additionally, these biodegradable materials need to be mechanically stable [5] at the site of coaptation to allow movement of the nerve which is especially required in the joint regions.

The aim of this rat study was to investigate the efficacy of a biodegradable polyurethane scaffold with uniaxial pore orientation in functional peripheral nerve regeneration in an 8 mm defect in the sciatic nerve. Focussing on nerve regeneration through the scaffold functional results are discussed in context with electrophysiological and histological data.

Material and methods

Experimental model

We used eighteen Sprague Dawley rats weighing 330–400 g. In each animal the right sciatic nerve was located midthigh. The nerve and its branches were microsurgically dissected from the sciatic notch to the hollow of the knee and isolated atraumatically from the surrounding tissue.

Tubular scaffolds

The experimental biodegradable polyurethane used in the study was based on hexamethylene diisocyanate, poly(ε-caprolactone) and dianhydro-D-sorbitol (K. Gorna, S. Gogolewski, AO/ASIF Research Institute, Davos, Switzerland). The porous scaffolds were prepared by extrusion of the polymer solution in DMF into water coagulation bath.

The implants have uniaxially-oriented pore structure with a size in the range of 2 µm of the pores in the wall and 75 µm × 700 µm of the elongated pores in the implant lumen (Fig. 1). The skin of the tubular implants was nonporous.

Before implantation the tubular scaffolds were coated with 1:8 diluted fibrin sealant.

Groups

The animals were randomly assigned to two experimental groups with immediate repair. Group (1) tubular scaffold. An 8 mm piece of the nerve was cut out and was replaced by a fibrin coated tubular scaffold. The nerve ends were coaptated under the microscope to the scaffold by two epineural sutures.

Group (2) (control group). An 8 mm piece of the nerve was cut out and the segment was rotated for 180° was replaced by a fibrin coated tubular scaffold. The nerve ends of the rotated segment were immediately coaptated under the microscope to the ischiadic nerve by two epineural sutures proximal and two coaptations distal.

Serial functional measurements (toe spread test, placing tests) were performed weekly from 3rd to 12th week after nerve repair. On week 12,

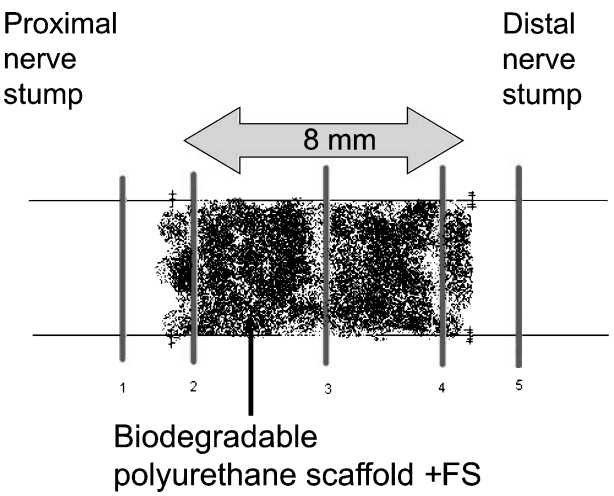

Fig. 2. Specimen zones

electrophysiological assessment was performed. Sciatic nerve and scaffold/nerve grafts were harvested for histomorphometric analysis. Collagenic connective tissue, Schwann cells and axons were evaluated in the proximal nerve stump, the scaffold/nerve graft (Fig. 2) and the distal nerve stump.

Results

Animals which underwent repair with tubular scaffolds of biodegradable polyurethanes coated with diluted fibrin sealant had no significant histological, electrophysiological, or functional differences compared with the nerve graft group.

There was a higher level of collagenic connective tissue within the scaffold and within the distal nerve stump. Schwann cells migrated into the polyurethane scaffold. There was no statistical difference to the nerve graft group although Schwann cell counts were lower especially within the middle of the polyurethane scaffold. In both groups axon counts showed a trend-wise decrease within the scaffold. In the distal zones there were slightly more vessels in the polyurethane scaffold group, but again no statistically significant difference (Fig. 3).

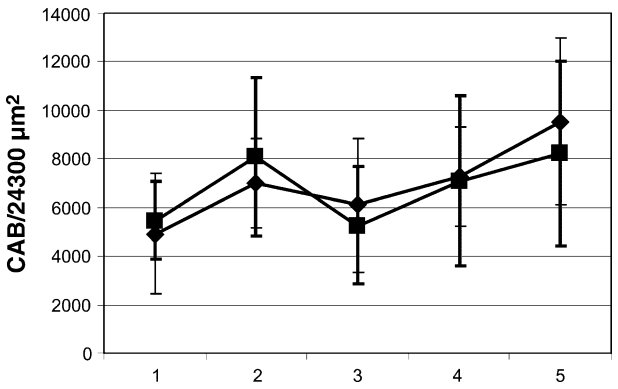

Fig. 3. In the distal zones slightly more vessels could be found (zone 3–5). ◆ PU scaffold FS1:8, ■ control

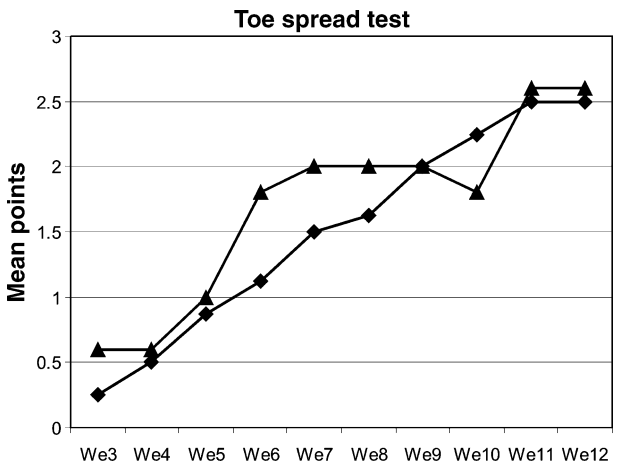

Fig. 4. Functional assessment of nerve regeneration by toe spread test. ◆ PU scaffold + FS, ▲ nerve graft 180°-control

The control group resulted in a trend-wise better electrophysiological recovery but did not show statistically significant differences.

The toe spread assessment as a functional test didn't show any statistically significant difference between both groups (Fig. 4). There was no contact placing in both groups.

Discussion

The results of this animal study demonstrates that using conduits from biodegradable polyurethane to bridge a nerve defect in a standard gap model in the rat leads to histological, functional and electrophysiological improvement up to 3 months.

No statistically significant differences were found compared to the control group with a 180° rotated sciatic nerve segment. We could find that these results were surprisingly good compared to a nerve graft. However, one should notice that the sciatic nerve graft at thigh level was rotated 180° and distally the nerve is divided into the three branches already. Rotation of the nerve graft in longitudinal axis of 180° may result in the major disadvantage of axonal misdirection.

The time of degradation of these polyurethane scaffolds was chosen for more than 6 weeks to create a mechanically stable environment for axonal regeneration and low inflammatory reaction. On the other hand resorption of the polyurethane scaffold should not be too fast, preserving mechanical properties, to keep the regenerative tissue in position.

However, polyurethane material did not show any elementary signs of degradation 3 months after surgery. One may hypothesize that the scaffolds will serve as a block for axonal regeneration at later stages of the regeneration process. We are convinced that using this type of polyurethane scaffolds in peripheral nerve tissue engineering degradation time has to be decreased, in addition to low inflammatory reactions leading to a low level of neural collagenization and improvement of functional results.

Despite the fact that the diameter of the scaffold was about 20% larger than the diameter of the rotated nerve graft no regenerative tissue could be found outside the graft indicating that there was no aberrant innervation. Additionally, it suggests that within the scaffold there is enough space for neural regenerative tissue enabling axonal sprouts advancing distally. Within the scaffold

the amount of collagenic and angiogenetic tissue was slightly higher than in the control group, indicating an increased inflammatory reaction which is essential for biodegradation of the polyurethane scaffolds.

The electrophysiological and functional results finally did not show statistical significant differences three months after surgery referring to high correlation of these data.

However, as a given fact the capacity of nerve regeneration in the rat is much higher compared to humans, the standard gap model has to be proven by a critical gap model.

Conclusion

These results suggest that biodegradable polyurethane tubular scaffolds coated with diluted fibrin sealant support peripheral nerve regeneration in a standard gap model in the rat up to 3 months.

References

1. Kannan RY, Salacinski HJ, Butler PE, Seifalian AM (2005) Artificial nerve conduits in peripheral nerve repair. Biotechnol Appl Biochem 41: 193–200
2. Millesi H (2003) Grundsätzliches zur operativen Behandlung peripherer Nervenläsionen. In: Mumenthaler M, Stöhr M, Müller-Vahl H (eds) Läsionen peripherer Nerven und radikuläre Syndrome, 8. Auflage, pp 95–98
3. Schmidhammer R, Van der Nest D, Redl H, Millesi H (2005) Synergistic terminal end to side nerve graft coaptation: investigation in a non-human primate model. Europ Surgery 38: 308–316
4. Schmidhammer R, Zandieh S, Hopf R, Mizner I, Pelinka L, Kroepfl A, Redl H (2004) Alleviated tension at the repair site enhances functional regeneration: the effect of full range motion mobilization on the regeneration of peripheral nerves – histologic, electrophysiologic, and functional results in a rat model. J Trauma 56: 571–584
5. Schmidhammer R, Zandieh S, Hopf R, Hausner T, Pelinka L, Kroepfl A, Redl H (2005) Effects of the alleviated tension at the nerve repair site using biodegradable tubular conduits: histological, electrophysiological and functional results in a rat model. Eur Surg 37/4: 213–219
6. Sluzky JD, Hentz VR (2006) Peripheral nerve surgery, practical applications in the upper extremity. Elsevier, Churchill Livingstone, p 40

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A new technique of autogenous conduits for bridging short nerve defects. An experimental study in the rabbit

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Summary

Backround. Nerve grafting is the most reliable used procedure to bridge a neural defect, but it is associated with donor site morbidity. In experimental surgery the search for an optimal nerve conduit led to the use of biological and artificial material. Nerve regeneration through epineural conduits for bridging short nerve defect was examined.

Methods. Four groups including 126 New Zealand rabbits were used. There were 3 study groups (A, B and C) and 1 control group (D). A 10-mm long sciatic nerve defect was bridged either with 3 variations of an epineural flap (Groups A, B and C) or with a nerve graft (Group D). Animals from all groups were examined 21, 42 and 91 days postoperatively to evaluate nerve regeneration employing light microscopy and immunocytochemistry. Nerve regeneration was studied in transverse sections at 3, 6 and 9 mm from the proximal stump. Using muscle stimulator the gastrocnemius contractility was examined at 91 days post surgery in all groups.

Findings. Immunohistochemical and functional evaluation showed nerve regeneration resembling the control group, especially in group A, were an advancement epineural flap was used.

Conclusion. An epineurial flap can be used to bridge a nerve defect with success.

Keywords: Defect; conduit; epineurium; nerve; gap.

Introduction

Nerve grafting is the most effective used procedure to repair a neural gap, but it is associated with donor site morbidity [4, 6, 7, 11]. In experimental models the search for an optimal nerve conduit led to the use of autogenous and artificial materials [5, 9, 10, 12]. Clinical implementation of conduits has focused on the use of autogenous tissue (veins, arteries, pseudoseaths, nerve grafts) and artificial conduits (polyglactine, silicon) [6, 7, 10].

Conduit materials does not seem to improve significantly the outcome. The major obstacle in the use of conduits is the limitation in the defect size that can be successfully bridged and is limited in humans to 2.5 cm. The epineurium carries the majority of the nerve vessels, i.e. vasa nervorum. Additional vascular supply comes from the intranervous vascular plexus and from various perforators. There is also another type of reverse vascularization from distal to proximal, involving shorter vessels [4]. In this study we used 3 variations of an epineural flap to bridge a short nerve defect and to study if the epineurium may serve successfully for this reason.

Materials and methods

One hundred and twenty six white New Zealand rabbits, weighing 3.5 kg were used. The animals were allocated to 4 Groups. In the 3 study groups (Groups A, B and C) 36 animals were included, while the rest 18 animals served as control (Group D). In all groups a 10-mm sciatic nerve defect was created and bridged either with 3 variations of an epineural flap (Groups A, B and C) or with a nerve graft (Group D). In all groups the sciatic nerve was exposed under general anaesthesia. The sciatic nerve was exposed under microscope. A 10 mm nerve defect was created using a sharp blade proximal of sciatic nerve bifurcation. An advancement epineural flap harvested from the proximal nerve stump and from the distal nerve stump were employed in Groups A and B, respectively. In Group C a specially designed reversed epineurial flap harvested from the proximal stump was employed. In the control Group D the defect was bridged using the excised portion of the sciatic nerve, which was sutured in its original site.

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Fig. 1. The epineurium harvested from the proximal nerve, forms the epineurial conduit

Following exposure of the sciatic nerve and creation of the defect a 10 mm was designed on the epineurium of the proximal stump in group A and a similar flap on the distal stump in group B. Surgical dissection started in a dorsal longitudinal direction and continued circumferentially to remove the epineurium (Fig. 1). Two millimetre of the epineurium located at the rim of the proximal (Group A) or distal (Group B) nerve stump was preserved to facilitate flap suturing. The excised epineurium was then used to bridge the nerve defect. To prevent collapse of the conduit and to facilitate suturing a 2 mm thick silicon tube was inserted temporarily within the conduit and between the two nerve stumps and removed before final closure. The proximal and distal edge of the nerve was secured on the proximal and distal nerve stumps using four 10–0 Ethilon stitches. The longitudinal flap edges were also approximated using 5–7 stitches. The space within the conduit was filled with a blood clot (Fig. 2), before completing epineurium suturing. In Group C the epineurium in the proximal nerve stump was not completely excised but its distal attachment was preserved. The epineurial flap was reversed pivoting on its distal attachment and sutured on the distal nerve stump with epineurial sutures. In this case the length of the flap was 12 mm.

In Group D the 10 mm defect was repaired using the previously resected nerve segment, which served as an autologous graft using 4 epineurial stitches at each suture line.

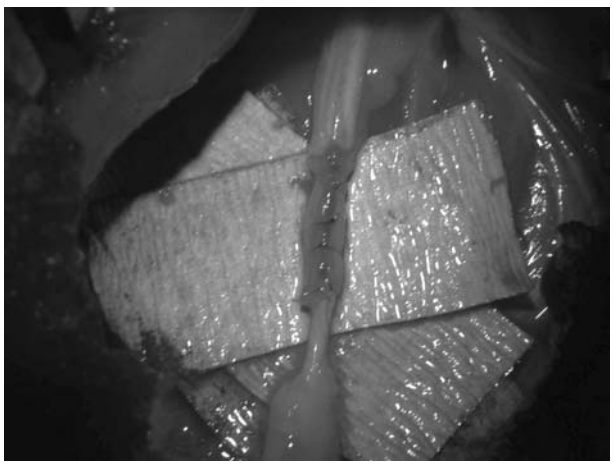

Fig. 2. The nerve defect bridged with the epineurial conduit filled by blood clot (e)

Postoperative evaluation

The nerve regeneration was studied at various intervals using immunochemistry, light microscopy and measurement of the Gastrocnemius contractility. Twelve animals from Groups A, B and C were sacrificed after 3, 6 and 12 weeks, while all control animals were sacrificed after 12 weeks. The conduit area was exposed and the grafted part was excised. Six specimens were used for light microscopy examination and 6 specimens for immunochemistry. After 12 weeks all animals underwent examination of the gastrocnemius contractility in both limbs.

Nerve regeneration was studied in 1 μm transverse sections at 3, 6 and 9 mm from the proximal stump (3 specimens for each group designated S3, S6, S9) and in longitudinal sections stump (3 specimens for each group). The epineurium conduit was resected and immersed in 2.5% glutaraldehyde. After fixation in 1% osmium tetroxide and dehydration in ethanol, the specimens were embedded in Agar 100. The specimens were stained with Toluidine blue and examined by light microscopy. Quantitative morphometry was performed measuring the number of myelinated axons per mm^2 and the mean axon diameter in every section.

In similarity with the light microscopy 6 specimens from each group were examined using immunochemistry. The harvested conduit was rinsed in ice-cold PBS and embedded in Tissue Tek O.C.T. Three micrometre thick transverse and three, 10 mm long longitudinal sections were cut on a cryostat. After fixation in 2.5% paraformaldehyde, the sections were exposed to primary antibodies (DAKO) to identify the components of the newly formed nerve, including 68 KD neurofilament protein, fibrinogen, fibrin and fibronectin. The immunocytochemistry and light microscopy findings at 3 and 6 weeks were only qualitatively analysed.

The isometric contraction force of the Gastrocnemius muscle, which is supplied by the tibial nerve, was measured 13 weeks after the defect bridging in all groups. The animals were anaesthetised and the sciatic nerves and the Gastrocnemius muscles were bilaterally exposed. Electric stimulators were placed proximally and distally to the defect and a recording electrode was placed in the Gastrocnemius muscle 10 mm below the tibial tubercle. A similar procedure was undertaken in the normal limb. Supramaximal electrical stimuli were delivered proximal to the nerve repair site or the respective intact nerve location by a Grass-SD-9 stimulator at a frequency of 100 Hz for 0.6 msec and the gastrocnemius electrode was recording transmitted evoked potentials.

The ratio of the compound muscle action potential between the operated and the normal limb (p-ratio) was recorded [1, 2]. Following contractility measurement the nerve specimens were excised and processed for light microscopy and immunochemistry as described above. The quantitative histomorphometric and electromyographic data were statistically compared using ANOVA and the significance level was set at $p = 0.05$.

Results

Regarding Gastrocnemius contractility the amplitude of the motor response in mV was expresses as the ratio between the operated and the normal side. The amplitude of the gastrocnemius muscle contraction ranged between 5.3 ± 1.2 and 21.8 ± 3.9 mV. The gastrocnemius contractility after 13 weeks compared to the contralateral normal leg was 60.3, 42.1 and 58.7% in groups A, B and C respectively, while in the control group D was 64.1%. The difference between these parameters was statistically not significant ($p = 0.10$).

Histomorphometric results

Employing light microscopy the number of myelinated axons was in group A and B 55 and 43% of the normal, contralateral nerve or 81 and 68% of the control group values. In the control group the respective value was 68% of the normal contralateral sciatic nerve. The difference was between groups A, B and D was statistically highly significant ($p < 0.001$). The mean axonal diameter was in group A and B 59 and 45% of the normal, contralateral nerve or 78 and 62% of the control group values. In the control group this parameter reached 71% of the normal nerve value. The difference was between groups A, B and D was statistically highly significant ($p < 0.001$). On microscopy examination several findings were evident. Three weeks after the operation on microscopy examination of the regenerated nerve presence of myelin sheaths was evident throughout the nerve section with extensive areas of connective tissue between the axons. In the longitudinal sections new myelinated axons could be seen throughout the conduit, which appeared thicker at the proximal third of the conduit. At 6 weeks the myelin sheaths were thicker than before and there was a clear tendency to mini-fasciculation in cross sections (Fig. 3). After 13 weeks the axons constituted a new structure closely resembling the normal nerve. Using immunocytochemistry the epineural conduit was filled with fibrin and fibronectin as part of the healing process, while S100 stain for Schwann cells was positive. Bunger bands (lSchwann cells) appeared in the third week along the conduit. Schwann cell proliferation preceded axonal growth. The proximal $2/3$ of the new structure showed staining of neurofilament proteins. The proximal third was well stained while the second one was slightly stained (progressive axon advancement). At 6 weeks fibrin and fibronectin are present. The conduit is occupied by stained neurofilaments at 13 weeks (Fig. 4).

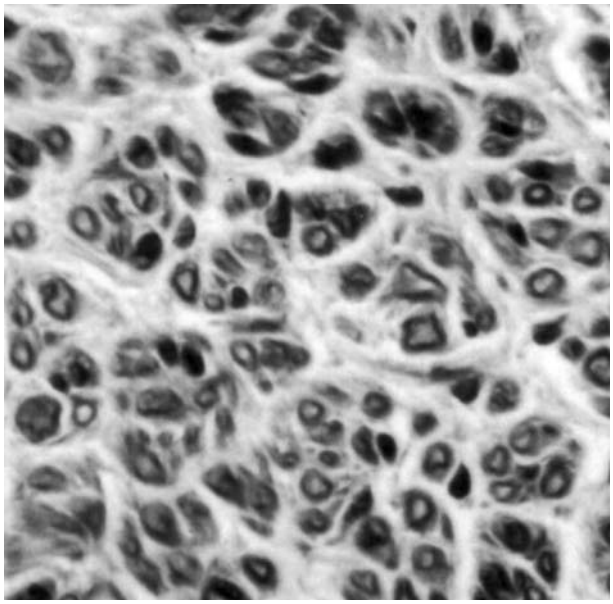

Fig. 3. Myelin sheaths stain with toluidine blue, 3 months postoperative. There is a tendency to mini-fasciculation in cross sections (magnification ×25)

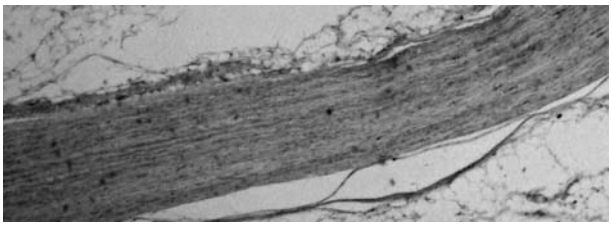

Fig. 4. The conduit is occupied by stained neurofilaments at 3 months (magnification ×25)

Discussion

A short nerve defect was bridged using various epineural flaps. The results of these conduits were comparable with those provided by nerve grafting. The principles of nerve injury have been refined based on an well understanding of nerve biology [4, 6–8]. Harvesting of the epineurium does not hinder nerve function. The epi-

neurium is a connective tissue, which surrounds the fascicles, while carrying the blood supply. From the epineurial plexus vessels arise, running between the nerve fibers. The epineurium defines fascicular groups [4]. To bridge a nerve gap represent a great challenge in surgery [1–7]. When the nerve defect was bridged with a free epineurial flap the resultant nerve regeneration approximated the results of the control group, achieving 93% for muscle contractility and 81% for the microscopy assessed parameters. In the same group the regenerated nerve reached 60.33% of the normal values concerning the contractility force and 55% concerning the parameters of the microscopy evaluation. The regeneration proceeded in a fashion with the progressive axonal maturation. When the epineurium flap was harvested from the distal stump the results were inferior compared to the proximally harvested flap, due to affecting nerve regeneration in significant way. In the latter flap the contractility of the injured side Gastrocnemius reached 42.1% of the normal gastrocnemius contractility. Using a distally attached epineural flap (C) did not improve the results.

Conclusions

The typical nerve grafting provides the best results regarding muscle response and neural regeneration. An epineurial flap may alternatively used to bridge short nerve defects and take advantage of the lesser donor site morbidity. The proximally harvested epineural advancement flap provides comparable results with the nerve graft.

References

1. Chiu DT, Lovelace RE, Yu LT, Wolff M, Stengel S, Middleton L, Janecka IP, Krizek TJ (1988) Comparative electrophysiologic evaluation of nerve grafts and autogenous vein grafts as nerve conduits: an experimental study. J Reconstr Microsurg 4(4): 303–312
2. Gravvanis AI, Tsoutsos DA, Tagaris GA, Papalois AE, Patralexis CG, Iconomou TG, Panayotou PN, Ioannovich JD (2004) Beneficial effect of nerve growth factor-7S on peripheral nerve regeneration through inside-out vein grafts: an experimental study. Microsurgery 24(5): 408–415
3. Karacaoglu E, Yuksel F, Peker F, Guler M (2001) Nerve regeneration through an epineural sheath: its functional aspect compared with a nerve and vein grafts. Microsurgery 21: 196–201
4. Lundborg G (1988) Nerve injury and repair. Churchill, Livingstone, pp 33–36
5. Mackinon SE, Dellon AL (1988) A comparison of nerve regeneration across a sural nerve graft and a vascularised pseudosheath. J Hand Surgery 13A: 935–942
6. Malizos KN, Dailiana ZH, Anastasiou EA, Soucacos PN (1997) Neuromas and gaps of sensory nerves of the hands: management using vein conduits. Am J Orthop 26: 481–485
7. Millesi H (1986)The nerve gap. Hand Clin 2: 651–663
8. Nicoli Aldini N, Fini M, Rocca M, Giavaresi G, Giardino R (2000) Guided regeneration with resorbable conduits in experimental peripheral nerve injuries. Int Orthop 24(3): 121–125
9. Pu LQ, Syed SA, Reid M, Patwa H, Goldstein JM, Forman DL, Thomson JG (1999) Effects of nerve growth factor on nerve regeneration through a vein graft across a gap. Plast Reconstr Surg 104(5): 1379–1385
10. Strauch B (2000) Use of nerve conduits in peripheral nerve repair. Hand Clin 16: 123–130
11. Suematsu N (1989) Tubulation for peripheral nerve gap: its history and possibility. Microsurgery 10(1): 71–74
12. Tang JB (1995) Vein conduits with interposition of nerve tissue for peripheral nerve defects. J Reconstr Microsurg 11(1): 21–26
13. Urabe T, Zhao Q, Danielsen N, Lundborg G (1994) Regeneration across a partial defect in rat sciatic nerve encased in a silicon chamber. Scand J Plast Reconstruct Hand Surg 30: 7–15

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End-to-side nerve neurorrhaphy: critical appraisal of experimental and clinical data

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Summary

End-to-side neurorrhaphy (ESN) or terminolateral neurorraphy consists of connecting the distal stump of a transected nerve, named the recipient nerve, to the side of an intact adjacent nerve, named the donor nerve, ''in which only an epineurial window is performed''. This procedure was reintroduced in 1994 by Viterbo, who presented a report on an experimental study in rats. Several experimental and clinical studies followed this report with various and sometimes conflicting results.

In this paper we present a review of the pertinent literature. Our personal experience using a sort of end-to-side nerve anastomosis, in which the donor nerve is partially transected, is also presented and compared with ESN as defined above.

When the proximal nerve stump of a transected nerve is not available, ESN, which is claimed to permit anatomic and functional preservation of the donor nerve, seems an attractive technique, though yet not proven to be effective. Deliberate axotomy of the donor nerve yields results that are proportional to the entity of axotomy, but such technique, though resembling ESN, is an end-to-end neurorrhaphy.

Neither experimental or clinical evidence support liberalizing the clinical use of ESN, a procedure with only an epineurial window in the donor nerve and without deliberate axotomy. Much more experimental investigation needs to be done to explain the ability of normal, intact nerves to sprout laterally. Such procedure appears justified only in an investigational setting.

Keywords: End to side coaptation; neurorrhaphy; epineural window.

Introduction

End-to-side neurorrhaphy (ESN) or terminolateral neurorraphy consists of connecting the distal stump of a transected nerve, named the recipient nerve, to the side of an intact adjacent nerve, named the donor nerve, when only an epineurial window is performed [34]. The cut end of the recipient nerve, therefore, is just put against the intact perineurium of the donor nerve. The technique has been utilized since the late XIX century, though it was abandoned with the introduction of microsurgical techniques when end-to-end nerve coaptation became the standard method of nerve repair. The procedure was reintroduced in 1994 by Viterbo *et al.* [34], who presented a report on an experimental study in rats. Several experimental and clinical studies followed this report with various and sometimes conflicting results.

In this paper we review the available data on ESN and its possible anatomo-physiological bases. Experimental and clinical applications are presented and discussed.

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Anatomo-physiological bases of ESN

Since its reintroduction by Viterbo *et al.* [34], ESN has been extensively studied and various questions have been raised, mostly concerning its anatomo-physiological bases. The efficacy of ESN, its ability to preserve donor nerve function, the necessity of disrupting the nerve connective sheaths of the donor nerve, the mechanism of reinnervation of the recipient nerve, and even the definition of the procedure have indeed all been questioned [27].

Are the nerve connective sheaths a barrier?

Epineurium

The epineurium is the outer connective sheath of the nerve, being areolar between the fascicles and more condensed around the nerve trunk. It is formed by bundles of collagen fibres, 60–100 nm in diameter, mostly

aligned longitudinally. The largest nerve vessels, lymphatics and nervi nervorum are in this sheath [12, 29].

In an experimental model of end-to-side nerve connection, using fibrin glue, Bertelli *et al.* [5] attached the cut end of a recipient nerve against the intact epineurium of a donor nerve. No functional motor or sensory recovery was obtained with such a model. Considering that fibrin glue is effectively used in end-to-end nerve coaptation [28], the epineurium should be considered a barrier to reinnervation.

A certain amount of reinnervation of the recipient nerve has been demonstrated in ESN when an epineurial window is created in the donor nerve at the level of the connection site. To improve the results of ESN, some Authors [24, 38] have indeed suggested the creation of a large epineurial window in the donor nerve.

Perineurium

The perineurium is a dense connective sheath, 1.3–100 μm thick, which surrounds the fascicles. It is composed of three concentric layers: 1) internal: a layer of flattened perineurial cells with tight junctions; 2) intermediate: 3–15 concentric lamellae of flattened perineurial cells with long processes, tight junctions and basement membrane fusion, interdispersed with packed collagen fibers, 40–65 nm in diameter, mostly longitudinal, forming a double, spiral compact network; 3) external: a layer of gradual transition from perineurium to epineurium with thicker collagen fibres, perineurial cells interdispersed and replaced by epineurium fibroblasts [29].

Although initial studies did not document any difference between end-to-side connections, with or without a perineurial window in the donor nerve [33, 41], subsequent studies suggested that a perineurial window is a prerequisite for effective nerve regeneration into the recipient nerve. Walker *et al.* [36] reported that a large (5 mm) perineurial window induced greater collateral sprouting or regenerative response than a small (1 mm) perineurial window, apparently without increasing cross sectional nerve injury or delaying functional recovery. Different Authors [3, 22, 39] reported a clear difference in the ultrastructural analysis of the site of nerve connection according to the presence or absence of an epi-perineurial window in the donor nerve. Regenerating axons in the recipient nerve were indeed seen only when an epi-perineurial window was performed in the donor nerve [3, 22].

Comparing epineurial and perineurial sutures, a significant increase in axonal regeneration was seen when perineurial sutures were used [3].

Endoneurium

The endoneurium is composed of fibroblasts and collagen fibres arranged mostly longitudinally, closely packed around axons and Schwann cells [29].

After making a simple perineurial window in the donor nerve, regenerated axons in the recipient nerve were significantly fewer than after using a perineurial window plus interruption of a number of axons, within their endoneurial tubes, in the donor nerve. Furthermore, the greater the number of axons injured in the donor nerve, the greater the axon regeneration response in the recipient nerve [22].

What type of axons regenerate?

After both experimental and clinical ESN [11, 15, 17, 19, 26, 30, 35], many Authors reported that sensory axon regeneration occurred alone or with significantly minor motor axon regeneration. Lutz *et al.* [17] reported that after ESN with a perineurial window, functional motor recovery was on average 70%, as compared to end-to-end neurorraphy, but satisfying functional results were unpredictable. When ESN was compared to end-to-end neurorraphy [16], the latter showed the best functional motor recovery.

When an axon count was performed after peroneal-tibial nerve ESN in the rabbit model, the number of regenerated axons appeared too low to permit any functional recovery [13].

What is the origin of regenerated axons in the recipient nerve?

Viterbo *et al.* [34] defined ESN as the connection of the cut end distal stump of the transected nerve (the peroneal) to an epineurial window opened on the side of an intact regional nerve (the tibial) in a rat model. This model, which is the most common in the literature, raised the possibility that regenerated axons could be provided by the proximal stump of the transected nerve, a phenomenon described as ''invasion'' or ''contamination'' [2] (Fig. 1A) and demonstrated by McCallister *et al.* [20]. To prevent such contamination, the proximal stump of the transected nerve has to be either directed away from the anastomosis site or sealed; the end-to-side coaptation site must also be sealed. Collateral sprouting of motor end units from other intramuscular nerves or cross innervation of the target muscle may be other possible sources of muscle reinnervation after ESN [26].

Table 1. *A summary of articles using double-labeling techniques to investigate the origin of regenerating axons in the recipient nerve*

Author, year	Donor/recipient nerves (rat model)	ESN technique	Double-labeled neurons (time of evaluation)
Zhang *et al.* (1999) [40]	tibial to peroneal nerve	epineurial window epi-perineurial window	double labelled motor and sensory neurons (8–12 months)
Lutz *et al.* (2000) [17]	median to muscolocutaneous nerve (1 rat)	perineurial window	double labelled motoneurons (6 months)
Xiong *et al.* (2003) [37]	tibial to peroneal	epineurial window	double labelled motor and sensory neurons (4–6 months)
Aszmann *et al.* (2003) [4]	tibial nerve to saphenous nerve	epineurial window	single labeling of sensory neurons (3 months)
Adelson *et al.* (2004) [1]	tibial to peroneal nerve	epineurial window	double-labelled motor neurons (3 months)
Matsuda *et al.* (2005) [18]	sciatic to peroneal	epineurial window	double-labelled motor and sensory neurons (3.2–5.5% of all labelled cells) (6, 12 and 24 weeks)
Bontioti *et al.* (2005) [7]	musculocutaneous nerve to radial (Group 1) or to both median/ulnar nerves (Group 2)	epineurial window epineurial sutures	*Group 1:* 3 double-labelled cells/380 labelled motoneurons 1.9% double-labeled/31% labelled sensory neurons (15000 cells evaluated) *Group 2:* 9 double-labeled/378 labelled motoneurons 3.3% double-labeled/43% labelled sensory neurons (25000 cells evaluated) (6 months)

Several studies tried to ascertain the origin of the regenerated axons in the recipient nerve after ESN by analyzing the donor nerve and using retrograde labeling techniques. In most studies, retrograde labeling has identified the dorsal ganglia as the origin of most of regenerated axons. Also, figures showing a few motoneurons with single or double labeling have been presented in some papers; though quantitative evaluation was only performed rarely (Table 1).

Is donor nerve function left intact after ESN?

When the issue has been specifically addressed, donor nerve fiber loss has been evident: Cederna *et al.* [8] identified denervated muscle fibres in the donor muscles after ESN; however, the long-term structure or function of the muscles were not affected by the procedure. In a median to ulnar nerve ESN model Papalia *et al.* [25] documented signs of nerve fibre atrophy in the ulnar nerve distal to the ESN point, suggesting the possible occurrence of secondary damage to the donor nerve.

Clinical applications

Results appear to be various. Mennen [21] reported on 56 patients with a variety of conditions, ranging from brachial plexus avulsion to digital nerve lesions, achieving the best results in proximal motor reinnervation (e.g. biceps muscle) and distal sensory reinnervation (e.g. hand skin); he also reported that results are still to some degree unpredictable. Bertelli and Ghizoni [6] reported no recovery after ESN with an epineurial window in five patients: two patients with a C5-C6 avulsion who underwent median nerve to brachialis motor branch ESN; one patient with a radial nerve lesion who underwent median nerve to posterior interosseus nerve ESN; and two patients with a common peroneal nerve lesion, who underwent tibial to peroneal nerve ESN. Bertelli and Ghizoni [6] also observed nerve recovery when a fascicular transfer was performed. Some Authors reported sensory reinnervation with a limited or absent motor recovery in patients with a median nerve lesion treated with an end-to-side median-to-ulnar neurorrhaphy through an epineurial window [23]. Sensory reinnervation in ten traumatic nerve defects at the palm or digit level treated by ESN with an epineurial window showed comparable results with those of nerve grafts or vein conducts [35].

Clinical results are therefore still, unfortunately, contrasting; while some Authors reported excellent results, others reported only sensory reinnervation or no reinnervation at all (Table 2).

Personal experience using a type of "end-to-side" neurorrhaphy

We have used a type of "end-to-side" neurorrhaphy for both experimental studies and for special clinical cases. We never performed solely an epineurial window in the donor nerve on any of these occasions, but always performed a cut of the epineurium, perineurium and a part of the entire contingent of axons. Our type of "end-to-

Table 2. *Major clinical series of ESN*

Author, year	Patients	Donor/recipient nerves	ESN technique	Results
Kayikcioglu *et al.* (2000) [14]	2	median to ulnar	epineurial window epineurial suture	no sensory or motor recovery
Bertelli *et al.* (1996) [5]	5	post interosseus to median: 1 peroneal to tibial: 2 brachialis motor branch to median: 2	epineurial window epineurial suture	no clinical, electrophysiologic and histologic recovery
Mennen (2003) [21]	56	Brachial plexus: 8 ulnaris to medianus: 33 medianus to ulnaris: 7 radialis to medianus: 1 digitalis to digitalis: 5 politeus to post tibialis: 2	epineurial window epineurial suture	successful: 16 partially successful: 10 unsuccessful: 4 early follow-up: 12 default/non compliant: 13 died: 1
Ogun *et al.* (2003) [23]	3	median to ulnar	epineurial window epineurial suture	sensory recovery: 3 motor recovery: 1
Voche and Quattara (2005) [35]	10	digital to digital: 15	epineurial window epineurial suture	point discrimination test: 9.1 (normal: 4.6) moving two-point discrimination test: 7 (normal: 2.6)

side'' neurorraphy was always, therefore, an end-to-end neurorraphy.

Experimental studies

Hypoglossal-facial (HFA) and hemihypoglossal-facial (HHFA) nerve anastomosis in rats

The facial nerve was cut and its distal stump was neurotized using the hypoglossal nerve as donor nerve, either entirely (HFA) or only half (HHFA). Then, a quantitative motoneuron innervation of the facial muscles was evaluated for each one of the two operative options [10]. As expected, when the donor nerve was all of the hypoglossal nerve, the number of motoneurons reinnervating facial muscles was higher than when the donor nerve was only half hypoglossus. It was therefore concluded that HHFA, compared with HFA, increased the risk of obtaining a reinnervation of facial muscles, which could prove insufficient for good functional results.

Facial-oculomotor nerve anastomosis in rats

This study was performed to evaluate the possibility of repairing the oculomotor nerve using part of the facial nerve as the donor nerve. The oculomotor nerve was transected and its distal stump was connected to one third of one branch of the ipsilateral facial nerve by using a nerve autograft [9]. Twelve weeks later the nerve autograft showed numerous regenerated axons that had reinnervated both the medial and superior rectus muscles. Facial-oculomotor nerve anastomosis resulted in a more complex brainstem motoneuron representation than that obtained after end-to-end repair of the transected oculomotor nerve.

Clinical application

Case 1 (BG)

A 29-year-old man with a bilateral post-traumatic facial and abducens palsy was evaluated in our department 15 months after the car accident that had caused an extensive bilateral skull base fracture. Neurological examination documented a complete facial nerve palsy on the left and a House-Brackmann scale grade III deficit on the right. The patient was therefore submitted, in December 2002, to surgery on the left facial nerve. To avoid deficits of other cranial nerves, and considering also the relatively long time of the facial palsy, an hemihypoglossal-facial nerve anastomoses was performed. A 6 cm-long sural nerve was interposed between the half transected hypoglossus and the totally transected facial nerve, at the stylo-mastoid foramen. The autograft was sutured end-to-end to both nerves. At 3 years follow-up the patient showed a House-Brackmann scale grade III deficit on both sides of the face, reaching a good facial symmetry.

Case 2 (BP)

A 48-year-old woman presented at our Centre of Peripheral Nerve Surgery with a post-traumatic radial nerve lesion which had occurred 9 months previously and was documented by EMG examination. Neurological examination documented: right extensor carpi mus-

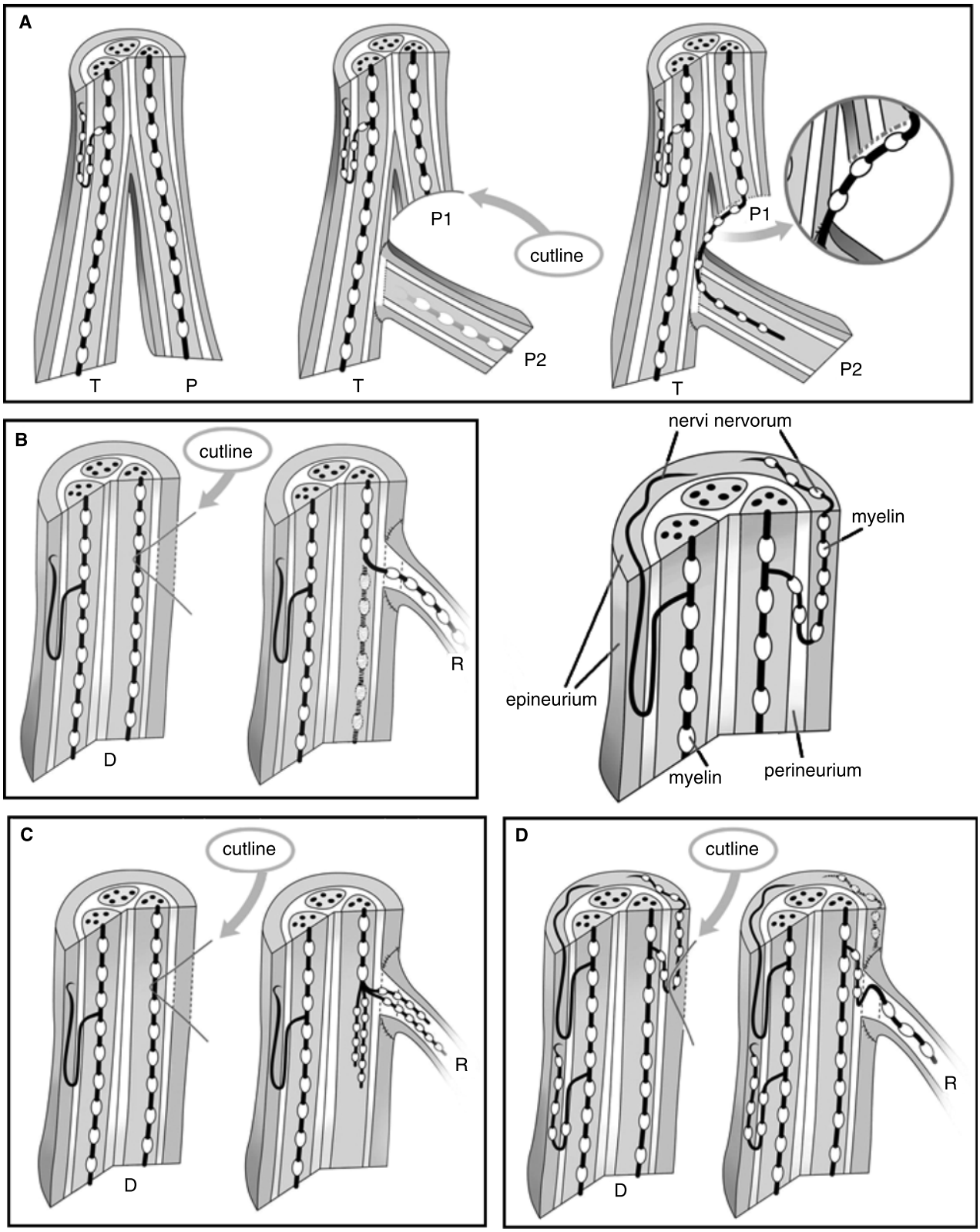

Fig. 1. Schematic drawing of a nerve and possible mechanisms of reinnervation in end-to-side nerve anastomosis. (A) ''Invasion'' or ''contamination'': the proximal stump (*P1*) of the peroneal nerve (*P*) might provide regenerating axons to the distal stump (*P2*) connected end-to side to the tibial nerve (*T*). (B) Section of some axons in the donor nerve (*D*) at the surgery site, during preparation or suture, is possible and can be a reason for regenerating axons in the recipient nerve (*R*). (C) Schematic possible explanation for double labelled motoneurons: transected axons in the donor nerve (*D*) regenerate in both the donor and the recipient nerve (*R*). Injection of two different axonal tracers in the donor and recipient nerve explain double labeling of ganglion neurons and motoneurons. (D) Even in the case of selective interruption of the epineurium in the donor nerve (*D*) nervi nervorum, that are sensory nerves located also in the epineurium, might regenerate into the recipient nerve (*R*)

cles hypotrophia, wrist and fingers extension deficit, right thumb abduction deficit and styloradial areflexia. When previous surgical exploration had been carried out in another institution, nerve repair had not been considered, as the gap between the proximal and distal radial nerve stumps was longer than 20 cm. It was decided, therefore, to neurotize the motor branch of the radial nerve using the median nerve as donor. The terminal motor branch of the radial nerve was dissected and cut proximally to the radial nerve division, after being separated from the superficial radial sensitive nerve. The distal stump of the motor radial nerve was sufficiently long enough to reach and be connected to the donor median nerve where a lateral fascicle was partially transected. At 4 years follow-up, the common extensor digitorum and abductor pollicis longus muscles showed motor (M2) recovery corresponding to an EMG examination documenting reinnervation and voluntary activity of both muscles, spontaneous activity being absent.

Discussion

Neurotization procedures are well known in peripheral nerve surgery. They are used when the proximal stump of a transected nerve is not available, and then, the distal stump can be reinnervated sacrificing an intact local nerve, named the donor nerve. The most known neurotization procedure is the classic hypoglossal-facial nerve anastomosis in which all the trunk of the hypoglossus is cut and its proximal stump is connected end-to-end to the distal facial nerve stump. In such a neurotization operation, the complete loss of the donor nerve function is counterbalanced by a very good recovery of facial nerve function. However, in the neurotization procedures, several attempts have been made to limit the loss of function of the donor nerve. The ESN represents the extreme of such attempts, leaving the donor nerve with intact axons and function and, more importantly, obtaining a functional recovery of the recipient nerve. In fact, it is claimed that an epineurial window in the donor nerve should be sufficient to permit the passage of regenerating axons from the donor to the recipient nerve with consequent effective function recovery. The appeal of such a technique appears evident but, in our opinion, no anatomical or experimental data support this view. Importantly, if we consider the rules that are followed for conventional microsurgical nerve repair, i.e. all the recommendations to align the fascicles of the proximal and distal stump to obtain the best coaptation, ESN should be the most contradictory technique of nerve repair in which a cut end of the recipient nerve is put perpendicular to the donor nerve where only an epineurial window is made. Therefore, either the rules for conventional nerve repair should be considered exaggerated or the anatomical bases of ESN should be further investigated.

It has been postulated [32] that the distal stump of a transected nerve, sutured to an epineurial window at the lateral side of an otherwise intact donor nerve, liberates neurotrophic factors or enzymes. These substances should cause the absorption of the intact connective sheaths, the perineurium and the endoneurium, serving also as a stimulus for lateral axon sprouting.

The distal stump of a transected nerve can be sutured to the lateral side of a donor nerve using a variety of techniques. In fact, in the donor nerve, the epineurium and the perineurium can be left intact or a window can be opened in one or both sheaths; also a variable number of axons can be transected within their endoneurial tubes [27]. As it would be expected, the more invasive and therefore disruptive the procedure is in the donor nerve, the more axon and function regeneration is evident in the recipient nerve [22]. Progressive injury to the connective layers and axons of the donor nerve proportionally increase axon regeneration across the end-to-side anastomosis. Some Authors have indeed suggested a distinction between ESN with or without deliberate axotomy. When a deliberate axotomy is performed, as, for example, in hemihypoglossal-facial nerve anastomosis, though the nerve connection is called end-to-side, in reality it is an end-to-end anastomosis between the hemisected donor hypoglossus and the recipient facial nerve.

After ESN, using an epineurial window in the donor nerve, axons regenerate along the recipient nerve. However, the amount of axon regeneration is variable among the reported papers. Furthermore, the source of such regenerating axons in the recipient nerve represents one of the major issues to be understood. On one side, regeneration is mainly, if not only, of sensory axons. On the other side, it has been demonstrated that the opening of epineurial window and use of stitches cause interruption of axons within the donor nerve [8]. If a partial neurectomy is obtained, ESN must be interpreted as a partial end-to-end neurorrhaphy (Fig. 1B) and the interrupted axons in the donor nerve can regenerate in both the donor and the recipient nerve (Fig. 1C). In this case, a double labeling technique, applying one axonal tracer on the recipient and the other on the donor nerve, can

lead to obtain ganglion neurons and motoneurons that are double labeling [37]. Even accepting the possibility of performing only an isolated epineurial window without any axon interruption in the donor nerve, it must be considered that *nervi nervorum* into the epineurium are axotomized and, therefore, they can give origin to regenerated axons that can enter the endoneurial tubes of the recipient nerve (Fig. 1D). Anatomically, nervi nervorum originate from: a) myelinated axons within the nerve; b) nonmyelinated axons of the perivascular plexus. Horsley described the former as running first at right angles to the nerve and then parallel to it [31]. Nervi nervorum are in plexuses of the epineurium, in the perineurium, and also in the endoneurium. They are sympathetic and sensory axons [12]. The prevalent sensory reinnervation evident after ESN might then also be explained by the disruption of nervi nervorum, as well as by the increased ability of sensory axons to regenerate.

Conclusions

When the proximal nerve stump is not available, ESN with anatomic and functional preservation of the donor nerve is an attractive technique, though yet not proven to be effective. Deliberate axotomy of the donor nerve yields results that can be expected, according to the entity of axotomy, and this technique should be considered a partial end-to-end neurorrhaphy.

Neither experimental nor clinical evidence support liberalizing the clinical use of ESN, a procedure with only an epineurial window in the donor nerve and without deliberate axotomy. Much more experimental investigation needs to be done to explain the ability of normal, intact nerves to sprout laterally. Such procedure is justified only in an investigational setting.

References

1. Adelson PD, Bonaroti EA, Thompson TP, Tran M, Nystrom NA (2004) End-to-side neurorrhaphies in a rodent model of peripheral nerve injury: a preliminary report of a novel technique. J Neurosurg 101 Suppl 1: 78–84
2. Al-Qattan MM (2001) Terminolateral neurorrhaphy: review of experimental and clinical studies. J Reconstr Microsurg 17: 99–108
3. Al-Qattan MM, al-Thunyan A (1998) Variables affecting axonal regeneration following end-to-side neurorrhaphy. Br J Plast Surg 51: 238–242
4. Aszmann OC, Korak KJ, Rab M, Grunbeck M, Lassmann H, Frey M (2003) Neuroma prevention by end-to-side neurorraphy: an experimental study in rats. J Hand Surg 28: 1022–1028
5. Bertelli JA, dos Santos AR, Calixto JB (1996) Is axonal sprouting able to traverse the conjunctival layers of the peripheral nerve? A behavioral, motor, and sensory study of end-to-side nerve anastomosis. J Reconstr Microsurg 12: 559–563
6. Bertelli JA, Ghizoni MF (2003) Nerve repair by end-to-side coaptation or fascicular transfer: a clinical study. J Reconstr Microsurg 19: 313–318
7. Bontioti E, Kanje M, Lundborg G, Dahlin LB (2005) End-to-side nerve repair in the upper extremity of rat. J Peripher Nerv Syst 10: 58–68
8. Cederna PS, Kalliainen LK, Urbanchek MG, Rovak JM, Kuzon WM Jr (2001) "Donor" muscle structure and function after end-to-side neurorrhaphy. Plast Reconstr Surg 107: 789–796
9. Fernandez E, Di Rocco F, Lauretti L, Gangitano C, Del Fa A, Massimi L, Maira G, Pallini R (2003) Reinnervation of extraocular muscles by facial-to-oculomotor nerve anastomosis in rats: anatomic nuclear changes. Neurosurgery 53: 409–414
10. Fernandez E, Lauretti L, Denaro L, Montano N, Doglietto F, Novegno F, Falchetti ML, Tufo T, Maira G, Pallini R (2004) Motoneurons innervating facial muscles after hypoglossal and hemihypoglossal-facial nerve anastomosis in rats. Neurol Res 26: 395–400
11. Goheen-Robillard B, Myckatyn TM, Mackinnon SE, Hunter DA (2002) End-to-side neurorrhaphy and lateral axonal sprouting in a long graft rat model. Laryngoscope 112: 899–905
12. Hromada J (1963) On the nerve supply of the connective tissue of some peripheral nervous system components. Acta Anat (Basel) 55: 343–351
13. Jaberi FM, Abbas BP, Nezhad ST, Tanideh N (2003) End-to-side neurorrhaphy: an experimental study in rabbits. Microsurgery 23: 359–362
14. Kayikcioglu A, Karamursel S, Agaoglu G, Kecik A, Celiker R, Cetin A (2000) End-to-side neurorrhaphies of the ulnar and median nerves at the wrist: report of two cases without sensory or motor improvement. Ann Plast Surg 45: 641–643
15. Liu HJ, Dong MM, Chi FL (2005) Functional remobilization evaluation of the paralyzed vocal cord by end-to-side neurorrhaphy in rats. Laryngoscope 115: 1418–1420
16. Liu K, Chen LE, Seaber AV, Goldner RV, Urbaniak JR (1999) Motor functional and morphological findings following end-to-side neurorrhaphy in the rat model. J Orthop Res 17: 293–300
17. Lutz BS, Chuang DC, Hsu JC, Ma SF, Wei FC (2000) Selection of donor nerves – an important factor in end-to-side neurorrhaphy. Br J Plast Surg 53: 149–154
18. Matsuda K, Kakibuchi M, Fukuda K, Kubo T, Madura T, Kawai K, Yano K, Hosokawa K (2005) End-to-side nerve grafts: experimental study in rats. J Reconstr Microsurg 21(8): 581–591
19. Matsumoto M, Hirata H, Nishiyama M, Morita A, Sasaki H, Uchida A (1999) Schwann cells can induce collateral sprouting from intact axons: experimental study of end-to-side neurorrhaphy using a Y-chamber model. J Reconstr Microsurg 15: 281–286
20. McCallister WV, Tang P, Trumble TE (1999) Is end-to-side neurorrhaphy effective? A study of axonal sprouting stimulated from intact nerves. J Reconstr Microsurg 15: 597–603
21. Mennen U (2003) End-to-side nerve suture in clinical practice Hand Surg 8: 33–42
22. Noah EM, Williams A, Jorgenson C, Skoulis TG, Terzis JK (1997) End-to-side neurorrhaphy: a histologic and morphometric study of axonal sprouting into an end-to-side nerve graft. J Reconstr Microsurg 13: 99–106
23. Ogun TC, Ozdemir M, Senaran H, Ustun ME (2003) End-to-side neurorrhaphy as a salvage procedure for irreparable nerve injuries. Technical note. J Neurosurg 99: 180–185
24. Ozmen S, Latifoglu O, Ayhan S, Yavuzer R, Nurlu G, Sezer C, Atabay K (2004) Impact of epineurial excision of the distal recipient nerve in terminolateral neurorrhaphy. J Reconstr Microsurg 20: 385–397
25. Papalia I, Geuna S, Tos PL, Boux E, Battiston B, Stagno D'Alcontres F (2003) Morphologic and functional study of rat median nerve re-

pair by terminolateral neurorrhaphy of the ulnar nerve. J Reconstr Microsurg 19: 257–264
26. Robillard BG, Mackinnon SE (2000) Invited Discussion to: Okajima S, Terzis JK Ultrastructure of early axonal regeneration in an end-to-side neurorrhaphy model. J Reconstr Microsurg 16: 323–325
27. Rovak JM, Cederna PS, Kuzon WM Jr (2001) Terminolateral neurorrhaphy: a review of the literature. J Reconstr Microsurg 17: 615–624
28. Sames M, Blahos J Jr, Rokyta R, Benes V Jr (1997) Comparison of microsurgical suture with fibrin glue connection of the sciatic nerve in rabbits. Physiol Res 46: 303–306
29. Sunderland S (1970) Anatomical features of nerve trunks in relation to nerve injury and nerve repair. Clin Neurosurg 17: 38–62
30. Tarasidis G, Watanabe O, Mackinnon SE, Strasberg SR, Haughey BH, Hunter DA (1998) End-to-side neurorraphy: a long-term study of neural regeneration in a rat model. Otolaryngol Head Neck Surg 119: 337–341
31. Vilensky JA, Gilman S, Casey K (2005) Sir Victor Horsley, Mr John Marshall, the nervi nervorum, and pain – more than a century ahead of their time. Arch Neurol 62: 499–501
32. Viterbo F (1999) Invited Discussion to: McCallister WV, Tang P, Trumble TE. Is end-to-side neurorrhaphy effective? A study of axonal sprouting stimulated from intact nerves. J Reconstr Microsurg 15: 603–604
33. Viterbo F, Teixeira E, Hoshino K, Padovani CR (1998) End-to-side neurorrhaphy with and without perineurium. Sao Paulo Med J 116: 1808–1814
34. Viterbo F, Trindade JC, Hoshino K, Mazzoni Neto A (1994) End-to-side neurorrhaphy with removal of the epineurial sheath: an experimental study in rats. Plast Reconstr Surg 94: 1038–1047
35. Voche P, Ouattara D (2005) End-to-side neurorrhaphy for defects of palmar sensory digital nerves. Br J Plast Surg 58: 239–244
36. Walker JC, Brenner MJ, Mackinnon SE, Winograd JM, Hunter DA (2004) Effect of perineurial window size on nerve regeneration, blood-nerve barrier integrity, and functional recovery. J Neurotrauma 21: 217–227
37. Xiong G, Ling L, Nakamura R, Sugiura Y (2003) Retrograde tracing and electrophysiological findings of collateral sprouting after end-to-side neurorrhaphy. Hand Surg 8: 145–150
38. Yan JG, Matloub HS, Sanger JR, Zhang LL, Riley DA, Jaradeh SS (2002) 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] 27: 484–492
39. Zhang Z, Soucacos PN, Beris AE, Bo J, Ioachim E, Johnson EO (2000) Long-term evaluation of rat peripheral nerve repair with end-to-side neurorrhaphy. J Reconstr Microsurg 16: 303–311
40. Zhang Z, Soucacos PN, Bo J, Beris AE (1999) Evaluation of collateral sprouting after end-to-side nerve coaptation using a fluorescent double-labeling technique. Microsurgery 19: 281–286
41. Zhao JZ, Chen ZW, Chen TY (1997) Nerve regeneration after terminolateral neurorrhaphy: experimental study in rats. J Reconstr Microsurg 13: 31–37

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Ingrowth of sensory axons into an end-to-side coapted nerve stump after donor nerve crush in the rat

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Summary

Background. Target innervation through an end-to-side (ETS) nerve coaptation depends on axonal sprouting from the donor nerve. Terminal axonal sprouting in a partially denervated target tissue is more extensive from a crushed donor nerve than from an intact donor nerve. We hypothesized that axonal sprouting into an ETS coapted recipient nerve could be stimulated by crushing the donor nerve.

Method. Twenty-seven rats were randomised into 3 groups. In all, the distal stump of the transected peroneal nerve was sutured to the side of the sural nerve in place of the epineural window. The control group received no additional treatment. In the experimental groups, the sural donor nerve was crushed either 8 mm proximal (proximal crush group) or 8 mm distal to the coaptation site (distal crush group). Sixteen weeks after the surgery, histomorphometric analysis of the recipient peroneal nerve stump 4 mm distal to the coaptation site was performed.

Findings. The number of myelinated axons in the recipient peroneal nerve stump was 758 ± 247 in the control group, 503 ± 246 in the distal crush group and 211 ± 96 in the proximal crush group. The differences between the groups were statistically significant ($p < 0.05$). The majority of myelinated axons were thin myelinated axons and the frequency distribution of their cross-sectional areas was similar in all groups.

Conclusion. Contrary to our expectations, a significantly lower number of myelinated axons were present in recipient nerves in the proximal and distal crush groups than in the control group. This suggests that sensory axon ingrowth into an ETS coapted nerve cannot be enhanced by crushing the donor nerve.

Keywords: Nerve regeneration; end-to-side nerve coaptation; collateral sprouting; nerve crush; rat; sensory neurons.

Introduction

The concept of end-to-side nerve (ETS) coaptation was revived over the last 15 years as a potential posttraumatic peripheral nerve repair technique. Motor as well as sensory axons grow from the donor nerve into the ETS coapted peripheral nerve stump and functionally reinnervate the respective target tissue [3]. As shown by retrograde double labeling, collateral sprouting is an important mechanism of axon growth into the recipient nerve [2, 6, 8, 11]. The other potential mechanism is the regeneration of the unintentionally injured axons of the donor nerve during the surgery [7].

After peripheral nerve crush, the number of regenerating axons is increased distal to the lesion site, and nerve cell bodies are shifted into a growth supporting state [1, 4]. Accordingly, the number of axons in the recipient nerve increased if the mixed donor nerve was crushed proximal to the site of ETS coaptation [7]. Similarly, the terminal sprouting of regenerating nociceptive axons was more extensive than collateral sprouting of an uninjured nerve in response to partial skin denervation in the rat [10].

We hypothesized that the crush injury of the donor nerve at the time of ETS nerve coaptation would increase the ingrowth of sensory axons into the recipient nerve by increasing the number of axons available for sprouting/regeneration and/or by increasing their capacity for growth by priming of nerve cell bodies of the donor nerve.

Methods and materials

Twenty-seven male albino rats (Wistar, Medical Experimental Center, Medical Faculty, Ljubljana, Slovenia), weighing 280–310 g at the beginning of the study, were used. All surgical procedures were performed under deep anesthesia with a mixture of dihydrothiazine and ketamin hydrochloride (Rompun, Bayer AG, Leverkusen, Germany, 8 mg/kg; Ketalar, Parke-Davis & GmbH, Berlin, Germany, 60 mg/kg; i.p.). The peroneal nerve was transected and its proximal stump was ligated (Ethibond 7/0, ETHICON, Edinburgh, UK) and implanted in the nearby muscles in order to prevent their regeneration. The distal stump was sutured to the side of the ipsilateral sural nerve (ETS nerve coaptation) at

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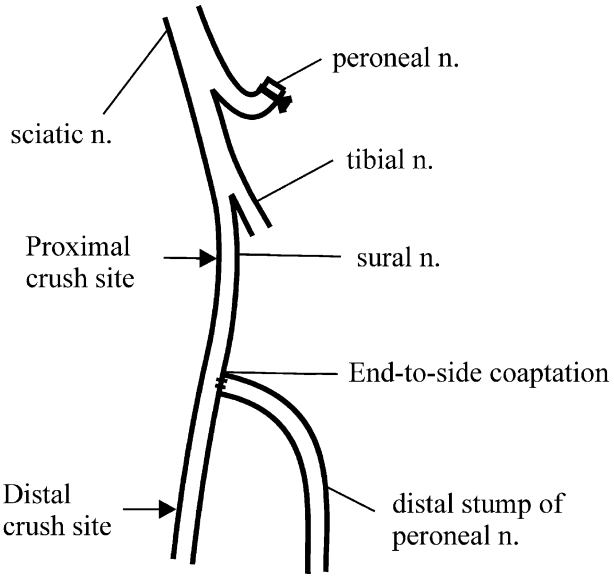

Fig. 1. Schematic drawing of the surgical procedures. The proximal end of the distal stump of the peroneal nerve was sutured to the epineural window on the side of the sural nerve (end-to-side nerve coaptation). The control group received no additional treatment. In the experimental groups, the sural donor nerve was crushed either 8 mm proximal (proximal crush group) or 8 mm distal to the coaptation site (distal crush group)

the level of the popliteal vein by using four epineurial sutures (Ethilon 11/0, ETHICON, Edinburgh, UK). An epineural window was created in the sural nerve at the site of coaptation. The control group received no additional treatment ($n=9$). In the experimental groups, the sural donor nerve was crushed either 8 mm proximal (proximal crush group, $n=9$) or 8 mm distal to the coaptation site (distal crush group; $n=9$) with watchmaker's forceps for 10 sec (Fig. 1). After 16 weeks, short nerve segments were taken from the recipient (peroneal) nerve 4 mm distal to the coaptation site. Semi-thin nerve cross-sections were processed for visualization of myelinated axons as described earlier [5]. Morphometric analysis of myelinated axons was performed by a Microcomputer Imaging Device program (Imaging Research Inc., Brock University, St. Catharines, Ontario, Canada), which allowed us to digitalize the picture obtained by a light microscope (Zeiss-Opton, Opton Feintechnik GmbH, Oberkochen, Germany). The differences among the mean values of the control and experimental groups were tested using ANOVA with post-hoc Bonferroni *t*-test.

Results

The number of myelinated axons in the ETS coapted peroneal nerve stump was 758 ± 247 in the control, 503 ± 246 in the distal crush group and 211 ± 96 in the proximal crush group (Fig. 2). The differences between the groups were statistically significant ($p<0.05$). The percentage of myelinated axons with their cross-sectional areas smaller than 20 μm^2 (diameter <5 μm) was 90% in the control, 86% in the distal crush group and 86% in the proximal crush group. The differences between the groups were not statistically significant ($p>0.05$).

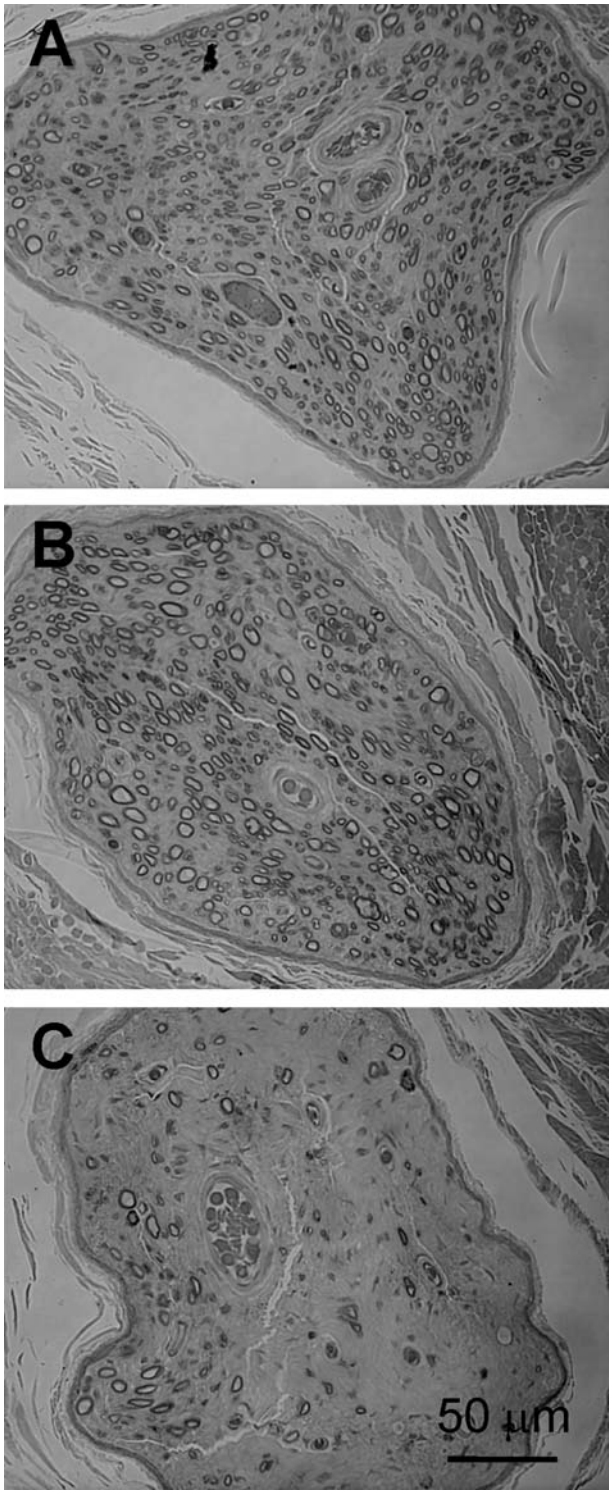

Fig. 2. Photomicrographs showing myelinated axons in the cross sections of the recipient peroneal nerve stump taken 4 mm distal from the site of its coaptation to the side of the sural nerve (end-to-side nerve coaptation) 16 weeks after surgery. The control group (A) received no additional treatment. In the experimental groups, the sural donor nerve was crushed either 8 mm distal (B-distal crush group) or 8 mm proximal to the coaptation site (C-proximal crush group)

Discussion

Contrary to our expectations, we found significantly less myelinated axons in the recipient nerve if the donor nerve had been crushed either proximal or distal to the ETS nerve coaptation site in comparison to the controls.

An earlier study demonstrated that the crush of the donor nerve proximal to the site of the ETS nerve repair did not enhance the recovery of the reinnervated muscle weight although it increased the axonal density in the recipient nerve [7]. In that study, the distal stump of the peroneal nerve was sutured to the tibial nerve which is a mixed nerve and results relate mostly to motor axons. In our study, the distal stump of the peroneal nerve was sutured to the side of the sural nerve, in which more than 93% of myelinated axons are sensory [9]. The ingrowth of sensory axons into the recipient nerve after ETS nerve repair is more extensive in comparison to motor axons [2, 8]. In many respects both kinds of axons behave similarly after injury [1, 4]. Therefore, it is less likely that the ability of the sensory axons to sprout their collaterals into the recipient nerve after a donor nerve crush is lower in comparison to the motor axons. Interestingly, we found more myelinated axon sprouts in the recipient nerve if the donor nerve had been crushed distal to the site of ETS coaptation than proximal to it. Therefore, it is possible that the collateral sprouting of sensory axons into an ETS coapted recipient nerve can begin only after the axons of the donor nerve had reinnervated their original targets.

We conclude that myelinated sensory axons ingrowth into an ETS coapted nerve cannot be enhanced, but may be hindered, by crushing the donor nerve at the time of nerve repair.

References

1. Bisby MA, Pollock B (1983) Increased regeneration rate in peripheral nerve axons following double lesions: enhancement of the conditioning lesion phenomenon. J Neurobiol 14: 467–472
2. Bontioti E, Kanje M, Lundborg G, Dahlin LB (2005) End-to-side nerve repair in the upper extremity of rat. J Peripher Nerv Syst 10: 58–68
3. Geuna S, Papalia I, Tos P (2006) End-to-side (terminolateral) nerve regeneration: a challenge for neuroscientists coming from an intriguing nerve repair concept. Brain Res Brain Res Rev (Epub ahead of print)
4. Jenq CB, Coggeshall RE (1987) Single vs multiple somatic nerve crushes in the rat. Brain Research 409: 250–258
5. Kovačič U, Sketelj J, Bajrović FF (2003) Sex related difference in collateral sprouting of nociceptive axons after peripheral nerve injury in the rat. Exp Neurol 184: 479–488
6. Kubek T, Kyr M, Haninec P, Samal F, Dubovy P (2004) Morphological evidence of collateral sprouting of intact afferent and motor axons of the rat ulnar nerve demonstrated by one type of tracer molecule. Ann Anat 186: 231–234
7. McCallister WV, Tang P, Trumble TE (1999) Is end-to-side neurorrhaphy effective? A study of axonal sprouting stimulated from intact nerves. J Reconstr Microsurg 15: 597–604
8. Sananpanich K, Morrison WA, Messina A (2002) Physiologic and morphologic aspects of nerve regeneration after end-to-end or end-to-side coaptation in a rat model of brachial plexus injury. J Hand Surg (Am) 27: 133–142
9. Swett JE, Torigoe Y, Elie VR, Bourassa CM, Miller PG (1991) Sensory neurons of the rat sciatic nerve. Exp Neurol 114: 82–103
10. Wiesenfeld-Hallin Z, Kinnman E, Aldskogius H (1988) studies of normal and expansive cutaneous innervation territories of intact and regenerating C-fibres in the hindlimb of the rat. Agents and Actions 25: 260–262
11. Zhang Z, Soucacos PN, Bo J, Beris AE (1999) Evaluation of collateral sprouting after end-to-side nerve coaptation using a fluorescent double-labeling technique. Microsurgery 19: 281–286

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Which myelinated sensory axons sprout into an end-to-side coapted peripheral nerve in the rat?

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Summary

Background. The high-threshold sensory afferents, which express trkA, are predominantly involved in terminal collateral sprouting in the skin of adult mammals. We explored which sensory axons are capable of sprouting into the end-to-side coapted nerve in the rat.

Method. The distal stump of the transected peroneal nerve was sutured to the side of the uninjured sural nerve. After 36 weeks, sprouting of sensory axons into the end-to-side coapted nerve was assessed by the electrophysiologic measurements of compound action potential and by counting the myelinated axons. The neurons in the dorsal root ganglia (DRG) L5 whose axons sprouted into the end-to-side coapted nerve were retrogradely labelled by the fluorescent dye Fluorogold. The expression of trkA in sprouting DRG neurons was investigated by immunohistochemistry.

Findings. Predominantly thin myelinated axons were found in the end-to-side coapted peroneal nerve. Their mean conduction velocity (CV) was between the average CVs of the Aδ and Aβ fibres in the normal sural nerves. About 90% of the sprouting DRG neurons were small and medium sized, and about 10% were large. About 85% of sprouting DRG neurons was immunoreactive to trkA, but the rest were not.

Conclusions. Mostly the high-threshold sensory afferents sprouted into the end-to-side coapted nerve, which resembles the collateral sprouting of sensory axons in the skin. However, our results suggest that also some low-threshold mechanoreceptors can sprout after the end-to-side nerve repair.

Keywords: Peripheral nerve; sensory neurons; collateral sprouting; rat.

Introduction

After a peripheral nerve injury, the uninjured adjacent motor as well as sensory axons are able to sprout their terminal collateral branches into a partly denervated foreign target tissue. This process, known as collateral sprouting, is the only mechanism by which reinnervation of the denervated tissue can occur in situations in which injured peripheral nerve axons are unable to regenerate. During the last decade, the retrograde double labelling of neurons in the dorsal root ganglia (DRG) and spinal cord revealed that uninjured sensory as well as motor neurons are able to sprout collateral branches also more proximally in the course of the peripheral nerve trunk into the end-to-side coapted nerve segment [1, 9]. However, it is not known which kind of sensory axons are able to sprout after the end-to-side nerve repair. Different ability of various types of sensory nerve fibres for collateral sprouting is of great importance for eventual clinical application of the end-to-side nerve repair in patients. It is interesting in this regard that in adult mammals only the unmyelinated and high-threshold myelinated afferents, which express trkA, are involved in terminal sprouting in the skin [4]. Therefore, we hypothesised that among myelinated axons only the thin Aδ fibres are able to sprout their collaterals into an end-to-side coapted peripheral nerve.

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Methods and materials

The peroneal, tibial, and saphenous nerves were transected and their proximal stumps were ligated in the right thigh of the adult female Wistar rats ($n = 13$). The distal peroneal nerve stump was sutured to the side of the intact ipsilateral sural nerve (end-to-side nerve coaptation) by four epineurial sutures (Ethilon 11/0), and the animals were left to recover for 36 weeks. At the end of the experiment, the presence of functional nociceptive axons in the end-to-side coapted nerve stump was assessed by the nerve pinch test [7, 8], and the animals with pinch positive response were randomly divided into two groups. In 5 animals, the segments of the sural nerve with the end-to-side coapted peroneal nerve were excised, placed on the wire electrodes and immersed in liquid paraffin ($37 \pm 1\,^{\circ}C$) in a recording

chamber. After stimulation of the distal end of the recipient peroneal nerve by a square wave supramaximal stimulus (12 V, 10 μs, 1 Hz), compound action potentials (CAPs) of the A fibres were recorded in the donor sural nerve by a data acquisition system (DaqBook 200, Iotech, Inc., USA) at the sampling frequency of 20 kHz. In 8 animals, sensory neurons that sprouted their axons into the distal stump of the recipient peroneal nerve were retrogradely labelled with 3% Fluoro-Gold (FG) (Fluorochrome Inc., USA). One week later, the ipsilateral dorsal root ganglia (DRG) L5 were removed and fixed either in 4% paraformaldehyde ($n=4$) or in buffered formaldehyde ($n=4$) for additional processing for histochemistry and immunohistochemistry, respectively.

The fluorescent FG labelled neurons in serial longitudinal sections of the frozen or paraffin embedded DRG were visualised under a fluorescence microscope (Olympus, IX81) using an appropriate U-MNU2 filter. The frozen DRG sections were then stained by hematoxiline and the FG labelled neurons with the nucleus were counted and their maximal cross areas were measured manually under a light microscope by a computer-based image analysis system. On the sections of the paraffin embedded DRG, trkA immunohistochemistry was performed with rabbit anti-trkA polyclonal antibody (Chemicon Int., USA) diluted 1:500 as described previously [6]. The percentages of FG labelled neurons displaying trkA immunoreactivity were evaluated in each DRG under a light microscope. The semi-thin cross sections of the recipient peroneal nerve in all experimental animals were cut 4 mm distal to the site of coaptation. Their myelinated axons were visualised and analysed morphometrically as described earlier [7, 8].

Results

The nerve pinch test confirmed the presence of functional nociceptive axons in the end-to-side coapted peroneal nerve stump in all the animals. Typically, CAPs recorded from the sural nerves after stimulation of the peroneal nerve segment showed one component corresponding to the myelinated sensory A fibres in all the examined animals ($n=5$). Their mean CV was 22.1 ± 4 m/s. The average number of myelinated axons in the peroneal nerve segments (Fig. 1) was 476 ± 162 ($n=5$) and 91 ± 3% of them had the cross-sectional area of less than 20 μm^2. The average number of sprouting FG labelled neurons in the ipsilateral DRG L5 was 496 ± 264 ($n=4$). About 90% of them were small or medium sized (cross-sectional area <800 μm^2), and about 10% of them were larger (Fig. 2). About 85% of the FG labelled neurons were immunoreactive to trkA antibody, but the rest were not.

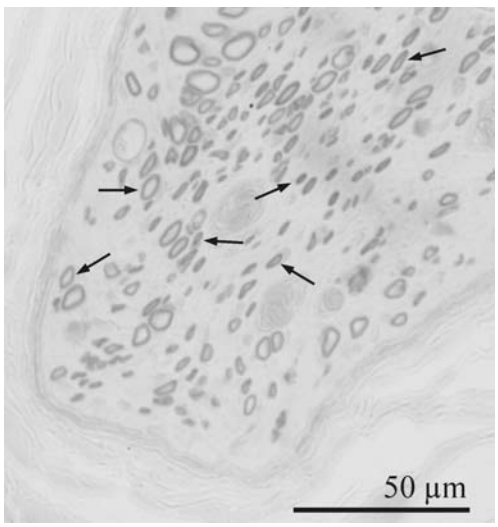

Fig. 1. Representative light photomicrograph of the cross-section of the distal peroneal nerve stump 36 weeks after end-to-side coaptation to the intact sural nerve. Cross-sections were taken about 4 mm distal from the site of coaptation and were stained with Azure Blue. Numerous thin myelinated axons (arrows) are present

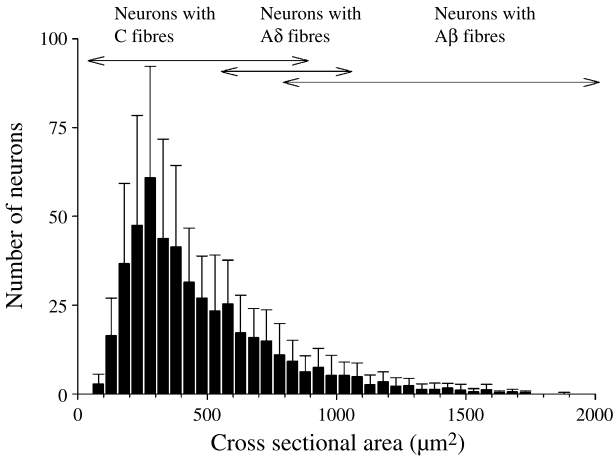

Fig. 2. Frequency histogram of the cross-section areas of FG labelled neurons in the DRG L5 ($n=4$) that sprouted their axons into the distal stump of the end-to-side coapted recipient peroneal nerve 36 weeks after surgery. Classes of neurons with C, Aδ, and Aβ fibres are in accordance with Lawson and co-workers [2, 5]

Discussion

In the rat sural nerve, more than 93% of myelinated axons are sensory ones [10]. Therefore, the majority of the myelinated axons found in the distal stump of the peroneal nerve, coapted to the side of the sural nerve, were sensory axons, which is in accordance with our previous observations [7, 8]. The cross-sectional area of about 90% of myelinated axons in the recipient peroneal nerve stump was less than 20 μm^2 (diameter less than 4 μm), and about 90% of the axons pertained to the small and medium sized (cross-sectional area <800 μm^2) retrogradely labelled DRG neurons. Therefore, the majority of myelinated sensory axons capable of extensive sprouting into the end-to-side coapted nerve segment are small myelinated Aδ axons, thus confirming our hypothesis. However, electrophysiological data suggest that also some thick myelinated Aβ axons can sprout into

the end-to-side coapted nerve since the average CV of the CAPs in the coapted peroneal nerve was higher than in the normal Aδ axons [2]. Accordingly, a small share of the retrogradely labelled sprouting DRG neurons observed in our study were large sized neurons (cross-sectional area $>800\,\mu m^2$), which are regarded as the low-threshold mechanoreceptors with Aa/β fibres [3]. However, there is a considerable overlap in the cell size among DRG neurons with C-, Aδ- and Aa/β-fibres, and there is strong evidence for the existence of myelinated nociceptive afferents with CVs in the Aβ range (for review see [2]). Recently, Fang *et al.* [5] demonstrated that trkA receptor is selectively expressed in nociceptive DRG neurons, both in those with slowly conducting (Aδ and C) as well as with more rapidly conducting (Aa/β) axons. The sprouting DRG neurons in our study were predominately trkA positive and mostly small and medium sized, however, about 15% of retrogradely labelled DRG neurons were trkA negative. The latter most probably belong to the low-threshold mechanoreceptor sensory neurons [5]. Therefore, we conclude that mostly the high-threshold sensory afferents sprouted into the end-to-side coapted nerve, which resembles the collateral sprouting of sensory axons in the skin [4]. However, our results also suggest that some trkA negative low-threshold mechanoreceptors can sprout into the end-to-side coapted distal nerve stump.

References

1. Bontioti E, Kanje M, Lundborg G, Dahlin LB (2005) End-to-side nerve repair in the upper extremity of rat. J Peripher Nerv Syst 10: 58–68
2. Djouhri L, Lawson SN (2004) Abeta-fiber nociceptive primary afferent neurons: a review of incidence and properties in relation to other afferent A-fiber neurons in mammals. Brain Res Brain Res Rev 46: 131–145
3. Doubell TP, Mannion RJ, Woolf CJ (1999) The dorsal horn: state dependent processing, plasticity and the generation of pain. In: Wall PD, Melzack R (eds) Textbook of pain. Churchill Livingstone, Edinburgh, London, pp 165–180
4. Dubovy P, Aldskogius H (1996) Degeneration and regeneration of cutaneous sensory nerve formations. Microsc Res Tech 34: 362–375
5. Fang X, Djouhri L, McMullan S, Berry C, Okuse K, Waxman SG, Lawson SN (2005) trkA is expressed in nociceptive neurons and influences electrophysiological properties via Nav1.8 expression in rapidly conducting nociceptors. J Neurosci 25: 4868–4878
6. Gasperšič R, Kovačič U, Cor A, Skalerič U (2006) Identification and neuropeptide content of trigeminal neurons innervating the rat gingivomucosal tissue. Arch Oral Biol Epub (ahead of print)
7. Kovačič U, Bajrović F, Sketelj J (1999) Recovery of cutaneous pain sensitivity after end-to-side nerve repair in the rat. J Neurosurg 91: 857–862
8. Kovačič U, Sketelj J, Bajrović FF (2003) Sex related difference in collateral sprouting of nociceptive axons after peripheral nerve injury in the rat. Exp Neurol 184: 479–488
9. Kubek T, Kyr M, Haninec P, Samal F, Dubovy P (2004) Morphological evidence of collateral sprouting of intact afferent and motor axons of the rat ulnar nerve demonstrated by one type of tracer molecule. Ann Anat 186: 231–234
10. Peyronnard JM, Charron L (1982) Motor and sensory neurons of the rat sural nerve: a horseradish peroxidase study. Muscle Nerve 5: 654–660

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Functional recovery and mechanisms in end-to-side nerve repair in rats

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Summary

Background. End-to-side nerve repair is attachment of a single distal nerve segment (recipient nerve) end-to-side to an intact donor nerve when there is a lack of proximal nerve segment after injury. The technique is currently used clinically but the mechanism(s) behind this technique are essentially unknown.

Methods. We have studied end-to-side nerve repair in the forelimb of rats, where a single distal radial nerve or an ulnar or a median, or both, nerves are attached end-to-side to an intact musculocutaneous nerve. We have studied functional recovery, origin of the regenerating axons and cell activation by the end-to-side nerve repair.

Findings. Functional recovery occurs after end-to-side nerve repair but is less sufficient than conventional end-to-end nerve repair or a nerve graft procedure. Sensory and motor axons grow from the musculocutaneous nerve out into the attached nerve segment(s). An injury is required to the musculocutaneous nerve to activate sensory and motor neurons as well as Schwann cells in the musculocutaneous nerve for initiation of regeneration.

Conclusions. End-to-side nerve repair may be an alternative method in specific cases of complex nerve injuries to reconstruct nerve trunks when no other repair options are possible. Some functional recovery does occur but regeneration of sensory and motor axons require an injury to the neurons of the donor nerve.

Keywords: End-to-side; nerve injury; paw print; nerve reconstruction.

Introduction

Peripheral nerve injuries in the upper extremity may vary from simple transection of a digital nerve to extensive laceration and avulsion of the spinal nerve roots in the brachial plexus. In many of these injuries conventional surgical methods are available for treatment such as end-to-end nerve repair, nerve grafting and neurotisation. However, in some patients the proximal nerve segment has disappeared and there is a need for other nerve trunks as sources of axons. During the last decade the end-to-side nerve repair has been brought into attention [20]. End-to-side nerve repair is attachment of a single distal nerve segment (the recipient nerve) end-to-side to an intact nerve trunk (the donor nerve) when there is no proximal nerve segment available. Although such procedure is used clinically in several different clinical situations [7, 8], the mechanisms behind the technique have been debated since the first reports by Despres, Kennedy, Balance *et al.* and Harris and Low more than 100 years ago (see review by Al-Qattan [1]). Since the technique was brought up into daylight again 1992 by Viterbo [18], many papers have been published regarding technical aspects [13], motor vs sensory reinnervation [15, 16], the origin of the regenerating axons [10, 14, 17] and various aspects on functional recovery [9, 19]. Various models have also been utilised to examine the merits of end-to-side nerve repair using both the fore- and hind limb in rats. In the present minireview we will summarise results and discuss mechanisms in end-to-side nerve repair in particularly the forelimb of rats based on published papers [2–5].

The surgical procedure

In the forelimb of rats an anterior approach is used to expose the musculocutaneous, the radial, the ulnar and the median nerves. The musculocutaneous nerve is left intact and used as the donor nerve while the radial or the ulnar and median nerves are transected and their distal segments are attached end-to-side to the intact musculocutaneous nerve (Fig. 1). In some experiments a piece of nerve from the hind limb was attached close to the intact musculocutaneous nerve. In all our experimental models an epineurial window was made in the musculocutaneous nerve without creation of a perineurial window.

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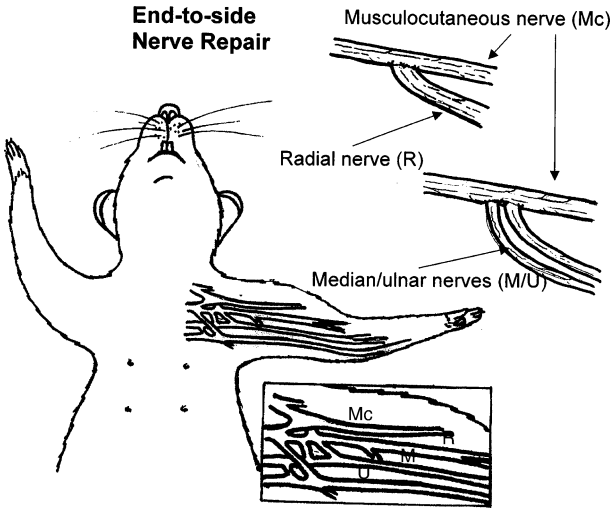

Fig. 1. Schematic drawing of the experimental design where the musculocutaneous nerve is used as a donor nerve. The distal radial or the distal median/ulnar nerve segments are attached end-to-side (Reproduced by kind permission of Journal of the Peripheral Nervous System, Bontioti *et al.* [5])

Axonal outgrowth and functional recovery following end-to-side nerve repair

As indicated above the effectiveness of end-to-side nerve repair has been the subject of discussions. In our model we can see that there is axonal outgrowth in the distal nerve segments of both the radial and the median/ulnar nerves where the axons are assembled into clusters. It is obvious that the musculocutaneous nerve can nourish two distal nerve segments (median/ulnar nerves). In such a model the total number of fibres are significantly higher than if only a single radial nerve is attached end-to-side [5]. These axons are also functionally active since electrical stimulation six months after the repair of the end-to-side attached radial or median/ulnar nerves result in contraction of muscles in the forearm innervated by these nerves. The tetanic muscle force in such muscles is up to 70% of the contralateral side and with a corresponding wet muscle weight of up to 76%. The functional recovery, investigated by walking track analysis, shows that the results do not reach the level of functional recovery after a conventional end-to-end nerve repair or a nerve graft procedure [3, 5], but is better if the nerves were not reconstructed at all.

The origin of the regenerating axons

Axonal sprouting into an attached recipient nerve can occur either by collateral sprouting or by terminal sprouting from axons in the donor nerve which are damaged during the repair procedure [10, 14, 17]. Retrograde labelling is one method that can be used to evaluate if collateral sprouting is the mechanism behind nerve regeneration in end-to-side nerve repair. Motor and sensory axons grow out from the musculocutaneous nerve since labelling of the radial or the median/ulnar distal nerve segments results in occurrence of dye in the spinal cord and in the dorsal root ganglia. However, six or eight months after end-to-side nerve repair the numbers of doubled labelled neurons in spinal cord and in dorsal root ganglia are meagre indicating that very few neurons have axons both in the original musculocutaneous nerve and in the attached distal nerve segment [4, 5]. This indicates that collateral sprouting is not the only mechanism by which fibres are recruited or that the pruning mechanism is active during a follow-up of six to eight months. However, one can also consider that the end-to-side nerve repair induces degeneration of fibres in the donor nerve at the time of repair. Such fibres can regenerate into the end-to-side attached nerve, i.e. as a conventional nerve transfer situation.

Cell activation by end-to-side nerve repair

Interestingly, end-to-side nerve repair can initiate cell activation, such as nuclear translocation of the transcription factor ATF3 [11], in sensory neurons in DRGs, in motor neurons in the spinal cord and in non-neuronal cells (i.e. Schwann cells) in the donor nerve trunk [2]. ATF3 is a marker of a stress response in a cell and occurs in neurons that regenerate [11]. Application of a piece of nerve alongside a donor nerve does not induce any activation, such as ATF3 expression, in cells. In contrast, creation of an epineurial window with or without attachment of a piece of a nerve induces ATF3 in the neurons and in the non-neuronal cells, which we have seen in both fore- and hindlimb models of rats. Taken together with the studies of retrograde labelling and the staining for ATF3 in nucleus of sensory and motor neurons, there seems to be a different sensitivity of motor and sensory neurons to respond and regenerate out into the attached nerve segment with a more profound regeneration of the sensory one [2, 5, 15, 16].

Induction of ATF3 in the lateral aspect of a donor nerve close to where nerve segment is attached end-to-side indicates that Wallerian degeneration of these fibres in the donor nerve may occur [6]. The prerequisition for activation of neurons and non-neuronal cells leading to terminal or collateral sprouting of axons into a recipient nerve may be an injury induced to the donor nerve by the end-to-side nerve repair technique.

Conclusions

Irrespective of the background, experimental and clinical studies indicate that end-to-side nerve repair can be an alternative method in nerve reconstruction where there are no other repair options possible in the upper or lower extremity. However, the technique may be more appropriate in reconstruction of some specific nerves in for example the upper extremity. Finally, the cortical reorganisation that occurs after peripheral nerve injuries during the early and late phase of rehabilitation of the injury is another aspect of end-to-side nerve repair. There may be a challenge to the brain of terminal and collateral sprouting in order to effectively handle the new situation with new reinnervation area after the repair [12].

References

1. Al-Qattan MM (2001) Terminolateral neurorrhaphy: review of experimental and clinical studies. J Reconstr Microsurg 17(2): 99–108
2. Bontioti E, Dahlin LB, Kataoka K, Kanje M (2006) End-to-side nerve repair induces nuclear translocation of the activating transcription factor 3 (ATF3). Scand J Plastic Reconstr Surg Hand Surg 40(6): 321–328
3. Bontioti EN, Kanje M, Dahlin LB (2003) Regeneration and functional recovery in the upper extremity of rats after various types of nerve injuries. J Peripher Nerv Syst 8(3): 159–168
4. Bontioti E, Kanje M, Dahlin LB (2006) End-to-side nerve repair: attachment of a distal, compared with a proximal and distal, nerve segment. Scand J Plast Rec Surg Hand Surg 40(3): 129–135
5. Bontioti E, Kanje M, Lundborg G, Dahlin LB (2005) End-to-side nerve repair in the upper extremity of rat. J Peripher Nerv Syst 10(1): 58–68
6. Cederna PS, Kalliainen LK, Urbanchek MG, Rovak JM, Kuzon WM Jr (2001) ''Donor'' muscle structure and function after end-to-side neurorrhaphy. Plast Reconstr Surg 107(3): 789–796
7. Frey M, Giovanoli P (2003) End-to-side neurorrhaphy of motor nerves: reinnervation of free muscle transplants – first clinical application. Eur J Plast Surg 26: 89–94
8. Frey M, Giovanoli P (2003) End-to-side neurorrhaphy of sensory nerves. Eur J Plast Surg 26: 85–88
9. Kalliainen LK, Cederna PS, Kuzon WM Jr (1999) Mechanical function of muscle reinnervated by end-to-side neurorrhaphy. Plast Reconstr Surg 103(7): 1919–1927
10. Kubek T, Kyr M, Haninec P, Samal F, Dubovy P (2004) Morphological evidence of collateral sprouting of intact afferent and motor axons of the rat ulnar nerve demonstrated by one type of tracer molecule. Ann Anat 186(3): 231–234
11. Lindwall C, Dahlin L, Lundborg G, Kanje M (2004) Inhibition of c-Jun phosphorylation reduces axonal outgrowth of adult rat nodose ganglia and dorsal root ganglia sensory neurons. Mol Cell Neurosci 27(3): 267–279
12. Lundborg G, Richard P (2003) Bunge memorial lecture. Nerve injury and repair–a challenge to the plastic brain. J Peripher Nerv Syst 8(4): 209–226
13. Noah EM, Williams A, Jorgenson C, Skoulis TG, Terzis JK (1997) End-to-side neurorraphy: a histologic and morphometric study of axonal sprouting into an end-to-side nerve graft. J Reconstr Microsurg 12: 99–106
14. Noah EM, Williams A, Fortes W, Terzis JK (1997) A new animal model to investigate axonal sprouting after end-to-side neurorraphy. J Reconstr Microsurg 13: 317–325
15. Tarasidis G, Watanabe OSEM, Strasberg SR, Haughey BH, Hunter DA (1998) End-to-side neurorraphy: a long-term study of neural regeneration in a rat model. Otolaryngol Head Neck Surg 119: 337–341
16. Tham SK, Morrison WA (1998) Motor collateral sprouting through an end-to-side nerve repair. J Hand Surg 23A: 844–851
17. Yamauchi T, Maeda M, Tamai S, et al (2000) Collateral sprouting mechanism after end-to-side nerve repair in the rat. Med Electron Microsc 33(3): 151–156
18. Viterbo F, Trindade JC, Hoshini K, Mazzonineto A (1992) Latero-terminal neurorrhaphy without removal of the epineural sheath. Experimental study in rats. Rev Paul Med 110: 267
19. Yuksel F, Karacaoglu E, Guler MM (1999) Nerve regeneration through side-to-side neurorrhaphy sites in a rat model: a new concept in peripheral nerve surgery. Plast Reconstr Surg 104(7): 2092–2099
20. Zhang F, Fischer KA (2002) End-to-side neurorrhaphy. Microsurgery 22(3): 122–127

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Synergistic terminal motor end-to-side nerve graft repair: investigation in a non-human primate model

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Summary

End-to-side nerve repair has re-emerged in the literature in recent years but clinical applications for this technique are not yet fully defined and clinical reports are rare and controversial. Hypothetically, there might be useful functional results performing peripheral end-to-side nerve graft repair using synergistic terminal branches with defined motor function. An end-to-side nerve graft repair bridging from the terminal motor branch of deep branch of the ulnar nerve to the thenar motor branch of the median nerve was performed in non-human primates.

The results in this non-human primate model demonstrate the efficacy of end-to-side nerve graft repair at the level of peripheral terminal motor branches. End-to-side neurorrhaphy may present a viable alternative in conditions of unsuitable end-to-end coaptation and inappropriate nerve grafting procedures.

Keywords: End-to-side; nerve regeneration; morphology.

Introduction

End-to-side coaptation has re-emerged in the literature recently. However, experimental studies are controversial and clinical applications of this technique are not well defined.

Hypothetically, there might be useful functional results performing very peripheral end-to-side nerve graft repair using synergistic tiny terminal motor branches. We created one possible application of such a peripheral synergistic terminal end-to-side nerve transfer in a median nerve defect model in non-human primates. An end-to-side nerve graft repair bridging from the terminal motor branch of deep branch of the ulnar nerve to the thenar motor branch of the median nerve was performed. The purpose of this study was to investigate the efficacy of this hypothesis.

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Material and methods

Experimental model

Experiments were carried out on 7 adult baboons weighing 23.9 kg on average. In general anaesthesia the median and the ulnar nerve were exposed on the right forearm at two different levels. The distal level was 3 cm proximal to the wrist. A distance of 10 cm of nerve exposure was chosen for electrophysiological evaluations. Through a *y*-shaped skin incision in the palm of the hand, the thenar muscles, the deep branch of ulnar nerve, and the thenar motor branch of the median nerve were exposed. Electrophysiological evaluations of the median and ulnar nerve and their thenar target muscles were performed for assessment and control of human anatomical innervations in non-human primates. Anatomical variation in innervation of the superficial and deep head of the flexor pollicis brevis muscle was excluded electrophysiologically in all animals.

Next, the motor branch of the median nerve at thenar level and the deep branch of ulnar nerve were exposed. A tunnel for a nerve graft dorsal to the digital flexor tendons was made from the ulnar to the radial part of the palm. For nerve grafting we used a sensory branch of the radial nerve harvested at the dorsum of the hand. End-to-side nerve coaptation for the graft and the very terminal deep branch of the ulnar nerve were performed by two epineural sutures after creating an epineural window (incision) under the surgical microscope. The nerve graft was placed into the prefabricated tunnel without any tension and end-to-end nerve repair was performed with the thenar motor branch of the median nerve. The length of the nerve graft was between 3 and 1.6 cm (mean 2.3 cm). The wound was closed in layers in all animals.

All animals in this study received humane care in compliance with the Guide for the Care and Use of Laboratory Animals published by the National Institute of Health.

Each animal was sacrificed 3 months postoperatively to follow progression of nerve and nerve graft morphology. The morphology of the left deep branch of ulnar nerve served as control.

Clinical assessment – opposition of the thumb

Hand function of all baboons was documented by recording the animal with a digital camcorder for several minutes. Assessment of opposition of the thumb was performed by slow motion mode when the baboon was grasping:

1) preoperatively
2) the day after the primary procedure
3) three months after surgery

Histologic analysis

Chromotrope aniline blue (CAB) was used to stain neural collagenous connective tissue.

Neurofilament Antibody (NFAB) was used to stain axons.

S100 Antibody was used to quantify the number of Schwann cells.

Results

Preoperatively grasping pattern was observed in all animals. When climbing inside their cages, all baboons grasped a thin rod opposing the thumb in a non-human primate fashion (short thumb-ray, full opposition like in human is not possible) or between thenar and hypothenar depending on the direction of the approach. When grasping thicker rods, grasping was performed by flexion of fingers and adduction of the thumb.

The day after the primary procedure no non-human primate opposition of the thumb could be observed in all animals.

Intraoperatively and postoperatively, no complications were seen in the 7 baboons (14 hands) studied.

Three months after surgery all animals clinically showed non-human primate opposition of the thumb and electrophysiologically reinnervation of median nerve innervated thenar muscles by an end-to-side nerve graft from the terminal motor branch of deep branch of ulnar nerve. Baboons preferred the left, non-operated hand for activities like scraping and fine grasping.

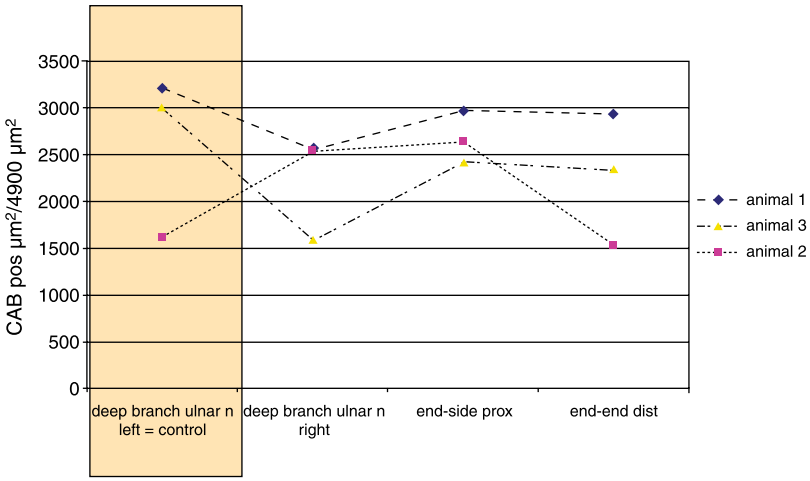

Fig. 1a. The values of neural collagenic tissue is depicted in the normal deep branch of ulnar nerve (control), the deep branch of ulnar nerve on the operated right side, at the end-to-side coaptation site and distal to the end-to-end coaptation site in animal 1–3

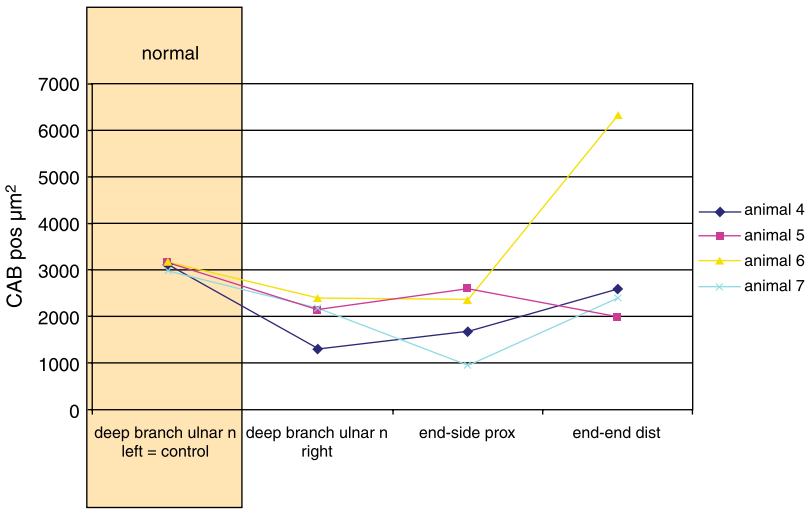

Fig. 1b. The values of neural collagenic tissue is depicted in the normal deep branch of ulnar nerve (control), the deep branch of ulnar nerve on the operated right side, at the end-to-side coaptation site and distal to the end-to-end coaptation site in animal 4–6. Animal 6 showed a very high collagenization at the end-to-end coaptation site

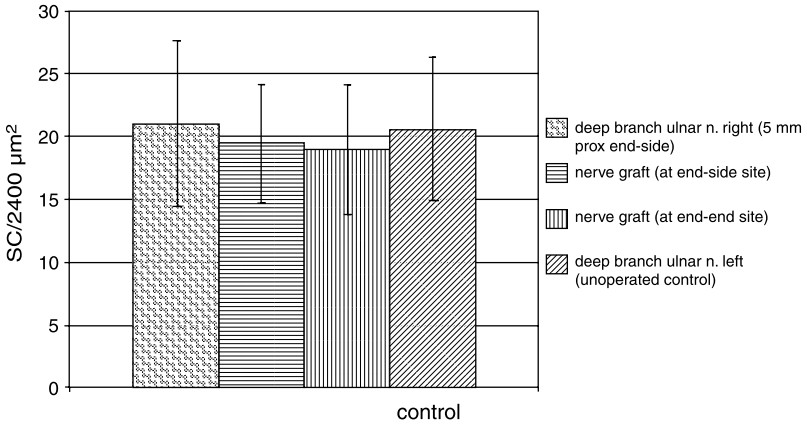

Fig. 2. Schwann cell counts on average 3 months after surgery are shown in the normal deep branch of ulnar nerve (control), the deep branch of ulnar nerve on the operated right side, at the end-to-side coaptation site and distal to the end-to-end coaptation site

Histology

CAB staining – neural collagenous connective tissue 3 months after surgery (Fig. 1a, b)

There were no statistically significant differences in the area of collagenic connective tissue between all nerve segments of end-to-side nerve graft repair animals and non-operated control sites. Although, the area of collagenic connective tissue was 6% larger 2 mm distal to the end-to-side repair site and 28% larger at the distal end of the nerve graft. Highest mean values were found at the distal site of the nerve graft, due to a very strong collagenization of the nerve graft of animal number six.

Neurofilament antibody staining – number of axon 3 months after surgery

The number of axons distal to the end-to-side repair site was smaller by 19% on average. Additionally, we found an average 23% decrease of axons 2 mm proximal to the end-to-end coaptation, compared to data of the deep branch of ulnar nerve ($^{**}p = 0.008$).

S100 Antibody – Schwann cells (SC) 3 months after surgery (Fig. 2)

Schwann cell counts showed similar values within all nerve segments. There was no difference to the non operated control.

Discussion

We demonstrated that synergistic end-to-side nerve graft coaptation from very terminal motor branches of the deep branch of ulnar nerve to the thenar motor branch of median nerve is possible and opposition of the thumb recovers. This was the first report on a new application of end-to-side nerve graft repair in median nerve defects, high median nerve lesions and brachial plexus injuries with preserved ulnar nerve function based on synergistic peripheral terminal motor branches. This new approach in end-to-side nerve repair may improve the quality of outcome by reducing time for reinnervation and by limiting end organ failure.

Our morphologic results indicate that synergistic terminal end-to-side nerve graft repair has a positive effect in axonal sprouting proceeding distally through the nerve graft and upon Schwann cell survival within the nerve graft. As expected we found a modest increase of neural collagenic connective tissue within the nerve graft in most animals (six out of seven).

Axons regenerate and reinnervate the denervated end plates by growing through the endoneural tubes that lead to these previous synaptic sites. However, the degree of functional restitution following peripheral nerve injury depends upon the functional connection between central nervous system, regenerating axons and target organs. The very important role of Schwann cells for axonal regeneration and particularly for the production of growth factors and basal laminar components is well-known [9, 14, 19, 38].

The survival of Schwann cells within a nerve graft has been shown [1, 28], but there is limited evidence on Schwann cell survival after synergistic terminal end-to-side nerve graft coaptation. Our results show a minimal decrease of Schwann cell counts within the nerve graft only. This may indicate good Schwann cell survival and Schwann cell multiplication processes within the nerve graft.

In a recent study Love *et al.* [23] found that stimulation of partially denervated rat soleus muscle did not affect the length or number of terminal Schwann cell processes, but did reduce the number of terminal Schwann cell bridges and altered the growth of axons along processes extended by terminal Schwann cells. Additionally,

it was shown that as a consequence of synaptic activity, terminal Schwann cells modulate the efficacy of the synapse by regulating transmitter release [11, 30]. In our study animals did not undergo muscle stimulation and there is limited evidence on training affecting muscle reinnervation processes. We know neither to what extent baboons passively exercised the denervated thenar muscles not to what extent baboons actively exercised the partial innervated thenar muscles. The effect on training affecting the ends organ after synergistic terminal end-to-side nerve repair is still unknown. However, though these new cellular insights in the hypothetical possibilities of regeneration are of major interest, this was not object of our study.

The process of scar formation at the nerve repair site and within the nerve graft after synergistic terminal end-to-side nerve repair has not been well studied. It is generally assumed that scar tissue, particularly at the nerve repair site, interferes with axonal sprouting [27]. This would be in accordance with our results. In our study, endoneural collagenic connective tissue showed a small increase within the nerve graft and a decrease of neurofilament positive axons. On the other hand, we do not know the original myelinated axon counts within the nerve graft. Hypothetically, we should have used two nerve grafts for enhanced axon counts advancing distally. This impact of the sensory nerve graft harvested at the dorsum of the hand on end-to-side nerve repair is not well defined. Nevertheless, our results in morphology showed that after synergistic end-to-side nerve graft repair fascicular alignment was in fact preserved and was prerequisite to functional recovery.

Conclusion

The results in this series demonstrate the efficacy of end-to-side nerve graft repair at the level of tiny very peripheral terminal motor branches in a non-human primate median nerve defect model. Applications of this technique may enhance functional results by avoidance of time dependent end organ failure.

References

1. Aguayo AJ, Attiwell M, Trecarten J, Perkins S, Bray GM (1977) Abnormal myelination in transplanted Trembler mouse Schwann cells. Nature 265: 73–75
2. Angelov DN, Gunkel A, Stennert E, Neiss WF (1993) Recovery of original nerve supply after hypoglossal-facial anastomosis causes permanent motor hyperinnervation of the whisker-pad muscles in the rat. J Comp Neurol 338: 214–224
3. Angelov DN, Neiss WF, Gunkel M *et al* (1997) Nimodipine-accelerated hypoglossal sprouting prevents the postoperative hyperinnervation of target muscle after hypoglossal-facial anastomosis in the rat. Restor Neurol Neurosci 11: 109–121
4. Angelov DN, Neiss WF, Streppel M, Andermahr J, Mader K, Stennert E (1996) Nimodipine accelerates axonal sprouting after surgical repair of rat facial nerve. J Neurosci 16: 1041–1048
5. Angelov DN, Skouras E, Guntinas-Lichius O *et al* (1999) Contralateral trigeminal nerve lesion reduces polyneuronal muscle innervation after facial nerve repair in rats. Eur J Neurosci 11: 1369–1378
6. Brown MC, Holland RL, Hopkins WG (1981) Motor nerve sprouting. Annu Rev Neurosci 4: 17–42
7. Brushart TM, Gerber J, Kessens P, Chen YG, Royall RM (1998) Contributions of pathway and neuron to preferential motor reinnervation. J Neurosci 18: 8674–8681
8. Brushart TM (1993) Motor axons preferentially reinnervate motor pathways. J Neurosci 13: 2730–2738
9. Bunge RP (1993) Expanding roles for the Schwann cell: ensheathment, myelination, trophism and regeneration. Curr Opin Neurobiol 3: 805–809
10. Carmignoto G, Finesso M, Siliprandi R, Gorio A (1983) Muscle reinnervation – I. Restoration of transmitter release mechanisms. Neuroscience 8: 393–401
11. Castonguay A, Robitaille R (2001) Differential regulation of transmitter release by presynaptic and glial Ca^{2+} internal stores at the neuromuscular synapse. J Neurosci 21: 1911–1922
12. Chen YG, Brushart TM (1998) The effect of denervated muscle and Schwann cells on axon collateral sprouting. J Hand Surg [Am] 23: 1025–1033
13. Dohm S, Streppel M, Guntinas-Lichius O *et al* (2000) Local application of extracellular matrix proteins fails to reduce the number of axonal branches after varying reconstructive surgery on rat facial nerve. Restor Neurol Neurosci 16: 117–126
14. Fu SY, Gordon T (1997) The cellular and molecular basis of peripheral nerve regeneration. Mol Neurobiol 14: 67–116
15. Gruart A, Gunkel A, Neiss WF, Angelov DN, Stennert E, Delgado-Garcia JM (1996) Changes in eye blink responses following hypoglossal-facial anastomosis in the cat: evidence of adult mammal motoneuron unadaptability to new motor tasks. Neuroscience 73: 233–247
16. Guntinas-Lichius O, Wewetzer K, Tomov TL *et al* (2002) Transplantation of olfactory mucosa minimizes axonal branching and promotes the recovery of vibrissae motor performance after facial nerve repair in rats. J Neurosci 22: 7121–7131
17. Harness D, Sekeles E (1971) The double anastomotic innervation of thenar muscles. J Anat 109: 461–466
18. Hennig R, Dietrichs E (1994) Transient reinnervation of antagonistic muscles by the same motoneuron. Exp Neurol 130: 331–336
19. Ide C (1996) Peripheral nerve regeneration. Neurosci Res 25: 101–121
20. IJkema-Paassen J, Meek MF, Gramsbergen A (2002) Reinnervation of muscles after transection of the sciatic nerve in adult rats. Muscle Nerve 25: 891–897
21. Ko CP, Chen L (1996) Synaptic remodeling revealed by repeated in vivo observations and electron microscopy of identified frog neuromuscular junctions. J Neurosci 16: 1780–1790
22. Koirala S, Qiang H, Ko CP (2000) Reciprocal interactions between perisynaptic Schwann cells and regenerating nerve terminals at the frog neuromuscular junction. J Neurobiol 44: 343–360
23. Love FM, Son YJ, Thompson WJ (2003) Activity alters muscle reinnervation and terminal sprouting by reducing the number of Schwann cell pathways that grow to link synaptic sites. J Neurobiol 54: 566–576
24. Love FM, Thompson WJ (1999) Glial cells promote muscle reinnervation by responding to activity-dependent postsynaptic signals. J Neurosci 19: 10390–10396

25. Lubischer JL, Thompson WJ (1999) Neonatal partial denervation results in nodal but not terminal sprouting and a decrease in efficacy of remaining neuromuscular junctions in rat soleus muscle. J Neurosci 19: 8931–8944
26. Lundborg G, Zhao Q, Kanje M, Danielsen N, Kerns JM (1994) Can sensory and motor collateral sprouting be induced from intact peripheral nerve by end-to-side anastomosis? J Hand Surg [Br] 19: 277–282
27. Millesi H (1985) Peripheral nerve repair: terminology, questions, and facts. J Reconstr Microsurg 2: 21–31
28. Pollard JD, McLeod JG (1980) Nerve grafts in the Trembler mouse. An electrophysiological and histological study. J Neurol Sci 46: 373–383
29. Reynolds ML, Woolf CJ (1992) Terminal Schwann cells elaborate extensive processes following denervation of the motor endplate. J Neurocytol 21: 50–66
30. Robitaille R (1998) Modulation of synaptic efficacy and synaptic depression by glial cells at the frog neuromuscular junction. Neuron 21: 847–855
31. Sanes JN, Suner S, Donoghue JP (1990) Dynamic organization of primary motor cortex output to target muscles in adult rats. I. Long-term patterns of reorganization following motor or mixed peripheral nerve lesions. Exp Brain Res 79: 479–491
32. Shawe GD (1955) On the number of branches formed by regenerating nerve-fibres. Br J Surg 42: 474–488
33. Son YJ, Thompson WJ (1995) Nerve sprouting in muscle is induced and guided by processes extended by Schwann cells. Neuron 14: 133–141
34. Son YJ, Thompson WJ (1995) Schwann cell processes guide regeneration of peripheral axons. Neuron 14: 125–132
35. Streppel M, Angelov DN, Guntinas-Lichius O *et al* (1998) Slow axonal regrowth but extreme hyperinnervation of target muscle after suture of the facial nerve in aged rats. Neurobiol Aging 19: 83–88
36. Streppel M, Azzolin N, Dohm S *et al* (2002) Focal application of neutralizing antibodies to soluble neurotrophic factors reduces collateral axonal branching after peripheral nerve lesion. Eur J Neurosci 15: 1327–1342
37. Sumner AJ (1990) Aberrant reinnervation. Muscle Nerve 13: 801–803
38. Terenghi G (1995) Peripheral nerve injury and regeneration. Histol Histopathol 10: 709–718
39. Valero-Cabre A, Navarro X (2002) Functional impact of axonal misdirection after peripheral nerve injuries followed by graft or tube repair. J Neurotrauma 19: 1475–1485
40. Valero-Cabre A, Tsironis K, Skouras E, Perego G, Navarro X, Neiss WF (2001) Superior muscle reinnervation after autologous nerve graft or poly-L-lactide-epsilon-caprolactone (PLC) tube implantation in comparison to silicone tube repair. J Neurosci Res 63: 214–223

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End-to-side coaptation – controversial research issue or important tool in human patients

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Summary

End-to-side coaptation is still a controversial procedure. Many authors reported surprisingly good results; others showed mediocre results only. There are also reports of complete failures. Apparently all authors are right. According to our experience the results depend on the level of end-to-side coaptation and on the nerve fiber composition. End-to-side coaptation between mixed nerves do have very poor expectations. The chances are much better if e.g. a small denervated pure motor nerve is coapted to a functioning small pure motor nerve. The same procedure may produce opposite results according to the circumstances. In our experience end-to-side coaptation is a reliable procedure of great use in selected cases. Main field of application are thin nerves with a well defined function and synergistic terminal motor branches.

Keywords: Peripheral nerve; end-to-side; regeneration; coaptation.

Introduction

My first contact with end-to-side coaptation in peripheral nerve surgery happened in 1993 in New York at a meeting when Fausto Viterbo [19] presented his concept of end-to-side coaptation in peripheral nerve surgery using an epineurial window in the donor nerve. There was a lot of scepticisms and nobody really believed him.

But early experiments demonstrated that in fact a denervated peripheral nerve coapted with transection of the epineurium is neurotizised by the donor nerve [8, 13, 17, 18].

A controversial discussion started and is still going on to answer the following questions:

1) How can axon sprout overcome the barrier function of the perineurium?
2) What type of axons sprout?
3) What stimulates the axons to sprout?
4) What is the origin of axons?
5) What about the donor nerve function?
6) Does it make sense to apply this technique in clinical cases?
7) If so, what functional value can be expected?

How can axon sprouts overcome the barrier function of the perineurium?

From the experiments of Spencer *et al.* [15] we know that a perineurial window causes an herniation of the endoneurial tissue due to higher intrafascicular pressure followed by a segmental demyelination of the herniated part. The perineurium regenerates around the herniated tissue which becomes remyelinated, and no functional loss occurs.

All surgeons know how thick the perineurium may be in nerve trunks. Sunderland [16] describes it as 1.3–100 μm thick consisting of a sequence of layers including lamellae of special collagen fibers with a diameter of 40–65 nm. Consequently a group of authors doubt the ability of axon sprouts to penetrate such a formidable barrier and insist on the creation of a perineurial window [9, 21].

But, is the perineurium equally well developed over the total length of a peripheral nerve?

Is it not much thinner in the terminal branches and in the nerve branches within a muscle?

Is it not possible that from such intramuscular branches sprouting arises to reinnervate a partially denervated muscle?

Motto: Research fellows should be flexible and open minded

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What type of axons sprout?

The view was held that only sensory nerve fibers sprout [6, 18]. Other authors [21, 22] described useful motor recovery in the rat model. Lutz *et al.* [9] observed motor recovery up to 70%.

Liu *et al.* [7] had in their experiments motor recovery but of course to minor degree as with end-to-end coaptation. Therefore it is evident that motor axons can regenerate under end to side conditions. However, all authors convene upon that they regenerate less well as sensible fibers and in an unpredictable way.

What stimulates the axons to sprout?

In this question the controversy is especially sharp. Authors who work with epineurial window only may believe that neurotropic factors from the denervated nerve triggers the sprouting. This factor must act across the intact perineurium. Authors who create a perineurial window change the equilibrium of the endoneurial space what may stimulate sprouting. Authors who do not believe in end-to-side coaptation at all have the suspicion that in all cases a trauma had been inflicted to some nerve fibers which stimulates them to sprout. In this case the end to side coaptation would be some kind of end to end coaptation with partial transection of a fascicle. This technique is well known and applied for instance in the baby sitter technique of Mersa *et al.* [12] for the facial nerve using the half of the hypoglossus nerve as donor. The next step in this line would be Oberlin's technique cutting one or two fascicles of a polyfascicular nerve and use them as donors by end to end coaptation hoping that the remaining fascicles of the donor nerve contain enough nerve fibers of all qualities to minimize the loss.

What is the origin of axons?

How can axons get into the denervated nerve coapted end to side to an innervated nerve?

One possibility is ''contamination'' [2, 11]: Axons originating from the proximal stump of the recipient nerve which after transection was not occluded sufficiently. Axon sprouts reach the donor nerve and grow along the epineurium to the site of the end to side coaptation and enter the recipient distal stump. Axons may sprout from the nervi nervorum in the epineurium of the donor nerve injured by creating the epineurial window.

If nerve fibers are hurt or stimulated in some other way they may give rise to axon sprouts:

Terminal sprouting

If the stimulus of end to side coaptation reaches the neuron in the dorsal root ganglion for sensible axons or the motor neuron in the ventral horn and stimulates the neuron to produce another axon besides the already existing we would talk of:

Collateral sprouting

Collateral sprouting could be proven by retrograde double labelling.

The evidence of double labelling is a strong argument that end to side coaptation according to the sense of the word exists regardless of the quantity and of the functional significance.

Double labelling was proven for motor and sensible neurons after end to side coaptation with epineurial and perineurial window by Zhang *et al.* [21] and Lutz *et al.* [9].

Double labelling was proven after end to side coaptation creating an epineurial window only by Xioing *et al.* [20], Adelson *et al.* [1], Matsuda *et al.* [10] and Bontioti *et al.* [3].

What about the donor nerve function?

Signs of degeneration of nerve fibers in the donor nerve have been mentioned by Cederna *et al.* [4] and Papalia *et al.* [14].

All authors agree upon that the functional loss in the donor nerve is nil or minimal.

Does it make sense to apply this technique in clinical cases?

The answer is: ''Yes''. Nobody would use end to side coaptation if functional recovery can be achieved by restoring continuity by end to end nerve repair or by nerve grafting. End to end coaptation is certainly the technique of choice if the loss is of the donor nerve is minimal or not important. However, if for an important function no less important donor nerve is available and new function should be gained and existing function preserved end to side coaptation would be essential. Of course a technique with unpredictable results is useless.

If so, what functional value can be expected?

End to side coaptation would be applied in cases in whom a given functional loss should be repaired without an available proximal stump or another expendable nerve which

could serve as an axon donor. The main point is that we want to gain something without sacrificing something else.

Clinical requirement: After being coapted end to side to an innervated nerve the coapted denervated nerve is neurotizised. Dependent muscles regain function independent from he donor nerve. The donor nerve retains its voluntary function independently from the recipient nerve.

Conclusion and consequences

End to side coaptation does work. An injury to the donor nerve is not necessary. There are functional results sometimes good results in experiments and clinical cases. Useful results occur in a very low percentage only.

Why? Is the end to side procedure as such unreliable or do we something wrong?

An analysis of the clinical cases and the experiments reveals that exclusively mixed nerve trunks had been used as the median, the ulnar or the radial nerve. If we consider that the sprouting occurs at a random chance and we cannot influence which nerve fibers should sprout we have to accept the fact that in the majority of cases we will have more sensible axons sprouting and if motor axons sprout they may not reach the target or arrive there in a very diluted number.

What is the consequence?

We have to use small nerves with one well defined function as donors and small nerve with one well defined function as a recipient.

Clinical cases

1) Dorsalis scapulae nerve as donor and long thoracic nerve as recipient in brachial plexus cases.
2) Phrenic nerve as donor and musculocutaneous nerve as recipient via nerve graft.
3) Phrenic nerve as donor and medial or lateral pectoral nerve as recipient in brachial plexus cases to reinnervate the major pectoralis muscle for later transfer as an external rotator (Table 1).
4) Based on animal experiments with baboons we have tried to reinnervate the thenar muscles in a high irreparable median nerve lesion with functioning ulnar nerve by transfer of motor fibers from the muscular branch of the deep head of the flexor pollicis brevis muscle (innervated by the ulnar nerve) to the motor thenar branch. The patient gained opposition and retained adduction.

At this level the perineurium is very thin and delicate. To create a window is impossible. The only thing we did was a longitudinal incision in the epineurium and fix the graft there with two stitches (10-0 nylon). The fascicle were not even touched.

Table 1. *Results of neurotization of the major pectoralis muscle. The donor nerve was the phrenic nerve. Nerve grafts were coapted end-to-side to the phrenic nerve and end-to-end to the medial and/or lateral pectoralis nerve. The length of the nerve grafts varied between 10 and 12 cm*

Clinical results (M0-M5 scale)	Cases	Average follow-up (months)	Length of nerve graft (cm)
M4 or better	4	54	8–10
M3-M3+	4	48	8–10
M2	1	16	10

References

1. Adelson PD, Bonaroti EA, Thompson TP, Tran M, Nystrom NA (2004) End-to-side neurorrhaphies in a rodent model of peripheral nerve injury: a preliminary report of a novel technique. J Neurosurg 101 Suppl 1: 78–84
2. Al-Qattan MM (2001) Terminolateral neurorrhaphy: review of experimental and clinical studies. J Reconstr Microsurg 17: 99–108
3. Bontioti E, Kanje M, Lundborg G, Dahlin LB (2005) End-to-side nerve repair in the upper extremity of rat. J Peripher Nerv Syst 10: 58–68
4. Cederna PS, Kalliainen LK, Urbanchek MG, Rovak JM, Kuzon WM Jr (2001) "Donor" muscle structure and function after end-to-side neurorrhaphy. Plast Reconstr Surg 107: 789–796
5. Dellon AL (1996) Nerve grafting and end-to-side neurorrhaphies connecting phrenic nerve to the brachial plexus. Plast Reconstr Surg 98(5): 905
6. Goheen-Robillard B, Myckatyn TM, Mackinnon SE, Hunter DA (2002) End-to-side neurorrhaphy and lateral axonal sprouting in a long graft rat model. Laryngoscope 112: 899–905
7. Liu K, Chen LE, Seaber AV, Goldner RV, Urbaniak JR (1999) Motor functional and morphological findings following end-to-side neurorrhaphy in the rat model. J Orthop Res 17: 293–300
8. Lundborg G, Zhao Q, Kanje M, Danielsen N, Kerns JM (1994) Can sensory and motor collateral sprouting be induced from intact peripheral nerve by end-to-side anastomosis? J Hand Surg [Br] 19(3): 277–282
9. Lutz BS, Chuang DC, Hsu JC, Ma SF, Wei FC (2000) Selection of donor nerves – an important factor in end-to-side neurorrhaphy. Br J Plast Surg 53: 149–154
10. Matsuda K, Kakibuchi M, Fukuda K, Kubo T, Madura T, Kawai K, Yano K, Hosokawa K (2005) End-to-side nerve grafts: experimental study in rats. J Reconstr Microsurg 21(8): 581–591
11. McCallister WV, Tang P, Trumble TE (1999) Is end-to-side neurorrhaphy effective? A study of axonal sprouting stimulated from intact nerves. J Reconstr Microsurg 15: 597–603
12. Mersa B, Tiangco DA, Terzis JK (2000) Efficacy of the "baby-sitter" procedure after prolonged denervation. J Reconstr Microsurg 16(1): 27–35
13. Noah EM, Williams A, Jorgenson C, Skoulis TG, Terzis JK (1997) End-to-side neurorrhaphy: a histologic and morphometric study of axonal sprouting into an end-to-side nerve graft. J Reconstr Microsurg 13: 99–106

14. Papalia I, Geuna S, Tos PL, Boux E, Battiston B, Stagno D'Alcontres F (2003) Morphologic and functional study of rat median nerve repair by terminolateral neurorrhaphy of the ulnar nerve. J Reconstr Microsurg 19: 257–264
15. Spencer PS, Weinberg HJ, Raine CS, Prineas JW (1975) The perineurial window – a new model of focal demyelination and remyelination. Brain Res 96(2): 323–329
16. Sunderland S (1970) Anatomical features of nerve trunks in relation to nerve injury and nerve repair. Clin Neurosurg 17: 38–62
17. Tarasidis G, Watanabe O, Mackinnon SE, Strasberg SR, Haughey BH, Hunter DA (1997) End-to-side neurorrhaphy resulting in limited sensory axonal regeneration in a rat model. Ann Otol Rhinol Laryngol 106: 506–512
18. Tarasidis G, Watanabe O, Mackinnon SE, Strasberg SR, Haughey BH, Hunter DA (1998) End-to-side neurorrhaphy: a long-term study of neural regeneration in a rat model. Otolaryngol Head Neck Surg 119: 337–341
19. Viterbo F, Trindade JC, Hoshino K, Mazzoni Neto A (1994) End-to-side neurorrhaphy with removal of the epineurial sheath: an experimental study in rats. Plast Reconstr Surg 94: 1038–1047
20. Xiong G, Ling L, Nakamura R, Sugiura Y (2003) Retrograde tracing and electrophysiological findings of collateral sprouting after end-to-side neurorrhaphy. Hand Surg 8: 145–150
21. Zhang Z, Soucacos PN, Bo J, Beris AE (1999) Evaluation of collateral sprouting after end-to-side nerve coaptation using a fluorescent double-labelling technique. Microsurgery 19: 281–286
22. Zhao JZ, Chen ZW, Chen TY (1997) Nerve regeneration after terminolateral neurorrhaphy: experimental study in rats. J Reconstr Microsurg 13: 31–37

Cerebral plasticity

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Cortical reintegration of a replanted hand and an osseointegrated thumb prosthesis

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Summary

Background. Following a peripheral nerve repair the injured nerve has to re-innervate its original cortical area, a process, which is poorly understood. Errors in this cortical re-innervation have been suggested as one key reason for the generally poor clinical outcome following nerve injuries in the hand.

Method. Functional magnetic resonance imaging (fMRI) was used to assess cortical reintegration following amputation and reattachment of bodyparts in two different situations: a patient with a hand amputation followed by immediate surgical replantation and a patient with an osseointegrated thumb prosthesis.

Findings. The primary motor cortex rapidly returns to a normal activation pattern after amputation followed by replantation or application of an osseointegrated prosthesis. The primary somatosenory cortex changes from an initial ipsilateral to a bilateral activation pattern. Sensory stimulation of an osseointegrated prosthesis also shows a bilateral activation pattern in the primary somatosenory cortex.

Conclusions. The primary motor cortex shows a more normal activation pattern possibly because most muscles controlling the hand are proximal to the injury and can be activated after an amputation. The primary somatosensory cortex reorganises more and the activation pattern is more bilateral compared to a healthy hand. This bilateral activation pattern could represent a compensatory mechanism for the inferior tactile function in the replanted hand and the osseointegrated prosthesis.

Keywords: Amputation; cortical reintegration; hand; osseointegrated; hand replantation.

Introduction

Amputation of the whole hand or part of the hand is a severe injury creating a large deafferentation as well as deefferentation in the cerebral cortex [7]. The cortical hand area is rapidly occupied by adjacent cortical areas especially the forearm and face, but also the contralateral hand seems to occupy part of the deafferented area [3, 14]. Clinical results following an amputation injury with surgical replantation or prosthesis are often poor especially regarding the return of functional sensibility. The reason for this has been suggested to be extensive cortical reorganisation, the outgrowing nerves navigate wrong and this has been shown to result in a changed, mosaic like activation pattern in the primary sensory cortex (S1) [6, 8].

Here we summarise our findings on cortical reintegration of an amputated and re-attached body part using functional magnet resonance imaging (fMRI) in two different situations: a patient with a hand amputation followed by immediate surgical replantation and a patient with an osseointegrated thumb prosthesis.

Methods and materials

The first patient is a 47 year old man with a traumatic amputation of the right dominant hand at wrist level. The hand was immediately surgically replanted. The patient received motor training according to standardised protocols, and also sensory re-education was started early to maintain the cortical representation of the hand, mirror training as well as the Sensor Glove were also used [11]. Cortical activation following sensory and motor stimulation was evaluated at 1, 2, 4, 8, and 12 months postoperatively using fMRI. The second patient is a 65 years old man who 16 years earlier had a traumatic amputation of his right dominant thumb at the MCP level. Three years after amputation he was treated with an osseointegrated thumb prosthesis. On functional testing he could feel deep pressure applied to the thumb from Semmes-Weinstein monofilament number 6.65 (450 g) when the thumb was firmly supported [1], and light stroke from a brush. Pulp pinch strength (digit I and II) was 8.5 kg in the right hand compared with 11 kg in the left hand. Fine manipulative tasks using pulp pinch and key grip (task number 4, 8, and 10) in Sollerman grip function test [12] were possible with the right hand, but took longer than with the left hand. fMRI was used to evaluate the cortical response to sensory stimulation.

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Imaging

MRI was performed on a 3T head scanner (Siemens Allegra). Gradient echo, and echo planar image (GRE-EPI) volumes of the whole brain (TE/TR/flip angle = 30 ms/3000 ms/90°, number of slices = 36, thickness = 3 mm, voxel size = $3 \times 3 \times 3\,mm^3$, gap = 0.9 mm) were acquired every three seconds [10, 13]. A T1-weighted three dimensional MP-RAGE sequence was also acquired as an anatomical reference.

Stimuli/paradigm

The fMRI study consisted of four paradigms. Each paradigm consisted of five blocks of alternating 30 sec task and 30 sec rest. During sensory stimulation, the patients could not visually detect the onset of task or rest epochs. In the patient with the replanted hand each fMRI study consisted of bilateral motor and sensory paradigms. Motor stimulation consisted of fist clenching at a frequency of 1 Hz. Sensory stimulation was performed on each fingertip using Semmes-Weinstein monofilament nr. 6.65 at a frequency of 1 Hz. In the patient with the prosthetic thumb two types of sensory stimulation were used: strokes across the thumb with the bristles of a toothbrush and pressure contact with a Semmes-Weinstein monofilament number 6.65 (filament) at a frequency of 1 Hz. Each stimulation was applied to the healthy left thumb and to the corresponding position on the right prosthetic thumb.

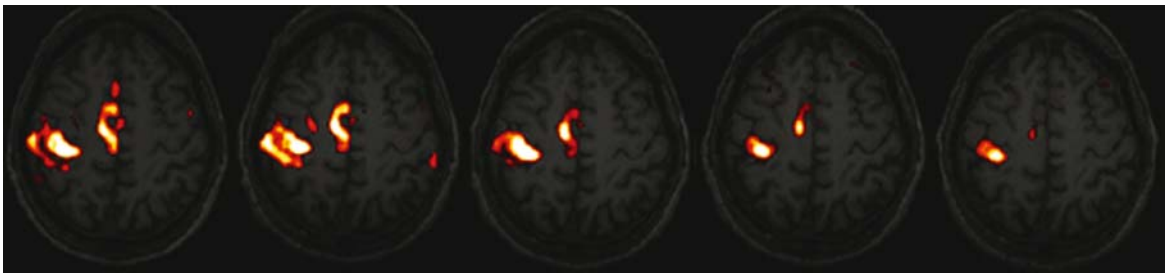
Data analysis

Functional images were analysed using SPM2 (www.fil.ion.ucl.ac.uk/spm), and statistical analysis based on the general linear model [4, 15] and involving correlation of voxel time courses with a reference function indicating the expected cortical response convolved with a haemodynamic response function (HRF) to correct for the delayed vascular response. Statistical parametric maps were then generated by assessing the probability of detecting the expected response level by chance, and the threshold used was at a possibility of less than 0.01, uncorrected for multiple comparisons. Left and right regions of interest (ROI) were drawn on the original EPI images to include the post central gyrus in the nine upper slices on each side of the brain, and the number of activated voxels (above the threshold) in each ROI and paradigm were recorded. The laterality index (*LI*) was used to reflect the hemispherical dominance of activation, and was calculated according to: $LI = (N_L - N_R)/(N_L + N_R)$, where N_L and N_R represent the number of activated voxels in the left and right ROIs, respectively. A laterality index of $+1$ indicates exclusively left-sided activation, whereas a value of -1 indicates right-sided activation. A laterality index of 0 therefore indicates that activation is equal on both sides.

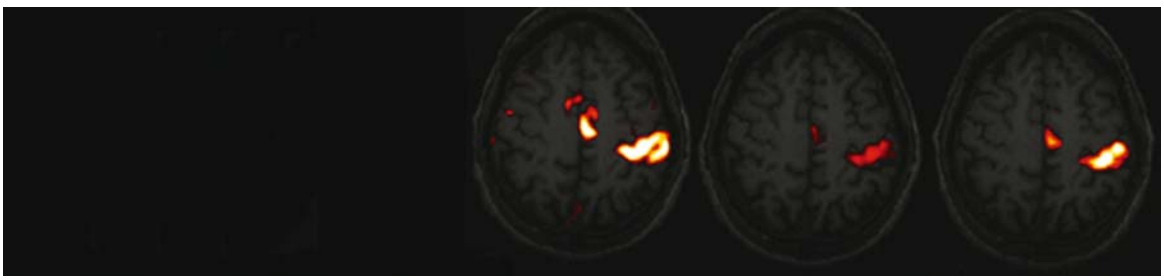
Results

In the hand replantation patient motor activation of the healthy hand showed activation predominately in the contralateral primary motor cortex. Motor activation of the replanted hand was first done after 4 months and at this time robust activation was seen predominately in the contralateral cortex and the activation pattern did not change on the following measurements (Fig. 1a and b).

Sensory stimulation of the healthy hand resulted in activation predominately in the contralateral primary somatosenory cortex. Sensory stimulation of the replanted hand resulted in a small ipsilateral activation of the primary somatosenory cortex at 4 weeks and from this time on the activation pattern gradually changed to a more bilateral and later a predominately contralateral activa-



a



b

Fig. 1. Cortical activation during motor stimulation of the healthy left hand and replanted right hand. fMRI Activation at a threshold of $p < 0.01$ is overlaid on transverse anatomical T1-weighted MR images. During motor stimulation of the healthy left hand robust activation is seen predominantly in the contralateral motor cortex in all five fMRI examinations at 1, 2, 4, 8, and 12 months, respectively (a). fMRI Experiments at 1, 2, 4, 8, and 12 months are shown in one row read from left to right. During motor stimulation of the replanted right hand at 4, 8, and 12 months postoperatively, activation was seen in the contralateral motor cortex (b)

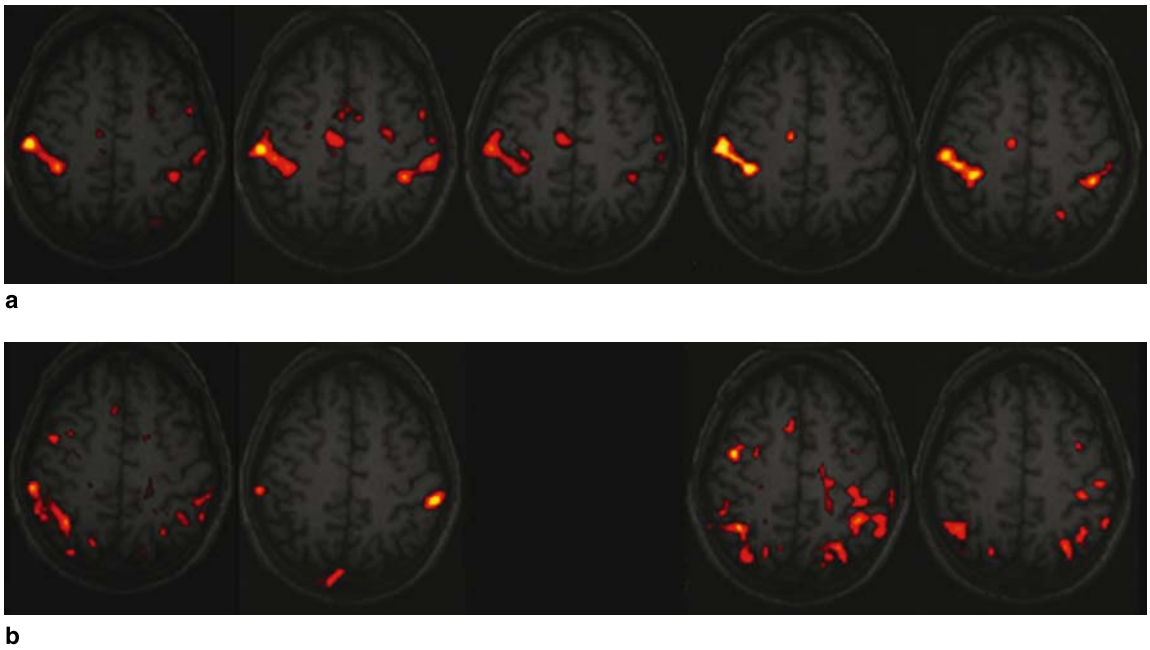


Fig. 2. Cortical activation during sensory stimulation of the healthy left hand and replanted right hand. fMRI Activation at a threshold of $p < 0.01$ is overlaid on transverse anatomical T1-weighted MR images. During sensory stimulation of the healthy left hand robust activation is seen predominantly in the contralateral somatosensory cortex in all five fMRI examinations at 1, 2, 4, 8, and 12 months, respectively (a). fMRI Experiments at 1, 2, 4, 8, and 12 months are shown in one row read from left to right. During sensory stimulation of the replanted right hand one month postoperatively, some activation in the ipsilateral somatosensory region but no contralateral activation could be detected. At two months, activation was seen in the contralateral as well as the ipsilateral somatosensory cortex. At four months the sensory activation could not be evaluated due to motion artefacts. At 8 and 12 months postoperatively the sensory activation became more robust and predominant on the left side (b). Laterality indexes for the right hand sensory stimulation at 1, 2, 8, and 12 months were −0.08, −0.01, 0.86, and 0.66, respectively. Laterality index +1 represents left activation, −1 represents right activation, and 0 represents equal activation on both sides

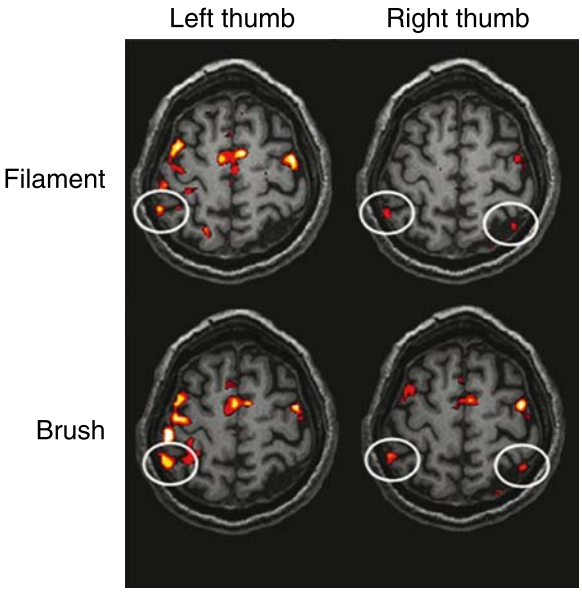


Fig. 3. Cortical activation during sensory stimulation of the left thumb and the right thumb prosthesis using a filament and brush. fMRI Activation is overlaid on transverse anatomical T1-weighted MR images. Sensory activation has been highlighted with white circles. Activation is shown at a statistical threshold of $p < 0.01$. Stimulation of the healthy, left thumb results in activation predominantly in the opposite somatosensory cortex. Stimulation of the osseointegrated thumb prosthesis in the right hand results in activation of somatosensory cortex bilaterally

tion pattern at 8 and 12 months (Fig. 2a and b). This is confirmed by the laterality indexes given in the figure text.

In the patient with an osseointegrated thumb tactile stimulation with a filament or a brush of the healthy thumb resulted in cortical activation predominately in the opposite hemisphere, laterality index −0.71 and −0.83. Tactile stimulation with a filament or a brush of the prosthetic thumb resulted in a more bilateral activation pattern compared to the healthy thumb, *LI* 0.14 and 0.43.

Discussion

Extensive cortical changes have been advocated as one major reason for the generally poor functional sensibility after amputation injuries [7]. Few studies have addressed this problem and several of them have investigated patients with hand transplantations showing in some cases impressing results with a good sensibility and a rapid normalization of cortical changes both in the sensory cortex and in the motor cortex [2, 5, 9]. In cases of amputation of a hand or part of the hand the extrinsic muscles controlling the hand are often proximal to the

injury and thus since the muscles are intact and can be activated the cortical changes in the motor cortex are minor and this likely results in a more rapid return to normal activation pattern in the cortical motorsystem as shown in transplantation and replantation cases [2, 5, 9] and also in our replantation case where the activation was normal at first investigation at 4 months. The sensory cortex, deprived of all input, is more likely to show profound reorganisation after the injury and later on when the peripheral nerve has healed it tries to reinnervate its original cortical area.

Patients with osseointegrated prosthesis often show some degree of perception of touch, which has been termed osseoperception, however the mechanism behind this has been unclear. In our study we noticed activation in the primary sensory cortex on fMRI when stimulating the prosthesis. Cortical activation was more bilateral than in sensory stimulation of the contralateral healthy thumb. Intraosseous free nerve endings may be transferring the vibrations in the prosthesis to the brain. Another possible mechanism are mechanoreceptors activated by the pressure on the prosthesis. Whatever the peripheral mechanism is, cortical activation is located in the primary sensory cortex and it is bilateral; the latter may be due to a compensatory mechanism, where the cortex recruits a larger area to compensate for the weak peripheral nerve signal from the prosthetic thumb. After hand replantation an ipsilateral activation is noted at 4 weeks. This is difficult to explain as the peripheral nerve has not grown to its targets at this time and as the activation is only ipsilateral. Further on the activation changes from an ipsilateral to a more contralateral activation pattern but even after 12 months the activation is more bilateral, measured as laterality index, compared to the healthy hand. This may also represent a compensatory gain, the peripheral nerve function is suboptimal and to compensate for this a larger cortical area is recruited.

We hypothesise that the rapid return to a normal activation pattern in the motor cortex is due to the fact that the muscles controlling the hand are located proximal to the injury and even if the muscles in these situations do not have a peripheral target to act on they can still be activated and contract and thus keep their cortical representation. The cortical changes seen in the sensory cortex after amputation and replantation or prosthesis are likely large but cortical changes occur also at normal nerve injuries, but to a less extent. A better understanding of the cortical reorganisation following deafferentation and also the mechanism by which the peripheral nerve tries to regain its projection in the sensory cortex is imperative for understanding the results after nerve surgery and also to design and optimise the sensory re-education following nerve repair in the hand.

References

1. ASHT (1992) Clinical assessment recommendation, 2nd edn. American Society for Hand Therapists
2. Brenneis C, Loscher WN, Egger KE, Benke T, Schocke M, Gabl MF, Wechselberger G, Felber S, Pechlaner S, Margreiter R, Piza-Katzer H, Poewe W (2005) Cortical motor activation patterns following hand transplantation and replantation. J Hand Surg [Br] 30: 530–533
3. Chen R, Cohen LG, Hallett M (2002) Nervous system reorganization following injury. Neuroscience 111: 761–773
4. Friston KJ, Holmes A, Worsley KJ, Poline JB, Frith CD, Frackowiak RSJ (1995) Statistical parametric maps in functional imaging: a general linear approach. Hum Brain Mapp 2: 189–210
5. Giraux P, Sirigu A, Schneider F, Dubernard JM (2001) Cortical reorganization in motor cortex after graft of both hands. Nat Neurosci 4: 691–692
6. Hansson T, Brismar T (2003) Loss of sensory discrimination after median nerve injury and activation in the primary somatosensory cortex on functional magnetic resonance imaging. J Neurosurg 99: 100–105
7. Lundborg G (2003) Richard P. Bunge memorial lecture. Nerve injury and repair – a challenge to the plastic brain. J Peripher Nerv Syst 8: 209–226
8. Lundborg G (2004) Nerve injury and repair. Regeneration, reconstruction and cortical re-modelling. 2nd edn. Elsevier, Philadelphia
9. Neugroschl C, Denolin V, Schuind F, Van Holder C, David P, Baleriaux D, Metens T (2005) Functional MRI activation of somatosensory and motor cortices in a hand-grafted patient with early clinical sensorimotor recovery. Eur Radiol 15: 1806–1814
10. Ogawa S, Lee TM, Nayak AS, Glynn P (1990) Oxygenation-sensitive contrast in magnetic resonance image of rodent brain at high magnetic fields. Magn Reson Med 14: 68–78
11. Rosen B, Lundborg G (2003) Early use of artificial sensibility to improve sensory recovery after repair of the median and ulnar nerve. Scand J Plast Reconstr Surg Hand Surg 37: 54–57
12. Sollerman C, Ejeskar A (1995) Sollerman hand function test. A standardised method and its use in tetraplegic patients. Scand J Plast Reconstr Surg Hand Surg 29: 167–176
13. Turner R, Le Bihan D, Moonen CT, Despres D, Frank J (1991) Echo-planar time course MRI of cat brain oxygenation changes. Magn Reson Med 22: 159–166
14. Wall JT, Xu J, Wang X (2002) Human brain plasticity: an emerging view of the multiple substrates and mechanisms that cause cortical changes and related sensory dysfunctions after injuries of sensory inputs from the body. Brain Res Brain Res Rev 39: 181–215
15. Worsley KJ, Poline JB, Vandal AC, Friston KJ (1995) Tests for distributed, nonfocal brain activations. Neuroimage 2: 183–194

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Cortical motor activation patterns following hand transplantation and replantation

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Summary

We studied cortical activation patterns by functional MRI in a patient who received bilateral hand transplantation after amputation 6 years ago. In the early post-operative period, the patient who had had the hand transplantation revealed strong activation of a higher motor area, only weak activation of the primary sensorimotor motor cortex and no activation of the primary somatosensory cortex. At one-year follow-up, a small increase in primary sensorimotor motor cortex activation was observed. Activation of the primary somatosensory cortex was only seen at the 2-year follow-up. Transplantation after long-standing amputation results in cortical reorganisation occurring over a two-year period.

Keywords: Cortical motor activation; hand transplantation; cortical reorganisation; somatosensory cortex.

Introduction

Cortical reorganisation after limb deafferentation is a well-known phenomenon which primarily results in a decrease of cortical representation of motor and sensory areas. In primates, an increase of the adjacent cortical areas has been observed [10]. Similarly, an enlargement of stump and shoulder cortical representation was found in human limb amputees [1, 2, 4, 7, 8]. However, little is known as to whether this reorganisation is reversible by hand transplantation several years after the primary injury. One study suggested that cortical reorganisation is quickly reversible after bilateral hand transplantation [5].

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To investigate the time course of cortical reorganisation after hand transplantation, we determined the activation pattern of simple finger movements in a patient who received bilateral allogenic hand transplantation after amputation 6 years ago.

Patient and methods

A 47-year-old, right-handed male received two hand allograft transplants 6 years after amputation at wrist level in a bomb injury [9].

The patient was included in an intensive rehabilitation program.

This patient was investigated by functional MRI after 6 weeks, 12 and 24 months postoperatively. Clinical examinations were performed before acquisition of MR images. The patient gave his informed consent to the study. He was scanned with the head immobilized and eyes closed. Both arms rested comfortably to prevent activity of the proximal arm muscles. He was instructed to perform a flexion/extension movement of all the fingers of each hand at 0.5–1 Hz. All tasks were under continuous visual control of one of the investigators.

Data were acquired with a 1.5 Tesla MR Scanner (Magnetom Vision, Siemens, Erlangen, Germany). The images covered the whole brain except the posterior lobe of the cerebellum. All active conditions were repeated 6 times in a pseudo-random order, each repetition contained five brain volumes. Acquisition time of the brain volume lasted 5.5 sec with 1.5 sec interval time between two scans. Between the conditions the patient were acoustically instructed of the next condition.

Results

Six week after surgery, the patient showed finger flexion and extension of grade 3, according to the British Medical Research Council (MRC; 3, active movement against gravity, 4, active movement against resistance and gravity; 5, normal power) in both hands. He was

unable to oppose the thumb to the little finger. At one year, finger flexion and extension improved markedly to grade 4 MRC without any improvement in intrinsic hand muscle weakness. Surface and deep sensation improved partially. The patient was able to identify light touch, painful pin prick stimuli and several finger positions. At two years, strength had improved further to MRC 4-5 for flexion and extension.

During left hand movement baseline fMRI revealed activation in the contralateral supplementary motor area (SMA), while only weak activation was seen in the right sensorimotor motor cortex (SM1). At one year, activation in the right SM1 was similar to that in the early post-operative period but the primary somatosensory area (SS1) was still not activated. Two years after transplantation, an increase of SM1 activation and activation of the right SS1 were observed. Similar activation patterns were observed during movements of the transplanted right hand.

Discussion

Functional MRI allows investigation of cortical activation in various tasks. In healthy controls, simple finger movements lead to an activation of the primary sensomotor (SM1) and somatosensory (SS1) cortex as well as in higher motor areas. The main higher motor region is the supplementary motor area (SMA) which is located in the mesial wall of the frontal cortex. This region is known to participate on programming motor plans. Additional function in preparing movements is based in the lateral premotor cortex which is close to the primary motor cortex in the frontal lobe. In complex motor tasks, activation is also observed in the parietal cortex which provides proprioceptive and visual informations for the movement planning and control.

In this study, we determined cortical activation pattern during a simple motor task after long-term (6 years) deafferentation. Six weeks post-operatively, the activation pattern in the patient after long-standing amputation contained activation of the SMA proper and only a small cluster of activity in SM1. Therefore, we assume that SMA activation is more resistant to the consequences of amputation. This finding might be related to the SMA function of motor programming [11] which can be trained by motor imaging or the use of myoprotheses. Furthermore, evidence exists from anatomical studies that direct connections exist from the SMA to the spinal cord [3]. This pathway could compensate for lost projections from the primary motor cortex after long-term amputation and would be in line with the clinical observation that motor recovery of our patients was similar.

However, over two years cortical reorganisation after hand transplantation led to activation of somatosensory areas and an increase of activation in primary motor cortex, indicating a shift of neuronal activity back from the 'programming area' to the executing SM1.

The patient with hand transplantation failed to show any activation in the lateral premotor regions, which are known to process sensory information for certain movement parameters [6, 12] possibly due to the fact that these areas received insufficient input from somatosensory areas.

One could speculate that the delay between amputation and transplantation critically influences the reversibility to a normal cortical activation pattern. However, it is not known whether there is a 'cut off' time between amputation and transplantation after which motor recovery is unsatisfactory. We suggest that patients who are eligible for hand transplantation should be included preoperatively in an extensive training program either with mental imaging of hand movements which is known to evoke cortical activity in motor areas or with forced use of myo-prothesis.

The observed results are based on one case. Future cases may show different patterns of activation and recovery due to age-related factors, variability of individual extent of activation size, circumstances of their hand lesion, surgical procedure or postoperative factors.

Our data suggest that cortical reorganisation after longstanding deafferentation and deefferentation is partially reversible but that reorganisation after hand transplantation continues for at least two years.

References

1. Cohen LG, Bandinelli S, Findley TW, Hallett M (1991) Motor reorganization after upper limb amputation in man. A study with focal magnetic stimulation. Brain 114 (Pt 1B): 615–627
2. Dettmers C, Liepert J, Adler T, Rzanny R, Rijntjes M, van Schayck R, Kaiser W, Bruckner L, Weiller C (1999) Abnormal motor cortex organization contralateral to early upper limb amputation in humans. Neurosci Lett 263: 41–44
3. Dum RP, Strick PL (1996) Spinal cord terminations of the medial wall motor areas in macaque monkeys. J Neurosci 16: 6513–6525
4. Fuhr P, Cohen LG, Dang N, Findley TW, Haghighi S, Oro J, Hallett M (1992) Physiological analysis of motor reorganization following lower limb amputation. Electroencephalography Clin Neurophysiol 85: 53–60
5. Giraux P, Sirigu A, Schneider F, Dubernard JM (2001) Cortical reorganization in motor cortex after graft of both hands. Nature Neurosci 4: 691–692

6. Jackson SR, Husain M (1996) Visuomotor functions of the lateral pre-motor cortex. Curr Opin Neurobiol 6: 788–795
7. Kew JJ, Ridding MC, Rothwell JC, Passingham RE, Leigh PN, Sooriakumaran S, Frackowiak RS, Brooks DJ (1994) Reorganization of cortical blood flow and transcranial magnetic stimulation maps in human subjects after upper limb amputation. J Neurophysiol 72: 2517–2524
8. Pascual-Leone A, Peris M, Tormos JM, Pascual AP, Catala MD (1996) Reorganization of human cortical motor output maps following traumatic forearm amputation. Neuroreport 7: 2068–2070
9. Piza-Katzer H, Ninkovic M, Pechlaner S, Gabl M, Ninkovic M, Hussl H (2002) Double hand transplantation: functional outcome after 18 months. J Hand Surg 27B: 385–390
10. Qi HX, Stepniewska I, Kaas JH (2000) Reorganization of primary motor cortex in adult macaque monkeys with long-standing amputations. J Neurophysiol 84: 2133–2147
11. Tanji J (1996) New concepts of the supplementary motor area. Curr Opin Neurobiol 6: 782–787
12. Wise SP, Boussaoud D, Johnson PB, Caminiti R (1997) Premotor and parietal cortex: corticocortical connectivity and combinatorial computations. Ann Rev Neurosci 20: 25–42

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Coordinated function oriented movements after multiple root avulsion

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Summary

In the early years of brachial plexus surgery the surgeon was happy if the patient developed voluntary movement of certain muscles especially of the biceps to flex the elbow joint. Degree of flexion and force of flexion were utilized to prove success.

For the patient, however, flexion alone is not of much use if he cannot do external rotation. The real goal for evaluation of brachial plexus surgery should be the ability to perform coordinated, complex movements. To achieve complex movements after a peripheral nerve lesion is rather easy if continuity of a nerve defect could be restored. This is much more difficult in cases of root avulsions. In such cases cerebral plasticity plays a decisive role. This is illustrated by typical cases.

Keywords: Brachial plexus; brain plasticity; nerve regeneration; root avulsion; peripheral nerve.

Introduction

The innervating nerve and its connection to the central nervous system defines the function and the functional qualities of the muscle, be it a far or a slow contracting muscle, etc.

The nerve and its representation in the cortex defines the topographic location of an afferent input.

In the fifties of the last century the surgeons were convinced that useful restoration of discriminative sensibility in the pulp of a digit in an adult patient was impossible after peripheral nerve lesion. In order to restore proper sensibility in the pulp of the thumb and index finger in a case of median nerve lesion, a so-called sensible island flap from the ulnar innervated part of the hand (ulnar side of the ring and radial side of the little finger) was transferred on their neurovascular bundle. This procedure provided not only a perfect cover of the finger tip with adequate skin but also a ''normal'' sensibility with good two-point discrimination etc. These patients learned very well to use their hand with now proper sensibility at strategic important locations. However, if a pin prick was done on the flap originating from the ring finger and now located at the thumb, the patients moved automatically the ring finger for protection without moving the thumb. This proved that even after years the reflectory reaction after an afferent stimulus corresponded still to the original representation zone of the ulnar nerve innervated digital nerve to the ring finger. Many authors including myself preferred to connect the digital nerve of the flap with the proximal stump of the nerve of the receiving finger in cases where the flap procedure was performed to reconstruct the pulp after injury in order to have a proper topographic representation.

In palliative surgery, after irreparable nerve lesions, muscles innervated by the radial nerve transferred for finger flexion or median or ulnar nerve innervated muscles were utilized for finger extension. But in these cases attention was paid that synergistic muscle e.g. wrist extensors be applied for finger flexion and wrist flexors for finger extension.

It was therefore a completely new experience when Seddon [1, 3] published a case of total brachial plexus paralysis where the ulnar nerve was transferred to connect the third and the fourth intercostal nerve with the distal stump of the musculocutaneous nerve to achieve active elbow flexion. In addition, the patient was treated by arthrodesis of the shoulder joint and by forearm amputation.

Since then intercostal nerve transfer has been applied by many surgeons throughout the world. In Japan, the

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intercostal nerve transfer was applied in many cases where myelography had indicated root avulsion as the only treatment [2]. Other surgeons including myself are still using this suggestion as one of the options to re-neuroticise part of the denervated territories. Since the intercostal nerves innervate the intercostal muscles important for breathing and the muscles of the abdominal wall, patients are encouraged during the rehabilitation period to exercise by deep breathing, coughing, and pressing the abdominal wall. For several months nothing will happen. Suddenly, one day, the patients realize that during deep breathing the biceps muscle shows contractions. Continuing the exercises patients learn to stimulate strong elbow flexion by deep breathing or by coughing. Apparently afferent impulses from the biceps and the brachialis muscle report the action to the central nervous system and a new pathway develops. Finally, patients learn to flex the elbow without stimulating the original function of the involved intercostal nerves.

It is not my intention to belittle the successes of the past. It is an extreme advantage for the patient if he or she can control in some way the shoulder joint and flex the elbow instead of having a flail arm. On the other hand elbow flexion alone does not really help the patient if he can do this just in front of his abdominal wall. External rotation in the shoulder joint is equally important for the patient to be able to move his arm in space. His movements should be function oriented. The patient should not think: ''flex the elbow by innervating the biceps'', but should grasp an object on a table in front of me, for example. For this task he needs a complex movement:

1) Adduct the arm, flex the elbow and do external rotation to bring the forearm in the sagittal plane of the object.
2) Move the arm forward to bring it in the frontal plane of the object.
3) Abduct the arm in the shoulder joint to bring the palmar side of the hand facing the object.
4) Lower the arm to close on the object by adduction in the present position.
5) Grasp the object using some kind of gripping function.

It is the brain that has to coordinate these movements based on constant input about muscle action and joint position.

After paralysis with regeneration the patient relearns coordinated function oriented movements by reactivation of existing or new pathways.

In cases of avulsion of all five roots of the brachial plexus, there are no pre-existing pathways.

With nerve fiber and tendon muscle transfers, respectively, a new situation is created and the question is whether the brain is able to deal with the new situation.

Studying special cases one is surprised how well the brain is coping with new challenges especially in children and young patients.

The following case is presented as an example

An eight-year-old boy suffered an avulsion injury of all five roots of the left brachial plexus due to a traction lesion by a traffic accident.

Primary surgery

Surgery was performed three months after the accident dealing with the plexus itself followed by a contralateral C7 nerve fiber transfer three months later.

The patient had intensive physiotherapy during the subsequent months.

He regained the following movements:

– Elbow flexion: innervation of biceps and brachialis muscle by intercostal nerves 3 and 4.
– Abduction of the shoulder joint, extension of the elbow finger and wrist extension by nerve fibers from C4 via nerve grafts to the posterior cord.
– Anteversion of the scapula by the serratus anterior muscle. Reinnervation of the long thoracic nerve by the dorsalis scapulae nerve.

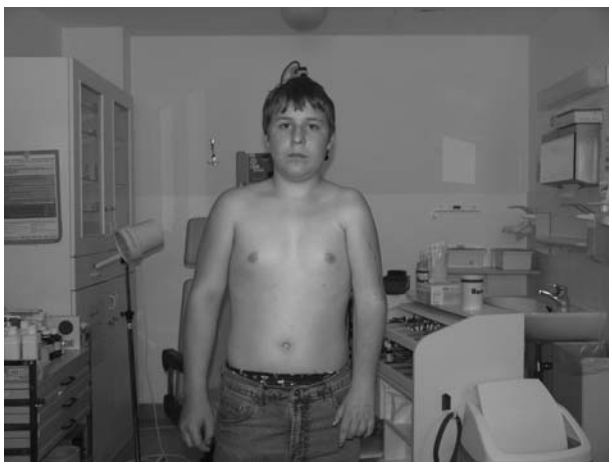

Fig. 1. An eight-year-old boy suffered a brachial plexus lesion on his left side. Multiple surgery was performed as indicated in the text. Result six years after surgery: position of rest

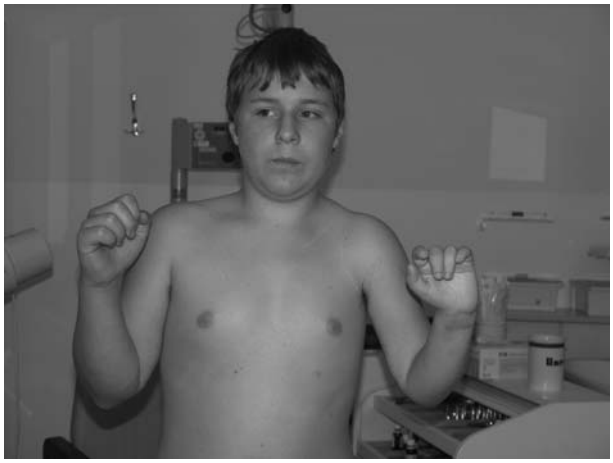

Fig. 2. Flexion of elbow joints

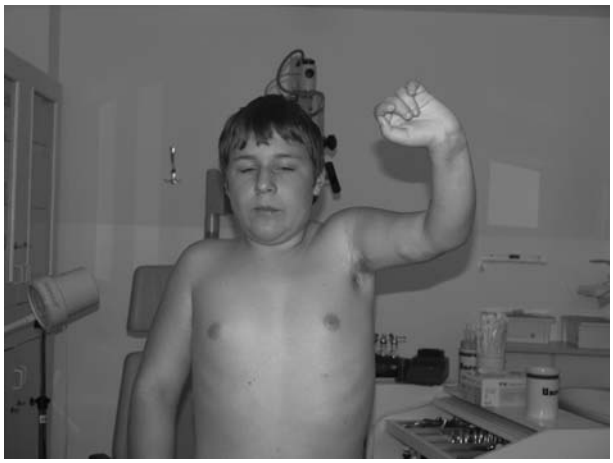

Fig. 3. Abduction and external rotation of the shoulder joint

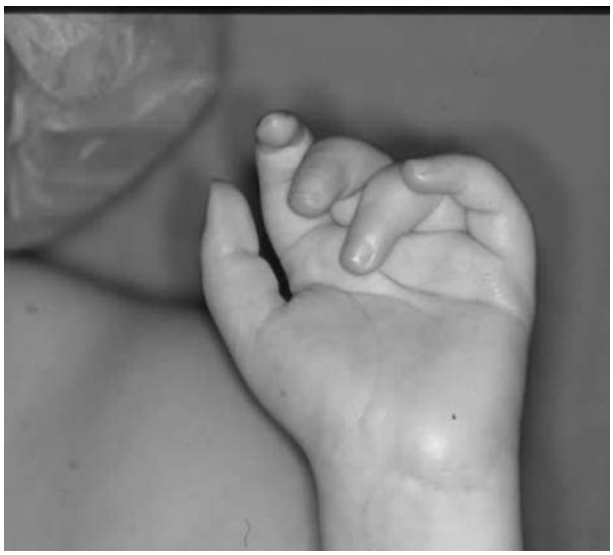

Fig. 4. Extension of the wrist by C4

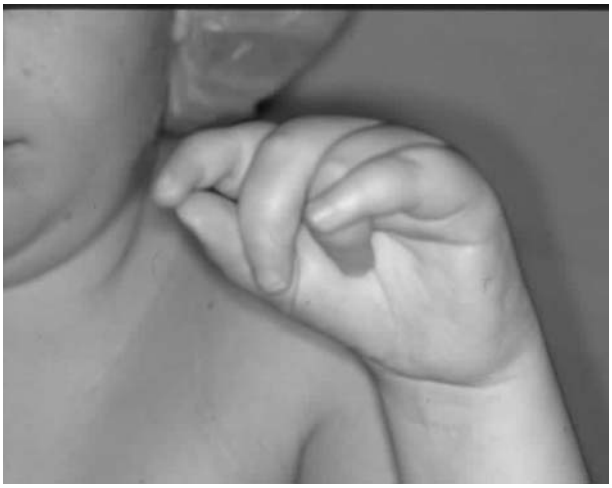

Fig. 5. Key grip function provided by contralateral C7

– Internal rotation and adduction by innervation of the major pectoralis muscle by nerve grafts from the phrenic nerve (end-to-side coaptation) to the medial and lateral pectoral nerve (end-to-end coaptation).
– Nerve fiber transfer from contralateral C7 to the median and the ulnar nerve by free nerve grafts (bilateral saphenous nerves).

Secondary surgery

Transposition of the insertion of the major pectoralis muscle to act as an external rotator. The phrenic nerve continued to innervate the diaphragma and the brain had learned in addition to innervate the major pectoralis muscle for internal rotation and adduction. Now the nerve had to transmit impulses of a different function provided by new cerebral pathways. The major pectoralis muscle has to be innervated for external rotation and adduction (Figs. 1–5).

Unfortunately, not all patients adapt so quickly and so well as this patient. It is our task to develop techniques to enhance cerebral plasticity in all patients.

References

1. Seddon HJ (1963) Nerve grafting. J Bone Joint Surg 45B: 447
2. Tsujama NR, Sagakuchi T, Har T, Kondo S, Kaminuma M, Tjichi M (1961) In: Ryn D (ed) Proc. 11th Ann. Meet Jap. Soc. of the Hand, Hiroshima
3. Yeoman PM, Seddon HJ (1961) Brachial plexus injuries; treatment of the flail arm. J Bone Joint Surg 43B: 493

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Enhanced sensory relearning after nerve repair by using repeated forearm anaesthesia: aspects on time dynamics of treatment

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Summary

Background. We describe a new principle for enhancing the effects of sensory re-education following nerve injury and repair. The outcome from nerve repair in adult patients is generally poor. One reason is the functional cortical reorganisation which always occurs because of axonal misdirection at the repair site. In healthy individuals selective anaesthesia of the forearm results in improved hand sensation. Here we hypothesised that this principle would be valid also after nerve injury and repair.

Method. In a prospective, randomised, double blind study we studied the effects of cutaneous forearm anaesthesia combined with sensory re-education on the outcome after median or ulnar nerve repair at wrist or distal forearm level.

Findings. EMLA-application four times over a two week period starting with beginning reinnervation of the fingers resulted in significantly improved sensory recovery (tactile gnosis) as compared to the placebo group and also at assessment four weeks after the last EMLA-session. However, at assessment 8–11 months after the first EMLA-treatment there was no difference between the groups.

Conlusions. Our findings indicate that repeated cutaneous forearm anaesthesia over a two week period can enhance the effects of sensory re-education at least over the four following weeks. However, the optimal time protocol for EMLA-treatment, aiming at a long-lasting or permanent effect on sensory recovery still has to be defined.

Keywords: Nerve injury; anaesthesia; sensory re-education; hand function.

Introduction

Injuries to major nerve trunks in the upper extremity may vary in level and severity from digital nerve injuries to brachial plexus lesions and from clean-cut nerve transection to severe nerve lacerations. Such injuries are usually seen in young males [32, 38] and may result in life-long hand function impairment, cold intolerance, dysaesthesia and pain [27, 28]. There is a substantial economic impact on society as well as patients with high probability of work loss [16, 46, 47]. Although evolving scientific concepts during the past decades have resulted in substantial new knowledge about basic biological mechanisms regulating nerve regeneration this had little impact on the treatment of nerve injuries. Technical aspects of nerve repair procedure have been much debated, but little has changed in this respect over the last decades [26, 28]. Still the tactile discriminative functions of the hand of an adult patient can usually not be fully restored following a median nerve lesion at wrist level [28].

During recent years there are accumulating data indicating that central nervous factors, rather than peripheral factors are responsible for the outcome after nerve repair. In adult patients specific cognitive capacities such as verbal learning capacity and visuo-spatial logic capacity help to explain variations in recovery of functional sensibility [53]. Comparing the outcome in adults and children there is a very obvious difference in results indicating that among all influencing factors the age of the patient is most important, children constantly showing superior functional results as compared to adults [1, 2, 12, 29, 40].

A nerve injury has immediate and long-standing consequences of the functional organisation of brain cortex [60]. Recovering sensory functions in the hand has much in common with learning a second language [29]. The background is the profound functional reorganisations which occur after nerve injury, and the learning process which is required to adapt to this new situation [28]. A nerve transection represents an acute de-afferentiation with immediate and long-standing influence on the cor-

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responding representational areas in brain cortex as well as in adjacent cortical territories [7, 11, 20, 26, 34, 55, 57, 58]. It is important to consider two phases in this reorganisational process with an early phase (phase one) representing the early period after nerve repair when the denervated body part is without sensation, and a later phase (phase two) when there is a beginning reinnervation of the denervated body part. For instance, transection of a median nerve at wrist level results in a silent area in the somatosensory brain cortex – corresponding to the projectional area of the thumb, index finger, middle finger and half ring finger that have been deprived of their central connections [33, 34, 55, 57]. As a result of this de-afferentiation adjacent cortical areas expand and gradually occupy the former median nerve territory. These processes, occurring during phase one, happen when the hand is still without sensation and when the cortical hand representation tends to disappear as a result of expanding adjacent cortical territories. After several months, when reinnervation of the hand starts, there is again a cortical reorganisation, this time reflecting the axonal misorientation at the repair site [60], parent axons reinnervating incorrect distal pathways so that skin areas of the hand are not reinnervated by their original axons (phase two). The original well-organised cortical representation of the hand is now transformed into a mosaic-like pattern [10, 15, 21, 57] and the nerve does not recapture all of its original territory.

Normally, the various body parts are represented in specific projectional sites in somatosensory and motor cortex. Already 70-year-ago it was demonstrated that body parts with especially well developed sensory functions – like the hand – have occupied a very large area of somatosensory cortex [39]. Thus, there is a correlate between sensory capacity and cortical representational area. We have previously demonstrated that sensory functions of the hand in normal individuals can be enhanced rapidly by anaesthetising a nearby body part – the forearm – by cutaneous anaesthesia [48]. The basis for the enhanced sensory function is probably a rapid expansion of the cortical hand representation as a result of the forearm de-afferentiation.

The purpose of the present study was to use the same principle in a nerve injury material, hereby testing a new concept in hand rehabilitation following nerve injury and repair. Our hypothesis was that de-afferentiation of the forearm would allow such an expansion of the cortical hand representation also in nerve injured patients, and that this might enhance the effectiveness of sensory re-education.

Patients and methods

The study included 13 patients with median ($n = 7$) or ulnar ($n = 6$) nerve injuries at wrist level aged 38.2 (range 19–75) years. There were 10 men and 3 women. A prerequisite for participation was some protective sensation at finger tip level as assessed with Semmes-Weinstein monofilament (SWM $\#4.31 = 2$ g pressure). Meantime since surgery was 22 months (range 11–52 months).

The study design was prospective, randomised and double blind. The patients were randomised to receive local anaesthetic agent containing 2.5% lidocain and 2.5% priolocain (EMLA®, AstraZeneca, Södertälje, Sweden) or placebo, a water and oil emulsion. The two agents could not be identified since they were identical in consistency, colour and packaging. The EMLA or placebo was applied under occlusive bandage for one hour to the volar side of the forearm (Fig. 1), in an area from the wrist and 15 cm proximally, on the same side as the nerve injury. Seven patients received EMLA (three women and four men, five median and two ulnar injuries, aged 29 (19–75) years) and six patients (six men, two median and four ulnar nerve injuries, age 39 years, range 29–50) patients received placebo.

The patients in both groups underwent an intense sensory re-education program with strictly scheduled home training and assisted supervised training during one hour while under the influence of EMLA or placebo (Fig. 2). Combined with the sensory re-education they received EMLA or placebo twice a week for two consecutive weeks.

In the sensory training we used a miniature Rubik's cube which have been manipulated in the way that one colour was removed and 8 mm holes were drilled instead giving one side with holes [48]. When practicing with the cube the patient could continuously change the sur-

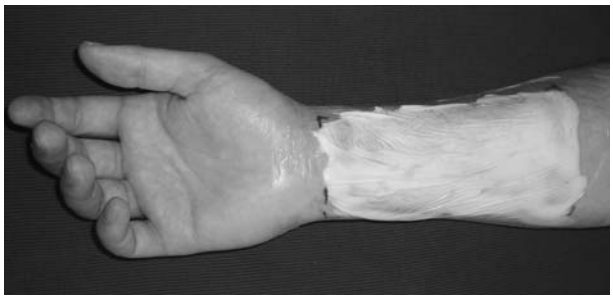

Fig. 1. The EMLA or placebo cream applied under occlusive bandage on the volar side of the forearm in an area form the wrist and 15 cm proximally on the same side as the nerve injury

Fig. 2. Patient during sensory relearning practice with the miniature Rubik's cube

face pattern by twisting the cube which easily could fit into a pocket. Each patient was provided with a training protocol which included a minimum training frequency of five sessions per day with the cube during the two-week intensive training. Thereafter the patient kept the cube and was encouraged to encompass the sensory relearning strategies in daily activities.

The protocol for the sensory training was according to the principles described by Wynn-Parry and Salter [62] and Dellon [8] under high concentration and with or without the use of vision. The training included exercises aiming at identification of textures, shapes and real objects. The forearm was covered with a dressing and the participants were instructed not to touch the forearm. After one hour's application, when the skin of the forearm was anaesthetised, the EMLA or placebo was carefully washed off.

The study was approved by the Local Ethics Committee at Lund University and written informed consent was obtained from all participants.

Follow-up

Assessment of hand sensibility was performed: 1) prior to the experiment; 2) after one week training, prior to the third EMLA/placebo application; 3) after the fourth application of EMLA/placebo after two weeks training; 4) four weeks after the last EMLA/placebo session; 5) 8–11 months after the first EMLA/placebo application.

Hand function was assessed in a standardised procedure according to the Model for Documentation of Outcome after Nerve Repair, including separated domains for sensory/motor/pain, discomfort and also a "total score" [52]. Special attention was paid to sensory recovery. Semmes-Weinstein monofilaments (SWM) were used for assessment of touch perception. The summarised SWM measure points were the three "critical sites" described by Bell-Krotoski [3, 4, 50, 52] i.e. pulp of digit I and II and base phalanx of digit II for the median nerve and pulp of digit V, base phalanx and proximal hypothenar for the ulnar nerve. For assessment of tactile gnosis the STI-test (Shape-Texture-Identification test) was used [18, 51] and classic static 2PD test (DiskCriminator [3]) was used for assessment of tactile gnosis. The 2PD test was performed according to procedures [36] as described by ASSH and ASHT [3] in a descending order starting with 15 mm to assess the level at which responses were correct (seven out of ten correct at just blanching of the skin) and quantification 0–3 ($0 \geq 16$ mm, $1 = 11$–15 mm, $2 = 6$–10 mm, $3 = 3 \leq 5$ mm).

Statistics

After calculation of the difference between results obtained before EMLA or placebo and results after one week, two weeks, four weeks after the last EMLA/placebo application/termination of sensory re-education, and mean 9 months (range 8–11 months), a group comparison with Mann–Whitney *u*-test was done. Level for significance was $p \leq 0.05$.

Results

We have recently reported the early results up to four weeks after the last EMLA/placebo session and termination of regular sensory re-education. Those results showed a significantly better improvement in functional sensibility (tactile gnosis) during that period in favour of the EMLA treated group [48].

In the present study we concentrated on a longer follow-up. At a mean time of nine months (range 8–11 months) after termination of the intensive training period we could see no lasting significant difference between the groups (Fig. 3).

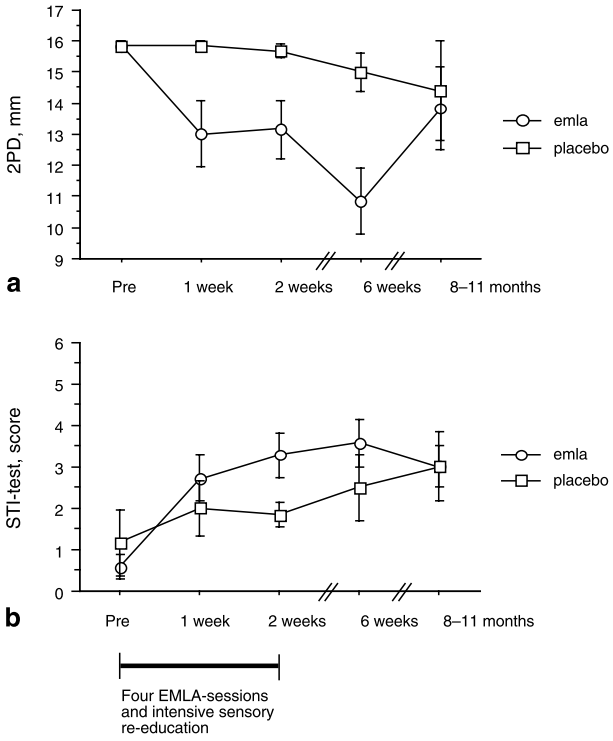

Fig. 3. Line chart illustrating the 2PD test result (a) and the STI-test result (b) over time in the EMLA/placebo groups with significant group difference at the 1, 2 and 6 week follow-up. EMLA/placebo treatment in combination with intensive sensory re-education was performed during the two first weeks

In both groups tactile gnosis was however improved both at the early and late follow-up as compared to initiation of treatment.

Discussion

In this study we focused on phase two after nerve injury and repair, i.e. when reinnervation of the hand is beginning and when the normal cortical hand representation is being transformed into a mosaic-like, disorganised pattern. "The hand speaks a new language to the brain" and processing of the new pattern of sensory impulses is difficult to perform for the adult brain. Our major finding is that this processing can be facilitated by cutaneous anaesthesia of the forearm, allowing the cortical hand representation to expand, hereby recruiting new cortical resources to participate in the processing of sensory information. We also have found that four sessions of cutaneous anaesthesia is not enough for a long-lasting effect of the improved functional sensibility.

We find it extremely important, when discussing sensory relearning after nerve repair, to separate between the early postoperative phase (phase one) characterised by no sensation in the denervated body part, and the late postoperative phase (phase two) characterised by beginning reinnervation of the denervated body part. From the viewpoint of the brain these two phases have fundamental differences: in phase one cortical representation of the denervated body part is taking over by adjacent expanding cortical territories so that the cortical projection of the denervated body part disappears. In phase two, the cortical projectional area is reappearing, however in a new and disorganised form. According to classical sensory re-education the protocol is started at first in phase two, leaving phase one without any attention at all.

We feel that the timing for initiation of sensory re-education is crucial, and should start immediately after nerve injury, aiming at maintenance of the cortical hand representation. In phase one following nerve repair in the upper extremity, maintenance of the cortical hand projection may be essential and important for facilitation of later sensory recovery when the hand is reinnervated – a sensory preparation of the brain. From experiments in healthy individuals many principles have been described to activate hand motor cortical areas, for instance by imaging hand movements (motor imagery exercises) [9, 17, 24, 25], observing movements of hands belonging to other individuals by activating mirror neurons [6, 43–45, 56] or reading or listening to action words, related to hand movements [14, 41]. In *tactile imagery* exercises the somato-sensory cortex is activated by imaging tactile stimulation of the hand [63]. An alternate way to activate somato-sensory cortex is to *observe* touch [13, 23].

Somato-sensory cortex can also be activated by using another sense – hearing – to substitute for absent sensibility. In the Sensor Glove System acoustic signals from miniature microphones are mounted at finger tip level in gloves, adapted to non-sensate hands [31]. The friction sound, induced by touch of various textures and materials, is transmitted to earphones making possible to ''listen to'' what the hand feels [31]. fMRI studies have shown that such a system induces activation not only of the cortical acoustic area but also the nearby somato-sensory area. Patients, subjected to median nerve repair and using the Sensor Glove System regain a better tactile gnosis after one year as compared to patients not using the glove [30].

Thus, there are various principles focusing at maintenance of the cortical hand representation in *phase one* – when the hand is still without sensation. In the present study, however, we focus on *phase two* i.e. the period starting 3–4 months after median nerve repair when reinnervation of the hand is beginning. This is when the cortical hand representation shifts into a mosaic-like pattern requiring relearning and adaptation to new cortical mapping of the hand. Our hypothesis was that increasing the cortical hand representation would recruit an extended number of nerve cells, and would hereby facilitate the processing of sensory impulses from the hand. It is well known from experimental studies that de-afferentiation of a body part is followed by a corresponding silent cortical area allowing a rapid functional expansion of nearby cortical areas. Accordingly, cutaneous anaesthesia of the forearm, as here performed by a crème (EMLA-crème) containing Lidocain and Prilocain, would allow for such an expansion of the cortical hand representation. Our concept was inspired by studies on motor function in stroke patients showing that selective anaesthesia of ventral root C5–C8 (motor innervation of shoulder and elbow) resulted in enhanced motor function of the hand (innervation C8–C11) [37].

We have previously demonstrated that selective cutaneous anaesthesia of the forearm in healthy patients results in a rapid improvement of sensory functions of the ipsilateral hand. Here we demonstrate that the same phenomenon occurs also in patients subjected to nerve injury and repair, provided the EMLA principle is applied in *phase two*, i.e. when reinnervation of the hand has started. Sensory re-educational programs, as described by Wynn-Parry [61] and Dellon [8] are essential for sensory recovery after nerve repair, but here we show that the effects of such sensory re-education can be much enhanced by selective cutaneous anaesthesia of the forearm. The optimal way to apply EMLA-crème in terms of frequency of application and duration of the EMLA-program remains to be defined. Here we used application twice a week for a two week period in combination of intense sensory training. We found a lasting effect of the EMLA-application for at least four weeks after the last treatment – at this time-point the patients subjected to EMLA-treatment still showed a superior sensory recovery as compared to the group not subjected to EMLA-treatment. When assessed 8–11 months later, however, the two groups showed almost identical results. The observations indicate that although the EMLA-treatment, as applied in this study, showed an effect lasting for at least four weeks, the effect did not last for 12 months. Studies, aiming at an analysis of the optimal

time dynamics in EMLA-treatment are now in progress. Hypothetically, an initial intensive EMLA-treatment should be repeated at defined intervals over a longer period of time for a maintained effect.

Our observations indicate a new principle for enhancement of the effects of sensory re-education after nerve injury and repair at wrist level, based on repeated sessions of forearm cutaneous anaesthesia. The concept that such selective cutaneous anaesthesia will increase the cortical hand representation is based on current concepts of brain plasticity mechanisms. In the somato-sensory cortex there is a complete somato-topic map of the entire body surface, the size of individual body parts reflecting the activity and sensory competence of that part [22, 42]. The cortical representation of the body parts is experience dependent, varying in size dependent on activity and sensory inflow. In this study we focus on rapid plasticity, occurring within hours. Merzenich et al. [35] demonstrated more than 20-year-ago that amputation of a middle finger in adult primates resulted in rapid expansion of cortical territories belonging to the adjacent fingers, and [54] demonstrated analogous functional reorganisations to occur after finger anaesthesia. Such rapid plasticity can certainly not be based on formation of new synapses or dendritic sprouting, but is rather associated with unmasking of the existing connections [5, 22, 42]. Normally, many connections between the periphery and the cortex as well as intracortical connections are physiologically ''silent'' because of inhibitory influences [59]. Sensory stimulation of a point of the skin activates neurons in the somato-sensory system which inhibits activity in neurons near the edges – a way to keep the receptive field smaller than its actual size. Such inhibition is due to activation of inhibitory interneurons near the edges of the receptive field. Decreased inhibition would be expected to increase the receptive field size an enable more neurons to be activated by the stimulus, a phenomenon which is sometimes referred to as unmasking of synapses. Gamma-amino-butyric acid (GABA) is the most important inhibitory neurotransmitter in the brain [19] and there is strong evidence that reduction of the GABAergic inhibition is crucial immediate in short term plasticity changes [7].

Thus, unmasking of normally existing but inhibited synapses can be achieved by selective de-afferentiation of appropriate skin areas. In a clinical perspective we feel that utilization of this phenomenon is a new principle of potential clinical importance in the rehabilitation process after peripheral nerve injury and repair.

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References

1. Almquist E, Eeg-Olofsson O (1970) Sensory-nerve-conduction velocity and two-point discrimmination in sutured nerves. J Bone Joint Surg Am 52: 791–796
2. Almquist EE, Smith OA, Fry L (1983) Nerve conduction velocity, microscopic, and electron microscopy studies comparing repaired adult and baby monkey median nerves. J Hand Surg 8: 404–410
3. ASHT (1992) Clinical assessment recommendation, 2nd edn. American Society for Hand Therapists
4. Bell-Krotoski J (2002) Sensibility testing with the Semmes-Weinstein monofilament. In: Mackin C, Skirven TM, Schneider LH, Osterman AL (eds) Rehab of the hand and upper extremity, 5th edn. Mosby, St Louis, pp 194–213
5. Björkman A (2005) Brain plasticity and hand function. Department of Clinical Sciences, Malmö – Hand Surgery, Lund University, Malmö
6. Celnik P, Stefan K, Hummel F, Duque J, Classen J, Cohen LG (2006) Encoding a motor memory in the older adult by action observation. Neuroimage 29: 677–684
7. Chen R, Cohen LG, Hallett M (2002) Nervous system reorganization following injury. Neuroscience 111: 761–773
8. Dellon AL (1981) Sensibility and re-education of sensation in the hand. Williams and Wilkins, Baltimore
9. Ehrsson HH, Geyer S, Naito E (2003) Imagery of voluntary movement of fingers, toes, and tongue activates corresponding body-part-specific motor representations. J Neurophysiol 90: 3304–3316
10. Florence SL, Garraghty PE, Wall JT, Kaas JH (1994) Sensory afferent projections and area 3b somatotopy following median nerve cut and repair in macaque monkeys. Cereb Cortex 4: 391–407
11. Garraghty PE, Hanes DP, Florence SL, Kaas JH (1994) Pattern of peripheral deafferentiation predicts reorganizational limits in adult primate somatosensory cortex. Somatosens Mot Res 11: 109–117
12. Hallin RG, Wiesenfeld Z, Lungnegard H (1981) Neurophysiological studies of peripheral nerve functions after neural regeneration following nerve suture in man. Int Rehabil Med 3: 187–192
13. Hansson T, Björkman A, Nylander L, Nyman T, Rosén B, Lundborg G (2005) Activation of the primary somatosensory cortex during stereoscopic observation of tactile stimulation of the hand. Proceedings, Xth FESSH Congress, Göteborg 15–18 June
14. Hauk O, Johnsrude I, Pulvermuller F (2004) Somatotopic representation of action words in human motor and premotor cortex. Neuron 41: 301–307
15. Jain N, Florence SL, Kaas JH (1998) Reorganization of somatosensory cortex after nerve and spinal cord injury. News Phsysiol Sci 13: 143–149
16. Jaquet JB, Luijsterburg AJ, Kalmijn S, Kuypers PD, Hofman A, Hovius SE (2001) Median, ulnar, and combined median-ulnar nerve injuries: functional outcome and return to productivity. J Trauma 51: 687–692
17. Jeannerod M, Frak V (1999) Mental imaging of motor activity in humans. Curr Opin Neurobiol 9: 735–739
18. Jerosch-Herold C (2005) Assessment of sensibility after nerve injury and repair: a systematic review of evidence for validity, reliability and responsiveness of tests. J Hand Surg [Br] 30: 252–264

19. Jones EG (1993) GABAergic neurons and their role in cortical plasticity in primates. Cereb Cortex 3: 361–372
20. Kaas JH (1983) What, if anything, is S1? Organization of first somatosensory area of cortex. Physiol Rev 63: 206–231
21. Kaas JH, Florence SL (1997) Mechanisms of reorganization in sensory systems of primates after peripheral nerve injury. Adv Neurol 73: 147–158
22. Kandel ER, Schwartz JH, Jessel TM (2000) Principles of neural science, 4th edn. McGraw-Hill
23. Keysers C, Wicker B, Gazzola V, Anton JL, Fogassi L, Gallese V (2004) A touching sight: SII/PV activation during the observation and experience of touch. Neuron 42: 335–346
24. Kosslyn SM, Ganis G, Thompson WL (2001) Neural foundations of imagery. Nat Rev Neurosci 2: 635–642
25. Lotze M, Montoya P, Erb M, Hulsmann E, Flor H, Klose U, Birbaumer N, Grodd W (1999) Activation of cortical and cerebellar motor areas during executed and imagined hand movements: an fMRI study. J Cogn Neurosci 11: 491–501
26. Lundborg G (2000) A 25-year perspective of peripheral nerve surgery: evolving neuroscientific concepts and clinical significance. J Hand Surg 25A: 391–414
27. Lundborg G (2003) Richard P. Bunge memorial lecture. Nerve injury and repair – a challenge to the plastic brain. J Peripher Nerv Syst 8: 209–226
28. Lundborg G (2004) Nerve injury and repair. Regeneration, reconstruction and cortical re-modelling, 2nd edn. Elsevier, Philadelphia
29. Lundborg G, Rosen B (2001) Sensory relearning after nerve repair. Lancet 358: 809–810
30. Lundborg G, Rosen B (2003) Enhanced sensory recovery after median nerve repair: effects of early postoperative artificial sensibility using the sensor glove system. J Hand Surg [Am] 28 Suppl 1: 38–39
31. Lundborg G, Rosén B, Lindberg S (1999) Hearing as substitution for sensation – a new principle for artificial sensibility. J Hand Surg 24A: 219–224
32. McAllister RM, Gilbert SE, Calder JS, Smith PJ (1996) The epidemiology and management of upper limb peripheral nerve injuries in modern practice. J Hand Surg [Br] 21: 4–13
33. Merzenich MM, Jenkins WM (1993) Reorganization of cortical representations of the hand following alterations of skin inputs induced by nerve injury, skin island transfers, and experience. J Hand Ther 6: 89–104
34. Merzenich MM, Kaas JH, Wall RJ, Nelson M, Sur D, Felleman D (1983) Topographic reorganization of somatosensory cortical areas 3B and 1 in adult monkeys following restricted desfferentiation. Neuroscience 8: 33–55
35. Merzenich MM, Nelson RJ, Stryker MS, Cynader MS, Schoppman A, Zook JM (1984) Somatosensory cortical map changes following digit amputation in adult monkeys. J Comp Neurol 224: 591–605
36. Moberg E (1991) The unsolved problem – how to test the functional value of hand sensibility. J Hand Ther 4: 105–110
37. Muellbacher W, Richards C, Ziemann U, Wittenberg G, Weltz D, Boroojerdi B, Cohen L, Hallett M (2002) Improving hand function in chronic stroke. Arch Neurol 59: 1278–1282
38. Noble J, Munro CA, Prasad VS, Midha R (1998) Analysis of upper and lower extremity peripheral nerve injuries in a population of patients with multiple injuries. J Trauma 45: 116–122
39. Penfield W, Boldrey E (1937) Somatic motor and sensory representations in the cerebral cortex of man as studied by electrical stimulation. Brain 60: 389–443
40. Polatkan S, Orhun E, Polatkan O, Nuzumlali E, Bayri O (1998) Evaluation of the improvement of sensibility after primary median nerve repair at the wrist. Microsurgery 18: 192–196
41. Pulvermuller F (2005) Brain mechanisms linking language and action. Nat Rev Neurosci 6: 576–582
42. Purves D, Augustine GJ, Fitzpatrick D, Hall WC, La Mantia A-S, McNamara JO, Williams SM (2004) Neuroscience (Sinauer Associates Inc. Sunderland, MA, USA, 3rd edn)
43. Rizzolatti G, Craighero L (2004) The mirror-neuron system. Annu Rev Neurosci 27: 169–192
44. Rizzolatti G, Fadiga L, Gallese V, Fogassi L (1996) Premotor cortex and the recognition of motor actions. Brain Res Cogn Brain Res 3: 131–141
45. Rizzolatti G, Fadiga L, Matelli M, Bettinardi V, Paulesu E, Perani D, Fazio F (1996) Localization of grasp representations in humans by PET: 1. Observation versus execution. Exp Brain Res 111: 246–252
46. Rosberg HE (2004) Hand Injuries – epidemiology, costs and outcome. Thesis, Lund University, Malmö
47. Rosberg HE, Carlsson KS, Hojgard S, Lindgren B, Lundborg G, Dahlin LB (2005) Injury to the human median and ulnar nerves in the forearm – analysis of costs for treatment and rehabilitation of 69 patients in southern Sweden. J Hand Surg [Br] 30: 35–39
48. Rosen B, Bjorkman A, Lundborg G (2006) Improved sensory relearning after nerve repair induced by selective temporary anaesthesia – a new concept in hand rehabilitation. J Hand Surg [Br] 31: 126–132
49. Rosén B, Dahlin L, Lundborg G (2000) Assessment of functional outcome after nerve repair in a longitudinal cohort. Scand J Plast Reconstr Surg Hand Surg 34: 71–78
50. Rosen B, Lundborg G (2003) A new model instrument for outcome after nerve repair. Hand Clin 19: 463–470
51. Rosén B, Lundborg G (1998) A new tactile gnosis instrument in sensibility testing. J Hand Ther 11: 251–257
52. Rosén B, Lundborg G (2000) A model instrument for the documentation of outcome after nerve repair. J Hand Surg 25A: 535–544
53. Rosén B, Lundborg G, Dahlin LB, Holmberg J, Karlsson B (1994) Nerve repair: correlation of restitution of functional sensibility with specific cognitive capacities. J Hand Surg 19B: 452–458
54. Rossini PM, Martino G, Narici L, Pasquarelli A, Peresson M, Pizzela V, Tecchio F, Torrioli G, Romani GL (1994) Short-term brain plasticity in humans: transient finger representation changes in sensory cortex somatotopy following ischemic anesthesia. Brain Res 642: 169–177
55. Silva AC, Rasey SK, Wu X, Wall JT (1996) Initial cortical reactions to injury of the median and radial nerves to the hands of adult primates. J Comp Neurol 366: 700–716
56. Stefan K, Cohen LG, Duque J, Mazzocchio R, Celnik P, Sawaki L, Ungerleider L, Classen J (2005) Formation of a motor memory by action observation. J Neurosci 25: 9339–9346
57. Wall JT, Kaas JH, Sur M, Nelson RJ, Fellman DJ, Merzenich MM (1986) Functional reorganization in somatosensory cortical areas 3b and 1 of adult monkeys after median nerve repair: possible relationships to sensory recovery in humans. J Neurosci 6: 218–233
58. Wall JT, Xu J, Wang X (2002) Human brain plasticity: an emerging view of the multiple substrates and mechanisms that cause cortical changes and related sensory dysfunctions after injuries of sensory inputs from the body. Brain Res Brain Res Rev 39: 181–215
59. Wall PD (1977) The presence of ineffective synapses and the circumstances which unmask them. Philos Trans R Soc Lond B Biol Sci 278: 361–372
60. Witzel C, Rohde C, Brushart TM (2005) Pathway sampling by regenerating peripheral axons. J Comp Neurol 485: 183–190
61. Wynn-Parry CB (1981) Rehabilitation of the hand. Ed, Butterworths, London
62. Wynn-Parry CB, Salter M (1976) Sensory re-education after median nerve lesions. Hand 8: 250–257
63. Yoo SS, Freeman DK, McCarthy JJ 3rd, Jolesz FA (2003) Neural substrates of tactile imagery: a functional MRI study. Neuroreport 14: 581–585

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Enhanced sensory re-learning after nerve repair using 3D audio-visual signals and kinaesthesia – preliminary results

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Summary

Sensory re-learning methods and basics on cortical reorganization after peripheral nerve lesion are well documented. The aim of enhanced sensory re-learning using 3D audio-visual signals and kinaesthetic training is the augmentation of cognitive memory (visual and acoustic sensory memory) and cognitive function for the improvement of cerebral plasticity processes and starts as soon as possible after nerve repair. Preliminary results are shown.

Keywords: Nerve regeneration; brain plasticity; reeducation; rehabilitation.

Introduction

Normal body sensations and feelings are the consequence of a constant and intimate interaction of all sensory organs and the cortex. Injuries to peripheral nerves cause sensory and motor dysfunctions that are thought to be attributable to functional changes in the cerebral cortical maps of the body. There is increasing evidence that the injury to the nerve triggers an immediate initiated mechanism that alters at multiple locations at subcortical and cortical level. Initial subcortical changes can be even more extensive than cortical changes.

Those peripheral injuries cause surprisingly rapid neurochemical and neuromolecular, functional and structural changes in peripheral, spinal, and brainstem regions. Additionally, a new balance between cortical and subcortical extents of changes develops over time. Mechanisms for these changes are ubiquitous in cortical and subcortical regions. They are a reflection of a global mechanism, beginning directly after injury and operate at multiple levels.

Sensory re-learning methods and basics in cortical reorganization after peripheral nerve lesion are well documented. But most investigations focus on changes observed many weeks until years after the injury. These longstanding injuries cause extensive cortical map changes. Merzenich *et al.* [8] showed that chronic section of the median nerve to glabrous hand skin caused enlargement of the cortical area 3b and 1 maps of intact radial nerve inputs from hairy dorsal skin on the hand, and these changes were persistent over weeks after the injury. These early findings were confirmed by subsequent studies [7].

It was suggested that chronic cortical changes developed progressively in at least two phases: an early chronic phase during the first 1–2 months that resulted in activation of cortical areas by new hairy inputs, and a subsequent chronic phase over about one year that resulted in additional modifications [4, 13]. No cortical changes were detected after about 2 years after the injury. This indicates that the first weeks and months are particularly crucial for cortical changes seen at later times. Related studies showed that these changes were not restricted to the cortex. Thalamic analyses after median and ulnar nerve injury indicated that hairy hand

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maps also expanded in the ventroposterior lateral nucleus [6]. More recent studies confirmed these findings and reported analogous functional changes in the brainstem cuneate nucleus 4–10 months after the injury [3]. These changes in the cuneate nucleus seem to start much earlier [15, 16].

With respect to magnetoencephalography (MEG) imaging of evoked responses to hand stimulation suggests that hand surgery and associated injury of hand inputs caused shifts in maps of the fingers of up to almost 1 cm in primary somatosensory cortex during the first week after the injury [9]. A recent study using steady state evoked responses (SSRs) to simultaneous multiple stimuli, provides further indications of cortical changes [5].

Cortical changes in humans are accompanied by subcortical functional changes. Cortical and subcortical somatosensory evoked potentials were studied in patients that had chronic unilateral carpal tunnel syndromes with confirmed abnormalities in median nerve inputs from the hand [14]. Stimulation of normal ulnar nerve inputs from the affected hand evoked larger brainstem, spinal, and primary somatosensory cortical potentials than stimulation of ulnar nerve inputs of the unaffected hand. This suggests that nerve injury caused increases in both cortical and subthalamic responses from uninjured ulnar nerve inputs in these patients. These increased responses to uninjured inputs may be related to expansions of maps of uninjured inputs.

In addition to functional changes in the subcortical central regions, chronic nerve injury in humans also changes functional activity in peripheral sensory neurons. Microneurography studies have shown that peripheral neurons that survive nerve injury often become sources of abnormal activity [2, 11]. This activity probably makes important contributions to central changes. Injured nerves can activate central substrates and maintain their sensory projection fields for long periods [1, 10, 12].

In consequence of the knowledge of these early changes after peripheral nerve injury immediate neurorehabilitation seems to be crucial. We developed an enhanced sensory re-learning program starting immediately after peripheral nerve repair which is using 3D audio-visual signals and kinaesthetic training and aims to augment cognitive memory (visual and acoustic sensory memory) and cognitive function for improvement of the global mechanism which is initiated by the trauma. It is hypothesized that 3D audio-visual signals and kinaesthetic training improves functional results after peripheral nerve repair.

Methods

In eight adult patients ulnar nerve repair was performed at forearm level immediately after the trauma.

Four patients were treated by an enhanced sensory re-learning program within 3 days after surgery. Minimal touch at the affected non sensible site of the hand was transformed into 3D acoustic and visual signals using a device constructed by Carbon-plates combined with an electronic impedance-device controlled by a MIDI-Sequencer (Fig. 1). Therefore, minimal touch is acoustically and visually perceptible in a 3 dimensional mode (Fig. 2). Additionally, change of pressure within the process of touching is acoustically and visually perceptible.

Also, patients were trained by kinesthetic stimuli for improvement of cognitive function (Fig. 3). Visual and auditive sensory memories were

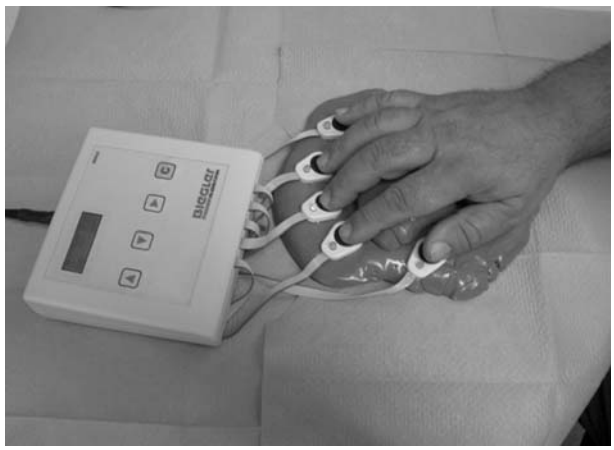

Fig. 1. The device for re-learning sensibility is shown. It is constructed by Carbon-plates combined with an electronic impedance-device controlled by a MIDI-Sequencer. The device distinguishes between the contact of the body part to the sensor and the degree of pressing the sensor. Preferably, the sensors are attached on a surface preshaped in the ergonomically optimal manner. According to a preferred embodiment, the sensors are attached to a soft and mouldable material

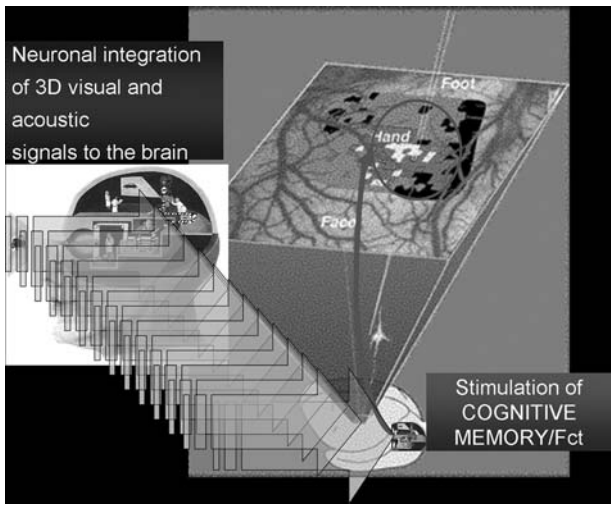

Fig. 2. The illustration shows how minimal touch at the affected non sensible site of the hand is transformed into 3D acoustic and visual signals thus stimulating the sensorimotor cortex resulting in an augmentation of brain plasticity processes

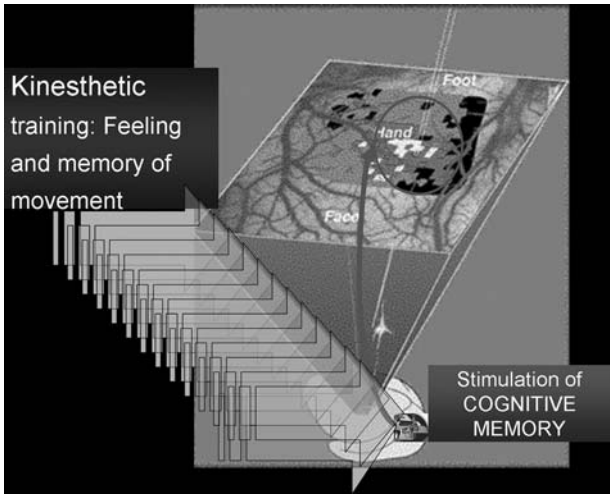

Fig. 3. Kinesthetic training was performed daily after nerve repair. The figure shows possible pathways for improvement of cognitive function

stimulated by visualization of the perception of sensory and motor patterns of daily life.

In four patients conventional sensory rehabilitation was performed.

Patients were evaluated using the hand function test according to Rosen and Lundborg 12 weeks after surgery.

Results

At the initial assessment, both groups started at zero tactile gnosis.

The enhanced sensory re-learning group using 3D audio-visual signals and kinesthetic training showed significant improvement compared to the conventional sensory rehabilitation group in the summarised outcome score ($p = 0.028$) 12 weeks after surgery. The outcome in the sensory domain is shown in Fig. 4.

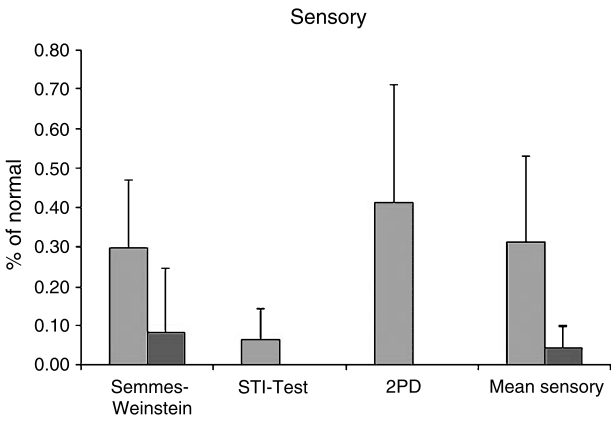

Fig. 4. The sensory enhanced re-learning group showed improved sensory results compared to the normal side in Semmes-Weinstein Monofilament test, STI test (Shape-Texture Identification), and 2-point discrimination (2PD). ☐ Virtual; ■ classic

Conclusion

These preliminary results show that enhanced sensory re-learning using 3D audio-visual signals and kinesthetic training can enhance recovery after ulnar nerve repair.

References

1. Campbell JN, Khan AA, Meyer RA, Raja SN (1988) Responses to heat of C-fiber nociceptors in monkey are altered by injury in the receptive field but not by adjacent injury. Pain 32(3): 327–332
2. Campero M, Serra J, Marchettini P, Ochoa JL (1998) Ectopic impulse generation and autoexcitation in single myelinated afferent fibers in patients with peripheral neuropathy and positive sensory symptoms. Muscle Nerve 21(12): 1661–1667
3. Churchill JD, Arnold LL, Garraghty PE (2001) Somatotopic reorganization in the brainstem and thalamus following peripheral nerve injury in adult primates. Brain Res 910(1–2): 142–152
4. Churchill JD, Muja N, Myers WA, Besheer J, Garraghty PE (1997) Somatotopic consolidation: a third phase of reorganization after peripheral nerve injury in adult squirrel monkeys. Exp Brain Res 118: 189–196
5. Diesch E, Preissl H, Haerle M, Schaller HE, Birbaumer N (2001) Multiple frequency steady-state evoked magnetic field mapping of digit representation in primary somatosensory cortex. Somatosens Mot Res 18(1): 10–18
6. Garraghty PE, Kaas JH (1991) Functional reorganization in adult monkey thalamus after peripheral nerve injury. Neuroreport 2(12): 747–750
7. Garraghty PE, Kaas JH (1991) Large-scale functional reorganization in adult monkey cortex after peripheral nerve injury. Proc Natl Acad Sci USA 88(16): 6976–6980
8. Merzenich MM, Kaas JH, Wall JT, Sur M, Nelson RJ, Felleman DJ (1983) Progression of change following median nerve section in the cortical representation of the hand in areas 3b and 1 in adult owl and squirrel monkeys. Neuroscience 10(3): 639–665
9. Mogilner A, Grossman JA, Ribary U, Joliot M, Volkmann J, Rapaport D, Beasley RW, Llinas RR (1993) Somatosensory cortical plasticity in adult humans revealed by magneto-encephalography. Proc Natl Acad Sci USA 90(8): 3593–3597
10. Moore CE, Schady W (2000) Investigation of the functional correlates of reorganization within the human somatosensory cortex. Brain 123 (Pt 9): 1883–1895
11. Nordin M, Nystrom B, Wallin U, Hagbarth KE (1984) Ectopic sensory discharges and paresthesiae in patients with disorders of peripheral nerves, dorsal roots and dorsal columns. Pain 20(3): 231–245
12. Schady W, Braune S, Watson S, Torebjork HE, Schmidt R (1994) Responsiveness of the somatosensory system after nerve injury and amputation in the human hand. Ann Neurol 36(1): 68–75
13. Schroeder CE, Seto S, Garraghty PE (1997) Emergence of radial nerve dominance in median nerve cortex after median nerve transection in an adult squirrel monkey. J Neurophysiol 77(1): 522–526
14. Tinazzi M, Zanette G, Volpato D, Testoni R, Bonato C, Manganotti P, Miniussi C, Fiaschi A (1998) Neurophysiological evidence of neuroplasticity at multiple levels of the somatosensory system in patients with carpal tunnel syndrome. Brain 121 (Pt 9): 1785–1794
15. Xu J, Wall JT (1999) Evidence for brainstem and supra-brainstem contributions to rapid cortical plasticity in adult monkeys. J Neurosci 19(17): 7578–7590
16. Xu J, Wall JT (1997) Rapid changes in brainstem maps of adult primates after peripheral injury. Brain Res 774(1–2): 211–215

Compression and irritation syndromes

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Anatomical structures to provide passive motility of peripheral nerve trunks and fascicles

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Summary

It is well known that tendons have to be able to move if the muscle contracts. It is still not generally known that any structure in the body has to be able to move passively against other structures. This is especially important for the movement of limbs. In a monoaxial joint like the humero-ulnar joint only structures in the plane of the joint axis remain fixed. Structures in a certain distance to the flexion or to the extension side have to be able to move against other structures in different levels. The amount of passive motion is dependent on the distance to the plane of the joint axis. Tissues which provide a frictionless passive motion are discussed.

Keywords: Peripheral nerve; brachial plexus; fascia; gliding tissue.

Introduction

All structures of any body have to be moveable passively against each other in order to follow the movements of the body. In regard of peripheral nerves this passive motion manifests itself at two different levels.

Movement of fascicles against each other, inside a nerve trunk

This movement is necessary to neutralize lateral compression by changing the shape. The movement is provided by the interfascicular epineurium. If the tissue is exposed to external irritation, it reacts with the development of an interfascicular fibrosis. In this case the motility is lost and with this also the ability to change shape and to adapt to different compression. This does not mean that the nerve does not function anymore, but the nerve is less resistant against external compression. Problems arise only when this fibrotic tissue starts to shrink, then compression is excerted to the fascicles and a pain syndrome may develop. These are the cases who need internal neurolysis. Only by removal of the interfascicular fibrotic tissue a decompression can be achieved. Fortunately, this situation is rather rare. Much more frequently the more externally located epifascicular epineurium and the paraneurium are involved.

The actual state of the interfascicular epineurium (Fig. 1), and the extent of such a fibrosis can be defined by surgery only. Magnetic resonance imaging gives us an idea of the interfascicular situation, and we expect from studies with high resolution MRI to receive a much better answer to this question.

Movement of the nerve against the surrounding tissue

This is provided by a special loose connective tissue which consists of several layers of loose tissue with capillaries and elastic fibres (Fig. 2). This tissue establishes the connection between the nerve and its surrounding. If we harvest a nerve graft, part of this tissue remains on the nerve and part of this tissue remains on the donor side. An exact microscopic description exists from Johannes LANG [2] who called this tissue ''conjuctiva nervorum''. In other papers the name ''adventitia'' is used [5].

The more precise name would be ''paraneurium'', a term which to my knowledge was first used by KRSTIC [1].

In a neuro-vascular bundle, the paraneurium fuses with the adventitia of the vessels and forms common layers

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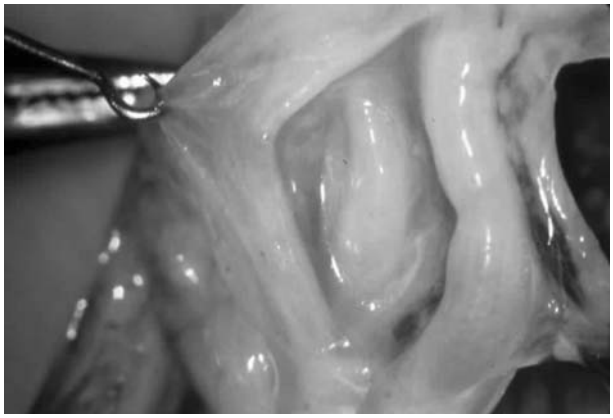

Fig. 1. Normal gliding tissue of a peripheral nerve consisting of loose connective tissue and fat tissue

Fig. 2. Thick collagenized gliding tissue of a peripheral nerve after trauma

around nerves and vessel, and for this tissue the term ''adventitia'' is appropriate. The ''paraneurium'' provides the ability for the nerve to move in longitudinal direction, if adjacent joints are extended or flexed. Any stimulus to this tissue is responded by the development of fibrosis [3]. The tissue might get thick and might be collagenized. If so, the nerve cannot move against the surrounding tissue and the nerve becomes irritated at entrapment sites.

Again, problems will start if a tissue shrinks. The paraneurium usually shrinks in longitudinal direction, causing an ondulating deformity of the fascicles inside the nerve. If progress occurs, there is a fusion with the epifascicular epineurium and from this moment onwards the two tissue layers cannot be differentiated.

This is also the reason why the paraneurium has escaped the attention of the surgeons who, when exploring a nerve, found the thickened epineurium.

If shrinkage occurs, and the whole nerve comes under compression, this pressure can be relieved by paraneuriotomy, as a first step. If this is not sufficient for the total circumference, then paraneuriectomy and – if the epineurium is involved – an epineuriotomy or an epineuriectomy, respectively, are to be done.

Movement in lateral direction, e.g. as it is necessary when lifting the arm, for the neurovascular bundle to the upper extremity is provided by large fascial spaces lined by fascias and filled with loose connective tissue and fat tissue. In this location, the lining of the spaces by fascial tissue provides the ability to glide within the space.

Lining tissues and fascias not only have to provide the ability for the nerve to glide against the surrounding tissues. They also protect the nerve tissue against structures which have to glide above. This is especially true for the clavicle. When lifting and abducting the arm in the shoulder joint, the clavicle glides several centimers along the superior trunk. This is possible in a frictionless way only because of the presence of the complicated cervical fascial tissue layers, with the omohyoideus muscle playing a decisive role. For these reasons we recommend to preserve as much as possible the fascial and gliding tissues.

Fortunately, the gliding tissue has a strong ability to regenerate, and very often an irritation of the gliding tissue has no clinical consequences. In other cases there are consequences. A recurrent fibrosis leading to severe pain syndrom may be the result. In these cases the fibrotic gliding tissue has to be replaced by a gliding tissue flap [4].

The problem of a gliding tissue flap is illustrated by the following case

A 42-year old female patient underwent surgery because of thoracic outlet syndrome. After this surgery a

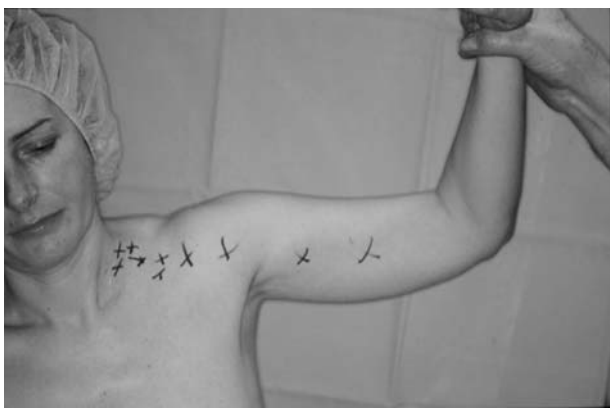

Fig. 3. A 42-year old female patient underwent surgery because of thoracic outlet syndrome. After this surgery a pain and irritation syndrome of the whole plexus brachialis area, from the superclavicular fossa to the middle of the upper arm developed. The patient could not lift the arm at all without pain, but there was also continuous pain

Fig. 4. A gliding tissue flap based on the pectoral branch of the thoracoacromial artery was formed. It was transposed to the brachial plexus and used to envelope the plexus

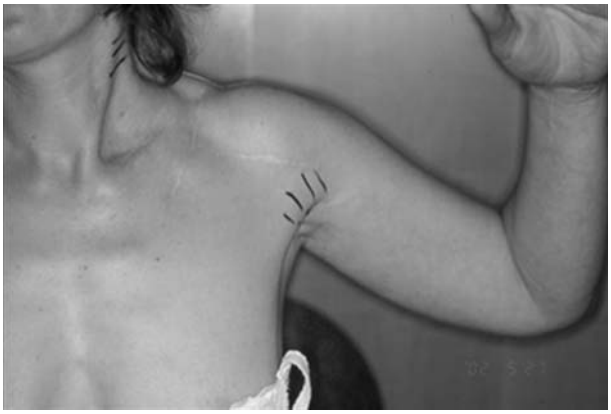

Fig. 5. Two years after surgery the patient is free of severe pain, but there is still some pain felt at the transient zone from the pectoral area to the upper arm indicated by the striated areas. Apparently, the gliding tissue flap should have been somewhat longer

pain and irritation syndrome of the whole plexus brachialis area, from the superclavicular fossa to the middle of the upper arm developed. The patient could not lift the arm at all without pain, but there was also continuous pain (Fig. 3).

During surgery the brachial plexus was explored. There was a severe fibrosis of the paraneurium, and a paraneuriectomy was performed. After that, a gliding tissue flap based on the pectoral branch of the thoracoacromial artery was formed, it was lifted and transposed to the plexus and was used to envelope the plexus (Fig. 4).

Two years after surgery the patient is free of severe pain, but there is still some pain felt at the transient zone from the pectoral area to the upper arm. Apparently, the gliding tissue flap should have been somewhat longer (Fig. 5).

References

1. Krstic R (1978) Die Gewebe des Menschen und der Säugetiere. Springer, Berlin Heidelberg
2. Lang J (1962) On connective tissue and blood vessels of the nerves. Z Anat Entwicklungsgesch 123: 61–79
3. Mazal PR, Millesi H (2005) Neurolysis, is it beneficial or harmful. Acta Neurochir Suppl 42: 2–6
4. Millesi W, Schobel G, Bohdanski T (1994) Subpectoral gliding tissue flap. Plastic Reconstructive Surg 93: 842–851
5. Van Beek A, Kleinert HE (1977) Practical neurorrhaphy. Orthopedic Clin North Am 8: 377–386

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Critical review of pathophysiologic mechanisms in thoracic outlet syndrome (TOS)

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Summary

Background. Thoracic outlet syndrome is a complex and multifactorial disease. There are multiple diagnostic steps and possible treatment options. The scientific literature not always contributes to a ''unifying vision''.

Method. We did an overview of the actual literature on TOS in the last 20 years and confronted these views with our surgical experience (about 50 cases and 10 operations).

After preparing a special issue in the German Journal of Hand Surgery (Handchirurgie Mikrochirurgie Plastische Chirurgie), where landmark papers were edited on anatomy, pathophysiology, diagnosis and treatment, we summarise our knowledge in this ''strategic'' paper.

Findings. To understand and treat TOS correctly, surgical experience in brachial plexus surgery is mandatory. The very well written basic papers on anatomy and its variations must be studied in detail. Neurophysiologic and vascular examinations are mandatory.

A conservative treatment always must be tried first. Postoperative outcome should be clearly correlated with the technical steps within the surgical procedure.

Conclusion. TOS diagnosis and treatment is complex, but rewarding. The symptom complex must be identified and no longer be considered as psychogenic. There is still need for better spread of information among neurologists, surgeons, and work compensation companies.

Keywords: TOS; thoracic outlet syndrome; pathophysiology; mechanisms; first rib.

Introduction

This is a personal comment by one ''manual worker'' who is a peripheral nerve surgeon dealing regularly with the brachial plexus and the thoracic outlet. My personal experience actually is about 10 operated TOS cases, but more than 200 direct reconstructive surgeries of the brachial plexus in children and adults.

When discussing the *pathophysiology of thoracic outlet* [3], we must assess vascular and/or nerve compression (tubular structures are deformed) or irritation (impairment of nerve microcirculation). There are anatomic keypoints one must know before entering the operative field; especially tissue variations and anomalies one should search for actively within the exploration. Some of them may be found by preoperative imaging techniques (cervical rib, hypertrophied lateral process of the 7^{th} cervical vertebra, callus after clavicular or rib fracture); others appear only by carefully study within the surgical exposure (scalenus muscle abnormalities, ligaments, aponeurotic sheaths described by Millesi since 2001 [7]).

As it comes to *diagnosis*, everybody would agree that diagnosis of thoracic outlet seems to be very difficult, subjective, variable, because the clinical patterns of vascular compression or nerve irritation are not homogeneous or static. They are dynamic, depending on limb positioning and the physical activity of the patient. This is not always reflected in the objective measurements we get. Wilhelm is the only author who proposed a systematic assessment which is helpful for diagnostic criteria and follow-up after surgery [12].

Anatomy

Topography

There are a few anatomic articles to read before performing this delicate surgery.

Atasoy [1] describes systematically three compression zones: the most proximal one in the scalenus triangle, then between the two participating bones- the so-called costoclavicular space, and finally a much more distal one behind the pectoralis minor muscle. When a surgical exposure is decided, all three zones should be exposed and surgery might only be stopped once the surgeon has checked that all these passages are easy to pass.

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Bone

In my personal experience of brachial plexus surgery in children, I have particular interest in the *cervical rib*. In about 150 cases of operated obstetric brachial plexus cases in the last 10 years, we clearly identified 10 cases where a complete cervical rib was present between the middle and lower trunk and certainly participated in the lesion as a hypomochlion to the trunks.

If this bone variance is a reality in the newborn, it maintains its reality and potential harmful character in the adult later on.

Gruber in 1869 [4] described the cervical rib and its low incidence in a general population.

Roos [9] considered various types of cervical ribs in his classification.

Scalenus muscle

Several variations are described in detail since a long time in the anatomic textbooks (e.g. Testut and Latarjet in 1948 [11]; von Lanz and Wachsmuth in 1955 [5]). One might find a detailed description about the normal insertions of the three parts of the scalenus, but also the so-called scalenus minimus muscle.

A recent anatomic work and discussion by Leijnse [6] allows a different thinking about this muscle variability: one might consider the scalenus as one single muscle perforated by nerves and vessels, thus giving different muscle parts and variations. Hereby, the growing and migrating nerves and vessels in foetal live are the active factors which will condition the muscle arrangement later on.

Ligaments

Poitevin [8] has published the most detailed anatomic study about the suprapleural ligaments. These ligaments also enter Roos' classification with nine types of anomalies.

In very rare cases, a vessel may compress a nerve even at the outlet, e.g. the arteria dorsalis scapulae might interfere with the upper trunk.

Diagnosis

We need to assess which exam we are asking for and what we want to get out of it.

In our Unit, we rely on Doppler ultrasonography and phlebography to assess subclavian (vein) compression, but always including dynamic positions. The exam is performed at rest and with different degrees of adduction and abduction.

The same should apply to the neurologic testing; but it is rather impossible to do neurophysiologic examination in a moving limb. Alteration of the conduction velocity in proximal sensory branches exiting the lower trunk might be a hint for irritation of the root Th1 by a ''pathogenic'' first rib.

In patients with defined distal nerve compression of the upper limb (median nerve suffering in the carpal tunnel, ulnar nerve irritated at the elbow level) with associated signs of proximal nerve irritation, with pain and dysesthesia in the arm and shoulder, one should consider the hypothesis of a ''double crush'' phenomenon (proximal and distal nerve compression) where the distal release alone might not be sufficient to relieve all symptoms.

Surgery

There is still a lot of debate about the approach and content of the procedure.

My personal approach is a single supraclavicular incision. This allows good control of all nerve structures and the subclavian artery. The proximal compression zones are visible; the retropectoral space might be explored separately if indicated by a delto-pectoral approach. Even the complete resection of the first rib is possible while protecting the lower trunk. We would advise a infraclavicular or axillar approach when the subclavian vein should be addressed for decompression or grafting. Obviously, different surgeons might have different experiences.

There is no such standard resection of bone or muscles: I would resect the first rib only when there is real pathologic interference with the vascular and/or nerve structures.

The same applies to the decision about scalenectomy.

I don't perform scalenotomy because this might be dangerous when the retraction of the muscle will adhere to the surrounding vessels and nerves. Therefore, I decide within the exploration about only anterior or anterior and middle scalenus muscle resection.

It gets technically more complicated when you ask about the extent of neurolysis, or if we should decide about local segmental sympathectomy.

Conclusion

We recently reviewed the literature for a special issue on thoracic outlet surgery of our German Hand Surgery Journal (Bahm [2, 3]). We included several large operative

series (Atasoy [1] and Sanders [10] in USA, Meyer and Stober in Switzerland).

When you focus on details about the surgery and when you compare the outcome criteria, it still remains very difficult to find a common language or conclusion.

We also found obvious recurrent cases too; mostly due to insufficient bone resection. The first surgery should be the good and only one- and therefore the TOS surgeon should find out the reality of symptoms, know the anatomic variants, and be customised with the brachial plexus region and its vessels to operate on a safe and experienced manner.

References

1. Atasoy E (2004) Combined surgical treatment of thoracic outlet syndrome: transaxillary first rib resection and transcervical scalenectomy. Hand Clin 20: 71–82
2. Bahm J (2006) On the problems of proximal vessel and nerve compression in the upper extremity. Handchir Mikrochir Plast Chir 38: 5–55
3. Bahm J (2006) Systematic and critical consideration of the thoracic outlet syndrome – clinical picture and therapy. Handchir Mikrochir Plast Chir 38: 56–63
4. Gruber W (1869) Über die Halsrippen des Menschen mit vergleichend-anatomischen Bemerkungen. St Petersburg
5. Lanz T von, Wachsmuth W (1955) Praktische Anatomie Band I Teil 2. Springer, Berlin Heidelberg
6. Leijnse JNAL (1997) The morphology of holes in aponeuroses caused by perforating nerves or vessels at the medial epicondyle of the elbow. Acta Anatomica 160: 42–50
7. Millesi H (2001) Chirurgie des zerviko-axillären Nervenplexus. In: Krupp S (ed) Plastische Chirurgie. Ecomed Landsberg
8. Poitevin LA (1995) Thoracic outlet syndrome (TOS). Research on anatomic variations: clinical relevance. In: Vastamäki M (ed) Current trends in hand surgery. Elsevier, Amsterdam, pp 323–329
9. Roos DB (1976) Congenital anomalies associated with thoracic outlet syndrome. Am J Surg 132: 771–778
10. Sanders RJ, Hammond SL (2004) Supraclavicular first rib resection and total scalenectomy: technique and results. Hand Clinics 20: 61–70
11. Testut L, Latarjet A (1948) Traité d'Anatomie Humaine. Doin Paris
12. Wilhelm A, Wilhelm F (1985) Das Thoracic Outlet Syndrom und seine Bedeutung für die Chirurgie der Hand. Handchir Mikrochir Plast Chir 17: 173–187

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TOS-surgery via a single supraclavicular incision

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Summary

Background. We report about our experiences using a single supraclavicular incision at the base of the neck for Thoracic Outlet Syndrome (TOS) surgery.

Methods. 10 patients aged between 12 and 59 years (mean 31 years) underwent 12 times a TOS procedure. Patients suffered from compression of their brachial plexus with main affection of the ulnar nerve (9 out of 12 cases). Electroneurography was positive for TOS 4 times in 3 patients, in other 3 patients additionally a distal nerve compression syndrome was evident. In 7 cases (5 patients) a cervical rib was present on X-ray. In 10 cases (8 patients) the subclavian artery showed a stenosis behind the clavicle on MRI-angiography. In all cases the brachial plexus was prepared and a complete scalenotomy was performed. Whenever present the cervical rib was resected and in 2 cases the first rib (1 with/1 without cervical rib) was taken out.

Results. The surgical procedures did not cause relevant complications. All patients were without discomfort within 6 months, including the nerve regeneration disturbances. One patient suffered from TOS recurrence 10 months after surgery (scalenotomy without resection of the 1st rib).

Conclusion. The single supraclavicular incision provided sufficient access to the structures of the brachial plexus, the subclavian artery and the cervical and 1st rib in all cases. The procedure produced not only sufficient pain relief and normalized extremity function but also a cosmetically acceptable, nearly invisible scar.

Keywords: TOS; supraclavicular incision; scalenotomy; first rib resection; cervical rib.

Introduction

The definition of the Thoracic Outlet Syndrome (TOS) summarizes problems caused by compression of nerves and vessels between neck and arm. There is evidence from the literature that the diagnosis indicates a surgical intervention to release the compressed structures. There are different surgical procedures [1] to perform the decompression, but up to now none of them represents the gold standard. We report about our experiences using a single supraclavicular incision at the base of the neck for TOS surgery.

Patients and methods

Ten patients (Table 1), mainly women, only one man, aged 12–59 years (mean 31 years) were diagnosed 12 times for TOS procedure. The mean duration of symptoms was 39 months. The mean pain value was 6 according to the Numeral Rating Scale (NRS).

In the clinical examination (Table 2) all patients suffered from disturbed sensibility. Nine suffered from compression of their brachial plexus with main affection of the ulnar nerve. Five out of 12 cases suffered from continuous pain. In 10 out of 12 cases patients had restricted movement of their upper extremity. The Tinnel Hoffman sign was present in the supraclavicular groove in 8 out of 12 cases.

In 7 cases (5 patients) a cervical rib was present on X-ray (Table 3). The electroneurographical investigation was positive for TOS in 2 patients, in 2 patients a distal nerve compression syndrome was additionally evident.

In 10 cases (8 patients) the subclavian artery showed a stenosis behind the clavicle on MRI- angiography.

Surgery was done in supine position. The upper extremity was left free. A small incision was made at the base of the neck, following the cleavage line of the skin from the sternoclavicular junction to the anterior boarder of the trapesius muscle. The platysma was cut, the supraclavicular nerves were prepared and protected carefully. The omohyoid muscle was explored in the supraclavicular groove, the phrenic nerve was recognized and used as a guideline to identify the brachial plexus structures. A scalenotomy of scalenus anterior, medius, and posterior muscle was performed. Trunks of the brachial plexus and the subclavian artery were caught in yellow straps to secure the structures during manipulation at the ribs. Either the cervical rib or/and the 1st rib was freed of periost, cut with a 6mm Raney rongeur at the rib-cartilage boarder, mobilised at the costo-vertebral joint and removed. After rib resection, the brachial plexus was covered with surrounding tissue. The platysma was reconstruced and the skin was closed with an absorbable, monofilament intracutaneous suture.

A follow-up examination was done approximately 14 months after surgery. At that time patients were asked for their post operative course and their actual status was evaluated by means of DASH score and evaluation of static two point discrimination as well as grip power and pain level by NRS.

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Table 1. *Details of 10 patients*

Pat.	Age	Sex	Side	Duration symptoms	NRS
1	38	f	left	48	10
2	38	f	left	6	9
3	26	f	right	72	9
4	16	f	left	36	5
5	59	f	right	84	5
6	16	f	left	5	7
7a	32	f	right	72	4
7b	30	f	left	60	4
8	49	f	left	24	8
9a	12	f	right	3	4
9b	12	f	left	25	5
10	44	m	left	30	7
MW	31			39	6
SD	15			28	2

Results

In all cases surgery was done via the presented supraclavicular incision (Table 4). In all cases scalenotomy was performed, which means detaching the scalenus anterior, medial and posterior muscle from the 1st rib and also removing the fascia covering the rib area. Whenever a cervical rib was present it was removed. In one case we removed the 1st rib as well as the cervical rib, and in 6 cases where there was no cervical rib, we removed the 1st rib. In 4 patients only a soft tissue release was performed. Due to compact fascia around the brachial plexus trunks we performed neurolysis in 10 of our cases. Additionally, in 4 of these cases an epineuriotomy was performed especially at the lower part of the brachial plexus.

Concerning surgical complications we could avoid damage of nerves and vessels in all cases. In 2 of our cases the pleura was opened and closed with stitches, nevertheless one case had to be treated with a Bülau drainage.

Immediately after surgery all patients suffered from disturbed sensibility of their hand, mainly concerning median and ulnar nerve, which ranged from mild dysaesthesia up to complete loss of sensation. Weakness of motor function was observed in some patients but no complete motor failure occurred. All patients reported more or less painful nerve regenerating symptoms.

The patients needed an average of 3 months to recover from nerve regeneration symptoms (Table 5). At the follow-up investigation, mean value of NRS was 1, compared with the mean value of 6 before surgery. In fact,

Table 2. *Clinical examination of 10 patients*

	1	2	3	4	5	6	7a	7b	8	9a	9b	10	MW
Disturbance of sensibility	x	x	x	x	x	x	x	x	x	x	x	x	12/12
Dysastheasia ulnar nerve		x	x	x	x	x			x	x	x	x	9/12
NRS	7	9	9	5	5	7	4	4	8	4	5	7	6
Continuous pain	x		x	x	x				x				5/12
Reduction of movement	x	x	x	x	x	x	x	x	x			x	10/12
TH pos	x	x	x		x	x			x	x		x	8/12

Table 3. *Diagnostic of 10 patients*

	1	2	3	4	5	6	7a	7b	8	9a	9b	10	MW
Cervical rib	x					x	x	x		x	x	x	7/12
NLG TOS		x							x				2/12
NLG SNUS		x										x	2/12
Stenose subclavian artery		x	x	x	x	x	x	x	x	x	x		10/12

Table 4. *Surgery*

	1	2	3	4	5	6	7a	7b	8	9a	9b	10	MW
Supraclavicular incision	x	x	x	x	x	x	x	x	x	x	x	x	12/12
Skalenotomie	x	x	x	x	x	x	x	x	x	x	x	x	12/12
Cervical rib resection	x					x	x	x		x	x	x	7/12
1st rib resection	x		x										2/12
Neurolyses		x	x	x	x	x		x	x	x	x	x	10/12
Epineuriotomy		x		x		x			x				4/12
Nerve damage													0/12
Vessel damage													0/12
Damage of pleura							x		(x)				2/12
Disturbed sensation	x	x	x	x	x	x	x	x	x	x	x	x	12/12
Motor faliure													0/12

Table 5. *Post operative examination*

	1	2	3	4	5	6	7a	7b	8	9a	9b	10	MW
FU months	6	49	7	5	6	10	6	20	23	13	17	3	14
Recovery symptoms	x	x	x	x	x	x	x	x	x	x	x	x	12/12
Duration of recovery (months)	4	6	2	1.5	3	2	2	3	4	1	1	3	3
NRS	3	0	2	0	3	4	0	0	5	0	0	0	1
NRS präop	10	9	9	5	5	7	4	4	8	4	5	7	6
Continuous pain					x				x				2/12
Sensitvity (2 PDK >6 mm)		x			x	x			x			x	5/12
Decrease ROM													0/12
Force (% of control)	30	47	85	91	29	82	100	100	21	100	100	56	74
DASH	66	25	23	8	73	14	13	13	89			32	36

10 of our 12 cases had no pain. Two patients were still suffering from continuous pain. Five patients showed reduced sensibility of their hands, defined by the static 2 point discrimination >6 mm. The force of the operated hand reached 74% of the control hand. Mean DASH amounted to an average value of 36 points.

Discussion

TOS surgery via single supraclavicular incision gave adequate access to all structures to be manipulated and yielded excellent results. Actually 8 out of 10 patients were freed from their symptoms and showed normalised nerve and motor function at the follow-up investigation. These good results are lowered by just 2 patients. Patient Nr. 5 suffered from a chronic neuropathic pain syndrome which was not influenced by surgery. In Patient Nr. 8, we performed TOS surgery as a soft tissue procedure without removing the 1st rib. Within one year after the operation the symptoms returned. The recurrence was re-operated including removal of the 1st rib.

Concerning the surgical approach, transaxillary incision does not give access to the structures of the brachial plexus [1]. Therefore we favour the supraclavicular access in accordance with other authors [4, 6, 7].

The question of an obligatory resection of the 1st rib in absence of cervical rib is discussed controversially in the literature [2–6]. There are indeed some strong arguments to perform 1st rib resection in order to prevent reoccurrence of the disease: a simple soft tissue procedure does not create more space under the clavicle, the presence of rib may lead to reattachment of the scalenius muscles to the rib, both facilitating recurrence of the symptoms. Considering the fact that surgery of recurrent TOS is really a difficult task, everything should be undertaken to reduce the risk of recurrence. However, a visible scare is the only disadvantage of the supraclavicular incision. The scar has been cosmetically unobstructive in all our cases up to now and been well accepted by the patients (Fig. 1).

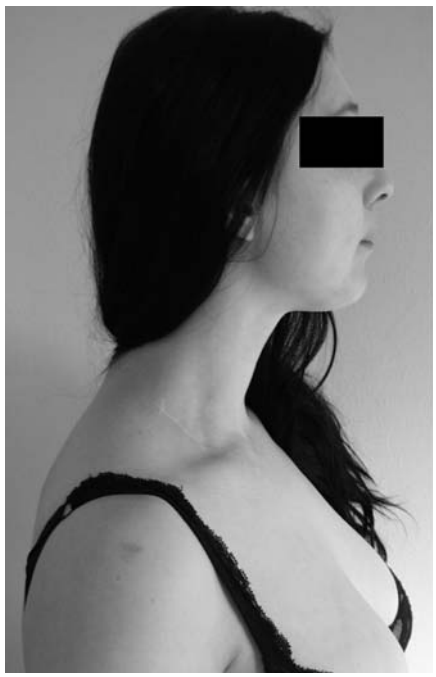

Fig. 1. Nearly invisible scar

References

1. Atasoy E (2006) Combined surgical treatment of thoracic outlet syndrome: transaxillary first rib resection and transcervical scalenectomy. Handchir Mikrochir Plast Chir 38: 20–28
2. Becker MH, Lassner F (2006) The asymptomatic thoracic outlet compression syndrome. Handchir Mikrochir Plast Chir 38: 51–55
3. Cappeller WA, Ukkat J, Winkler M, Taute BM (2001) Vascular complications in thoracic outlet syndrome: combined transaxillary revascularization and rib resection. Chirurg 72: 298–304
4. Hug U, Kilgus M, Neff P, Burg D, Meyer VE (2006) Long-term follow-up after surgery for thoracic outlet syndrome (TOS). Handchir Mikrochir Plast Chir 38: 37–41
5. Samarasam I, Sadhu D, Agarwal S, Nayak S (2004) Surgical management of thoracic outlet syndrome: a 10-year experience. ANZ J Surg 74: 450–454
6. Sanders RJ, Hammond SL (2006) Supraclavicular total scalenectomy with or without first rib resection: technic and results. Handchir Mikrochir Plast Chir 38: 29–36
7. Stober R (2006) The thoracic outlet syndrome – diagnostic tips, operative technique and results. Handchir Mikrochir Plast Chir 38: 46–50

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Thoracic outlet syndrome: a multidisciplinary problem with a perspective for microsurgical management without rib resection

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Summary

Background. Thoracic outlet syndrome (TOS) refers to a group of complex symptoms in the upper extremity caused by compression of the brachial plexus, subclavian artery and vein. Different surgical approaches were described for the management of TOS.

There is, however, no ''gold standard'' procedure for this complicated and multidisciplinary problem.

Objectives. This study evaluated the effectiveness of a microsurgical neurovascular decompression in the treatment of TOS.

Methods. 11 patients suffering from TOS (for 1.3 to 15 years after the beginning of the symptoms) were selected for a treatment of the complex symptoms of pain (diffuse or irradiated to the arm and hand), aching or paresthesia in the neck, shoulder, anterior chest, upper extremity and hand. Four of the 11 patients were suffering from signs of vascular compression. Eight patients showed slow progressive neurological deterioration (distribution of the ulnar nerve) with partial muscle atrophy. Patients underwent a microsurgical treatment using a supraclavicular approach followed by brachial plexus neurolysis, scalenectomy and release of the subclavian artery and vein without rib resection. Postoperative results were classified, using Am. J. Surg. (176: 215–218, 1998) scale (4), as good, fair and poor.

Results. Surgical results were studied, with a follow-up of 24 to 48 months. Prior to surgery, all patients had partial or severe limitation in physical activities. Post-operative follow-up showed that 9 (82%) of the 11 patients returned to normal everyday physical activities with a complete or significant relief of the symptoms (good results). In 2 patients (18%) the pain decreased and the use of medication was reduced (fair results). Eight of the 11 patients returned to full or partial employment. There were no cases of poor results in the study.

Conclusion. Microsurgical neurovascular decompression of TOS without a removal of the cervical or first rib using a supraclavicular approach is an effective treatment method for a relief or complete release from symptoms and allows most patients to return to an active normal life.

Keywords: Thoracic outlet syndrome; microsurgery; prognosis.

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Introduction

Most of the patients suffering from TOS show symptoms evolving from a brachial plexus compression. Patients suffering from vascular symptoms such as arterial compromise or venous stasis, comprise only 10% of all TOS patients [9]. However, if vascular compression exists it may result in a significant long-term disability [3]. The most common symptoms are pain and parasthesia (88–83% respectively) in the area of the hand and forearm. Amongst the various surgical approaches described over the years, only the transaxillary and the supraclavicular ones are currently in use.

Materials and methods

This prospective study evaluates the functional recovery of 11 patients (nine women and two men) who suffered from TOS for 1.3–15 years after the onset of the symptoms, and who underwent a microsurgical release of the brachial plexus, subclavian artery and vein. Two of the patients had previous sympatectomy procedures; two had carpal tunnel release and one underwent an ulnar nerve release. Patients ranged in age from 16 to 40 years old. Patients were carefully selected according to a continuous follow up (prior to the surgery) for a minimum of 6–18 months, with a minimum of at least 4–6 months of physical therapy and swimming. Possible sources of their symptoms, other than the brachial plexus, were excluded using neuroradiological (X-ray, MRI, ultrasound) and neurophysiological (EMG, NCT) investigations. All the patients suffered from complex symptoms of pain (diffused or irradiated to the arm and hand), aching or paresthesia in the neck, shoulder, anterior chest, upper extremity and hand. Four of the 11 patients were suffering from signs of vascular compression. All patients had a markedly positive *Roos' sign* (rapid onset of symptoms when the hand is elevated above the head), *Tinel's sign* (pain or tingling on plexus percussion) over the brachial plexus beneath the scalene muscles and positional *Adson's test or hyperabduction* of the upper extremity show-

ing diminution or loss of radial artery pulse. Eight patients showed slow progressive neurological deterioration (distribution of the ulnar nerve) with partial muscle atrophy.

Surgery

The operation was performed under a general anesthesia without myorelaxant agents to prevent neuromuscular blocking. An obliquely 7 cm transverse incision was made 2 cm above the clavicle. Using surgical microscope, neurolysis of the C5, C6 nerve roots and upper trunk of the plexus was completed to the point where the anterior and posterior divisions of the upper trunk were visible. Then, the anterior scalene muscle was divided inferiorly. The C7 root and the middle trunk were then apparent. The dissection and neurolysis continues medially and deeper until the lower trunk and then C8 and T1 roots were identified and released. The fibrous bands between the first or cervical rib (if such existed) and the brachial plexus elements, the subclavian artery and the vein were removed.

Intraoperative electrophysiological recording

During neurolysis, Compound Muscle Action Potentials (CMAPs) were recorded from APB and FDI muscles at the beginning of operation, during the operation and after the release of the nerve scar tissue.

Post-operative clinical evaluation

An analysis of the results was done using the scale after surgical treatment of the TOS [4]. Results were categorized as "*good*" if there was complete or near-complete resolution of the symptoms, as "*fair*" if there was a partial resolution of the symptoms, and as "*poor*" if there was no improvement or a worsening of the symptoms. Surgical results were studied with a follow-up period of 24 to 48 months.

Results

During surgery compound muscle action potential (CMAP) was recorded from APB and FDI muscles at the beginning of the operation, during operation and after the release of the nerve scar tissue. After the releasing of the nerve tissue an increasing amplitude of CMAP was discovered (Fig. 1).

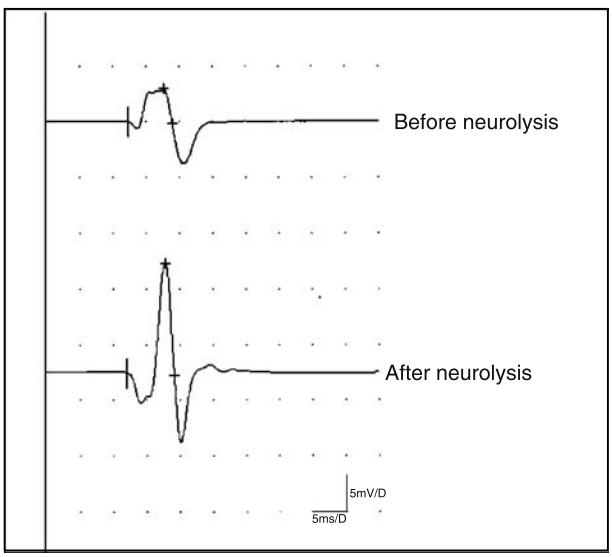

Fig. 1. Intraoperative compound muscle action potential before and after neurolysis

Duration of surgery

The surgery lasted approximately 3.5 h. Patients received antibiotics during surgery and no antibiotics before or afterwards.

Post-operative care

Patients usually left the hospital 1–2 days after the surgery. Immobilization continued for up to 4 days. Physiotherapy usually began after this period and swimming was generally recommended one month after the surgery. Generally, on the first day after the surgery, the patient felt a decrease in pain, warmth in the hand and received good radial pulsation. There were no early or delayed complications after the surgery in any of the operated patients.

Post-operative results

Were determined on 11 patients, with a follow-up of 24 to 48 months. According to the clinical analysis, 9 (82%) of the 11 TOS patients showed good results with a complete or significant relief of symptoms and returned to work (Table 1). Two patients (18%) showed fair results, and though still partially functionally limited, became much more independent in their everyday motor activities after the surgery. There were no cases of poor results.

Before surgery, all 11 patients had partial or severe limitation in their physical activities. Eight of these patients were either only partially employed or fully unemployed. After the surgery, 9 of the 11 patients showed a normal physical activity without any restriction in their everyday life (Table 1). Eight of the 11 patients returned to full or partial employment (Table 2). After the surgery the severity of the pain significantly

Table 1. *Restriction of the physical activity before and after surgery*

Restriction of physical activity	Before surgery	After surgery
No restriction	0	9 (82%)
Limited activities	7 (64%)	2 (18%)
Greatly limited	4 (36%)	0

Table 2. *Results of surgery in emplyoment of patients*

Employment	Before surgery	After surgery
Fully employed	3 (27.3%)	8 (73%)
Partially employed	4 (36.3%)	2 (18%)
Unemplyoed	4 (36.3%)	1 (9%)

Table 3. *Results of surgery in pain relief*

Relief of pain (%)	Patients	Medication
76–100	9 (82%)	stopped
26–75	2 (18%)	reduced
<25	0	

decreased in all patients (Table 3). In 9 of the 11 patients, post-operative follow-up showed a complete or significant pain relief and their use of medication stopped. In 2 patients the pain intensity decreased and their use of medication was reduced.

Discussion

A variety of surgical approaches is widely used for surgical decompression of the neurovascular structures in TOS. Although the rate of complications may be as high as 14–34.5% [1, 6], most of these complications are minor and self limiting. However, other complications such as hypertrophic scars following rib resection may cause a recurrence of TOS. The recurrence of symptoms following scalenectomy or scalenotomy performed via supraclavicular exposure is reported to be 20% [5, 11]. In the case of first rib resection the reason for the recurrence may be an incomplete resection which leads to massive scarring [10, 12, 14]. During our follow up period of 24 to 48 months no recurrences occurred, but bearing in mind that most recurrences occur in the first six months after the surgery [13], we feel that this less traumatic technique will cause fewer recurrent cases. The rational behind our microsurgical approach draws on the assumption that it is less traumatic, and thus reduces the amount of tissue damage and bleeding that can potentially cause scarring and recurrence. This approach is based on performing a decompression in all potential sites of pressure along the plexus and the subclavian vessels, and was prompted by Dellon [2] who recommended to adopt the supraclavicular approach without the rib resection and to perform a thorough microsurgical neurolysis. It is believed by some authors [7] that the pathomechanism of TOS may include more than one site of compression, each increasing the influence of the others, causing a ''Multiple crush phenomenon''. This theory explains the need for an extensive neurolysis rather than routine approaches which are based on the decompression of a specific anatomic structure [i.e the rib or the scalenous muscle].

Microsurgical neurolysis is not possible through transaxillary approach due to poor visualization of the brachial plexus, especially the C8-D1 roots.

Conclusion

The supraclavicular approach provides a convenient and wide view of the brachial plexus. The use of microsurgical techniques minimizes the risk of nerve or vascular damage during the neurolysis and allows extensive removal of adhesions, scar tissues and fibrous bandages followed by vascular decompression [8]. Microsurgical decompression of the brachial plexus and subclavian vessels without cervical or first rib resection through the supraclavicular exposure seems to be an elegant surgical solution for TOS which is both safe, effective and significantly decreases the rate of recurrence.

Although the present series is not extensive enough, the long follow up period, the high rates of patients' satisfaction, the short hospitalization and the lack of surgical complications and/or of recurrence, together with the theoretical rational for this approach suggest that it has a role in the thoracic outlet surgery.

References

1. Davies AH, Walton J, Stuart E, Morris PJ (1992) Surgical management of the thoracic outlet compression syndrome. Br J Surg 79: 372–377
2. Dellon AL (1993) The results of supraclavicular brachial plexus neurolysis (without first rib resection) in management of post-traumatic ''thoracic outlet syndrome''. J Reconstr Microsurg 9: 11–17
3. Durham JR, Yao JST, Pearce WH *et al* (1995) Arterial injuries in the thoracic outlet syndrome. J Vasc Surg 21: 57–70
4. Goff CD, Parent FN, Sato DT, Robinson KD, Gregory RT, Gayle RG *et al* (1988) A comparison of surgery for neurogenic thoracic outlet syndrome between laborers and nonlaborers. Am J Surg 176: 215–218
5. Green RM, McNamare J, Ouriel K (1991) Long-term follow-up after thoracic outlet decompression: an analysis of factors determining outcome. J Vasc Surg 14: 739–746
6. Mingoli A, Feldhaus RJ, Farina C, Cavallar N, Sapienza P, Marzo L, Cavallaro A (1995) Long-term outcome after transaxillary approach for thoracic outlet syndrome. Surgery 118: 840–844
7. Novak CB (2003) Thoracic outlet syndrome. Clin Plastic Surg 30: 175–188
8. Rochkind S, Alon M (2000) Microsurgical management of old injuries of the peripheral nerve and brachial plexus. J Reconstr Microsurg 16: 541–546
9. Sanders RJ, Haung C (1991) Review of arterial thoracic outlet syndrome with a report of five new intances. Surg Ginecol Obstet 173: 415–425
10. Sanders RJ, Haung CE, Pearce WH (1990) Recurrent thoracic outlet syndrome. J Vasc Surg 12: 390–400
11. Sanders RJ, Pearce WH (1989) The treatment of thoracic outlet syndrome: a compression of different operations. J Vasc Surg 10: 626–634
12. Sellke FW, Kelly TR (1998) Thoracic outlet syndrome. Am J Surg 154–156
13. Sessions RT (1989) Reoperation for thoracic outlet syndrome. J Cardiovasc Surg 30: 434–444
14. Urshel HC Jr, Razzuk MA (1986) The failed operation for outlet syndrome: the difficulty of diagnosis and management. Thorac Surg 42: 523–528

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Neurosurgical prevention of ulceration and amputation by decompression of lower extremity peripheral nerves in diabetic neuropathy: update 2006

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Summary

Background. A triad of metabolic abnormalities are known that render the peripheral nerve in diabetes mellitus susceptible to chronic compression: conversion of glucose to sorbitol increases the intraneural water content, slowing of axoplasmic transport of proteins hinders structural repair, glycosylation of endoneurial collagen reduces perineurial gliding. In the early 1990s, Dellon et al demonstrated that removal of a site of anatomic narrowing of the tibial nerve in the rat model prevented neuropathic walking.

Method. Scientific literature related to this concept was reviewed. Through the end of 2006, there have been 15 peer-reviewed studies that used the inclusion criteria of 1) presence of symptomatic neuropathy, 2) positive Tinel sign over the tarsal tunnel demonstrating a site of compression, 3) no previous history of ulcer or amputation and 4) used the Dellon Triple Decompression technique (neurolysis of the peroneal nerve at the knee and the dorsum of the foot, and neurolysis of the tibial nerve in the four medial ankle tunnels).

Findings. These studies demonstrated relief of pain in 88% and restoration of sensation in 79% of patients. One study demonstrated that the natural history of diabetic neuropathy can be changed by observing no ulcers/amputations in the operated leg of 50 diabetics followed for a mean of 4.5 years, while 12 ulcers and 3 amputations occurred in the non-operated contralateral limb ($p < 0.001$). Results of a multi-centered prospective study are available at NeuropathyRegistry.com, demonstrating a reduction in the prevalence of ulceration in 665 diabetics at 2.5 years from 15 to 0.6% in those diabetics without a previous history of ulceration and from 50 to 2.2% in 44 patients with a previous history of ulceration.

Conclusions. Decompression of superimposed nerve compressions in the patient with symptomatic neuropathy reliably relieves pain, restores sensation, and thereby prevents ulceration and amputation.

Keywords: Neuropathy; diabetes; nerve compression; neurolysis.

Introduction

Diabetic neuropathy (DN), a diffuse, distal, symmetrical mixed sensorimotor polyneuropathy, will effect at least half of all diabetics [12, 14, 21], at a time when an epidemic in diabetes is occurring world-wide (300 million diabetics by 2030) [9]. Diabetic neuropathy, traditionally, is considered progressive and irreversible, predictably causing loss of sensation, then ulceration, infection and, finally, amputation [12, 14]. In the United States, 15% of diabetics develop an ulcer, and more than 80,000 amputations occur per year unrelated to vascular occlusion. Health care costs related to neuropathy are enormous [3]. Seminal research in a diabetic rat model demonstrated in the early 1990s that in the absence of a site of anatomic narrowing of lower extremity nerves, a neuropathic walking track pattern did not occur, suggesting that the natural history of diabetic neuropathy could be changed [7]. It is the purpose of this paper to review briefly the clinical and basic science begun a quarter century ago that permits us to say definitively today that a neurosurgical approach to multiple sites of chronic nerve compression in the lower extremity can relieve pain, restore sensation, and, thereby, prevent ulceration and prevent amputation in diabetics with symptoms of neuropathy.

Methods

A "meta-analysis" of the scientific literature identified 15 articles related to the concept of relief of symptoms of neuropathy by decompression of multiple superimposed nerve compressions in the lower extremity. The index articles were the first description of this clinical optimism in 1988 by Dellon [5], that described the metabolic abnormalities that render the peripheral nerve susceptible to chronic compression, 1) conversion of glucose to sorbitol increases the intraneural water content, 2) slowing of axoplasmic transport of proteins hinders structural repair, and 3) glycosylation of endoneurial collagen reduces perineurial gliding. This subject was reviewed recently [15]. The second index article was the retrospective study by Dellon in 1992 that identified the inclusion parameters for this meta-analysis: [6] 1) presence of sympto-

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Table 1. *Diabetic neuropathy: results of posterior tibial nerve decompression*

Study	Number of		Improvement (%)	
	Patients	Nerves	Pain	Sensibility
Dellon [6]	31	22	85	72
Wieman and Patel [20]	33	26	92	72
Caffee [4]	58	36	86	50
Aszmann *et al*. [1]	16	12	n.a.	69
Tambwekr [18]	10	10	80	70
Wood and Wood [22]	33	33	90	70
Biddinger and Amend [2]	15	22	86	80
Valdivia *et al*. [19]	60	60	85	85
Lee and Dellon [10]	46	46	92	92
Nelson and Little [11]	6	6	86	86
Steck [17]	25	25	84	72
Rader [13]	49	49	90	75
DiNucci [8]	36	36	80	80
Yao *et al*. [23]	70	70	95	5
Siemionow *et al*. [16]	37	37	90	90
Total	516	464	88	79

matic neuropathy, 2) positive Tinel sign over the tarsal tunnel demonstrating a site of compression, 3) no previous history of ulcer or amputation and 4) used the Dellon Triple Decompression technique (neurolysis of the peroneal nerve at the knee and the dorsum of the foot, and neurolysis of the tibial nerve in the four medial ankle tunnels). Table 1 lists the studies reviewed.

Results

Review of the many studies listed in Table 1 show a uniformity of success in relieving pain in 88% of the 515 patients evaluated for that outcome, and restoration of sensation in 79% of the 464 patients evaluated for that outcome. There were no new ulcerations reported in those studies that included patients without a previous history of ulceration. In the two studies [4, 20] that included a total of 30 patients with a previous history of ulcers and/or amputation, there was just one patient (3%) with a new ulceration. A multi-centered, prospective study utilizing the same inclusion criteria given above is on-line at NeuropathyRegistry.com. This group of more than 737 operations in more than 430 neuropathy patients further documents the ability of the Dellon Triple Nerve Decompression procedure to prevent ulcers: with the expected incidence of ulceration being 15%, there were just 0.6% new ulcers at 2.5 years after surgery in 665 decompressed legs. In those patients with a previous history of ulceration, where the expected recurrence rate is 50%, there was recurrence in 2.25% of 44 patients at 2.5 years. These surgeons each were trained Dellon's diagnostic patient selection and surgical technique.

Discussion

This review is limited by the studies included, and author bias. The implications of this study are that there is hope to prevent ulceration and amputation in patients with neuropathy. The natural history of neuropathy can be altered by identification of superimposed nerve compressions along the peroneal and tibial nerves, and decompressing these nerves in the appropriate anatomic locations. Relief of pain and restoration of sensation can be expected in about 80% of these individuals.

References

1. Aszmann O, Kress KM, Dellon AL (2000) Results of decompression of peripheral nerves in diabetics: a prospective, blinded study. Plast Reconstr Surg 106: 816–821
2. Biddinger KR, Amen KJ (2004) The role of surgical decompression for diabetic neuropathy. Foot Ankle Clin N Amer 9: 239–254
3. Boulton AJ, Vileikyte L, Ragnarson-Tenvall G, Aplequist J (2005) The global burden of diabetic foot disease. Lancet 366: 1719–1724
4. Chafee H (2000) Decompression of peripheral nerves for diabetic neuropathy. Plast Reconstr Surg 106: 813–815
5. Dellon AL (1988) Optimism in diabetic neuropathy. Ann Plast Surg 20: 103–105
6. Dellon AL (1992) Treatment of symptoms of diabetic neuropathy by peripheral nerve decompression. Plast Reconstr Surg 89: 689–697
7. Dellon ES, Dellon AL, Seiler WA IV (1994) The effect of tarsal tunnel decompression in the streptozotocin-induced diabetic rat. Microsurg 15: 265–268
8. DiNucci K (2005) Results of decompression of multiple lower extremity peripheral nerves in diabetic with symptomatic neuropathy, Presented at the Amer College of Foot and Ankle Surgery meeting, New Orleans
9. International Diabetes Federation (2006) http://www.eatlas.idf.org/
10. Lee C, Dellon AL (2004) Prognostic ability of Tinel sign in determining outcome for decompression surgery decompression surgery in diabetic and non-diabetic neuropathy. Ann Plast Surg 53: 523–527
11. Nelson SC, Little ER Jr (2006) Pilot study of the use of the SF 36 questionnaire outcome evaluation for multiple lower extremity nerve decompressions in diabetic peripheral neuropathy. J Amer Pod Med Assoc (in press)
12. Pirat J (1978) Diabetes mellitus and its degenerative complication: a prospective study of 4400 patients observed between 1947 and 1973. Diabetes Care 1: 168–252
13. Rader A (2005) Surgical decompression in diabetic neuropathy. J Amer Pod Med Assoc 95: 446–450
14. Rather HM, Boulton AJ (2005) Recent advances in the diagnosis and management of diabetic neuropathy. J Bone J Surg 87B: 1605–1610
15. Siemionow M, Demir Y (2004) Diabetic neuropathy; pathogenesis and treatment: a review. J Reconstr Microsurg 20: 241–252
16. Siemionow M (2006) Clinical outcome of peripheral nerve decompression in diabetic and non-diabetic peripheral neuropathy. Ann Plast Surg (in press)
17. Steck J (2005) Results of decompression of lower extremity peripheral nerve for treatment of symptomatic neuropathy, Presented at Am Soc Peripheral Nerve Meeting, Puerto Rico

18. Tambwekar SR (2001) Extended neurolysis of the posterior tibial nerve to improve sensation in diabetic neuropathic feet. Plast Reconstr Surg 108: 1452–1453
19. Valdivia JMV, Dellon AL, Weinand MD, Maloney CT Jr (2005) Surgical treatment of peripheral neuropathy: outcomes from 100 consecutive decompressions. J Amer Pod Med Assoc 95: 451–454
20. Wieman TJ, Patel VG (1995) Treatment of hyperesthetic neuropathic pain in people with diabetes; decompression of the tarsal tunnel. Ann Surg 221: 660–665
21. Witzke KA, Vinik AI (2005) Diabetic neuropathy in older adults. Rev Endocrin Metab Disord 6: 117–127
22. Wood M, Wood W (2003) Decompression of peripheral nerve for diabetic neuropathy in lower extremity. J Foot Ankle Surg 42: 268–275
23. Yao Y, Wang RZ, Shang B, Liu FY, Wei UK (2005) Treatment of symptomatic diabetic peripheral neuropathy by surgical decompression of three peripheral nerves. Chin Med J 242: 35–37

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IMF®-Therapy (Intention controlled Myo-Feedback) – an innovative method in the treatment of peripheral nerve lesions

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Summary

Physiotherapy is a well established part of the rehabilitation of peripheral nerve paralysis. The aim of this type of treatment is to re-establish arbitrary functions by improving the patients' active and passive mobility as well as their strength and stamina.

IMF®-Therapy (Intention controlled Myo-Feedback) is an innovative method in the treatment of peripheral nerve lesions that goes beyond the purely neuro-scientific framework and also takes into account methods and concepts of the psychology of learning. The essential assumption is that things learnt in the past are firmly established in the long term motor memory and can be reactivated by the patient.

From results achieved in 32 patients treated with this therapy it can be concluded that IMF®-Therapy may be a promising additional rehabilitation tool in peripheral nerve lesion.

Keywords: Physiotherapy; IMF®-Therapy; peripheral nerve lesion.

Introduction

Problematic definition

"What do I have to do to be able to pick up a glass again?" That is the burning question for patients who suffer from motor and sensory defects in their arms and hands after brachial plexus lesion and have lost movement in those limbs. Despite the reconstruction of nerves there is often no recovery.

Physiotherapy is a well established part of the rehabilitation of peripheral nerve paralysis. The aim of this type of treatment is to re-establish arbitrary functions by improving the patients' active and passive mobility as well as their strength and stamina. This is achieved through the activation of peripheral motor processes, such as proprioceptive neuromuscular facilitation (PNF) or passive muscle stimulation. Despite regular treatment sessions chronic patients exposed to IMF®-Therapy sometimes suffer not only from limited motor functionality but also severely impaired surface and depth sensitivity. Many chronic patients are also unable to imagine a movement learnt prior to becoming disabled. This phenomenon often only becomes apparent when patients are asked to simulate a specific arbitrary movement in their mind, for example pulling a folder from a shelf in their old office. As long as there is some remaining activity in the affected limb and patients are encouraged to use physical strength to carry out the movement, they will establish pathological movement patterns with every attempt. This leads to their positive learning experiences from the time before the illness being overshadowed by post-lesional, negative experiences.

Theories about channelling movement agree that planning and carrying out arbitrary movements is highly influenced by anticipating the specific aims of movements [10, 13, 14]. Not being able to imagine a movement means that the patient cannot remember the effects of previously carried out movements, resulting in an inability to reach similar desired results in the present situation. Planning and carrying out purposeful movements therefore becomes impossible. This poses two problems for the rehabilitation process. Finding solutions for these problems could significantly improve rehabilitation:

– How do chronic patients acquire the ability to reactivate positive learning experiences despite not being able to remember previously learnt movements? Literature does not offer any answers.

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– How do patients acquire the ability to inhibit negative learning experiences? Literature does not offer any answers to this problem either.

IMF®-Therapy's approach goes beyond the purely neuro-scientific framework and also takes into account methods and concepts of the psychology of learning. The essential assumption is that things learnt in the past are firmly established in the long term motor memory and can be reactivated by the patient [19].

Learning aims

The aim of IMF®-Therapy is the *re-learning* of sensory-motor functions.

– The therapy focuses on the ability to reactivate a positive learning experience whilst inhibiting the negative experience.
– As soon as the patient is able to remember the old experiences, he or she shall learn to make use of these sensory-motor experiences and to re-establish an association of the imagined anticipated aim and the movement to be carried out with the affected limb. This is achieved through internal simulation of sensory consequences of individual actions.
– The assumption is that these learning processes activate various mechanisms related to neuro-plasticity. Scientific research provides evidence for the theory that improvements to sensory-motor functions are based on neuro-plasticity and the ability of injured peripheral neural pathways to regenerate, which can be influenced by learning processes.
– Therefore plastic changes could cause a reorganisation on the cortical level, which in turn could improve regeneration on the peripheral level.

Learning mechanisms

Sensory-motor experiences, anticipated aims, changes to the neural activity and amplification are based on learning mechanisms.

Reactivating previously learnt movements

To relearn sensory-motor functions means to be able to refer to cognitive concepts of movements acquired before the injury. These contain the positive learning experiences gained through putting arbitrary movements in relation to the aims of the movements. The lack of an expected effect of the movement caused by a lesion might reduce the ability to carry out the movement but it doesn't eliminate it completely [1]. Visual scientific results from cerebral research show that previously established neuronal structures are being transferred to other areas of the brain. This means they are still available and could be reactivated [18, 19].

Apart from representations of positive learning experiences there are also those with negative experiences acquired after the injury or illness. These include pathological patterns, a result of repeated active attempts of movements with unwanted effects. The basic prerequisite for relearning lost functions is to inhibit these representations and to reactivate old experiences.

IMF®-Therapy suggests mental training as a solution to the problem of reactivating pre-lesional experiences. The method is based on the knowledge that actions can be carried out mentally [5]. It is therefore possible to encourage the patient to imagine different situations from the time before falling ill and to search for movement patterns that used to lead to the anticipated effects in the affected limb. Memory is usually retrieved after a few repeated attempts.

We can assume that unwanted effects can be inhibited once the memory of the positive experience has been retrieved. The choice of specific movements depends on the power of the association and the relative importance of the movements [5–8]. Regular mental training reinforces the selection criteria in favour of the anticipated movement and against the unwanted effects.

Anticipation

Once the patient has succeeded in remembering movements learnt prior to the lesion, the anticipation of a specific arbitrary movement becomes possible again. Anticipation is ''a memory of consequences of actions experienced in the past'' [3]. Simply ''thinking of the intended effects of an action or imagining its effects is sufficient to trigger a movement which will result in exactly these effects'' (''ideo-motoric principle'', [9, 11]). Hence movement is initiated through anticipation, or cognitive expectation, of its intended aims [3, 10, 13, 14]. Neuro-scientific research backs the theory of anticipated aims. Brain research on monkeys showed that even before a movement is carried out certain neuronal areas in the brain are active, representing the anticipated aims of the action [16]. For example, when the intention is to grasp an object, the opening of the hand is being imagined and prepared on the cortical level, while the hand is still in the process of moving towards the object [10].

Changes in the neuronal activity through learning

The movement concept assumes that learning results in representations of actions, which can be activated through anticipation. Anticipation triggers stimulation patterns. Each stimulation pattern causes adaptive changes in the connections involved. These changes are rather minor and rarely strong enough to fully reconstruct specific connections between different patterns of activity after just processing them once. Only very frequent and repeated triggering of the same patterns will result in minimal changes to the synaptic connections [15]. These changes are related to behavioural patterns and increase the neural activity on the cortical level. According to Hebb [4] these ultimately lead to plastic changes, because synaptic connections are strengthened and new connections are being formed. IMF®-Therapy aims to repeatedly activate the same stimulation patterns through mental training, which makes it easier for the patient to actively carry out the movement.

This increased neuronal activity on the cortical level continues on the spinal level. An EMG can prove that this kind of activation can influence the frequency and amplitude of the small alpha-motoneurones in the spinal cord (Type-I fibres). The actual number of motor units being recruited depends on the regenerative state of the affected peripheral nerves [17]. The strong activation of alpha-motoneurones achieved through mental activity as part of IMF®-Therapy is intended to directly influence the regenerative process of the affected nerves as well as the atrophic process of the Type-I muscle fibres.

Amplification

Mental activity escapes conscious perception and control [12]. IMF®-Therapy assists in achieving perceptible effects with the help of an external mechanism, an EMG-driven muscle stimulating device called MfT Z^2. This technical innovation allows a mental connection of MfT Z^2 to arbitrary nerve impulses, provided an EMG rest activity is detectable in the paretic muscle. The EMG-activity, which correlates with the imagined movement, triggers a low-frequency muscle stimulation, which in return provides an intrinsic feedback. The patient may or may not be able to feel this, depending on his or her post-lesional sensory qualities. However, if the sensory stimulation triggers the anticipated aim in the patient's mind through either proprioceptive or exteroceptive effects, then the same stimulation patterns of the cognitive representation are activated again, leading to the plastic mechanisms described above.

Muscle stimulation activates Type-II fibres. Just as with an arbitrary movement an activation of Type-I muscle fibres is followed by an activation of Type-II fibres, which has a positive effect on the intra-muscular co-ordination.

At the beginning of the treatment many patients are not quite sure whether the reaction and effect experienced close together means the reaction came first and was followed by the effect (amplification) or vice versa. After continued practice the patients learn that perceptible effects relate to the intention and the anticipated aims.

Method

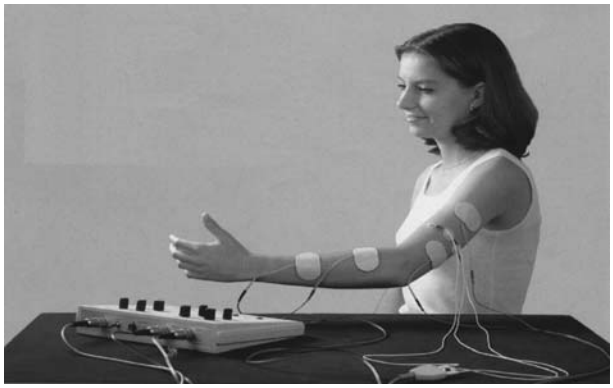

Fig. 1. IMF®-Therapy contains mental, sensory and repetitive training, supported by the medical device MfT Z^2. An interface between the patient and MfT Z^2 allows the mental activation of the device via arbitrary nerve impulses

Mental training

At the beginning of the therapy the therapist develops an individual "tailor-made" imaginary movement with the patient, which is directly aimed at the paralyzed muscles. Relevant electric activity in the form of EMG-potentials can be detected. The medical device MfT Z^2 picks up these potentials via sensors (surface-EMG-electrodes), amplifies them and feeds them back to the paralyzed muscles in the form of a two-channeled muscle stimulation.

With each imagined movement the arbitrary movement is channeled in order to trigger actual physical changes in the central nervous system, which enables and improves arbitrary movement.

The result of this channeling is the activation of the paralyzed muscle fibers (Type I), which are most seriously affected by atrophy.

Sensory training

Imagining movements sensitizes the sensory system for feedback from peripheral structures. Contrary to actual movements imagined movement does not trigger a movement in the joints which will give relevant feedback noticeable to the patient. In IMF®-Therapy artificial feedback is created with the help of the MfT Z^2.

Stimulation electrodes are placed on the paralyzed muscles and they are innervated through imagined movement (e.g. for the abduction and exterior rotation of the arm: M. supraspinatus, deltoideus, teres minor, infraspinatus). The stimulation activates the remaining muscle fibers (Type II).

Every time the patient imagines a movement involving the affected limb he or she received feedback from the paralyzed muscles.

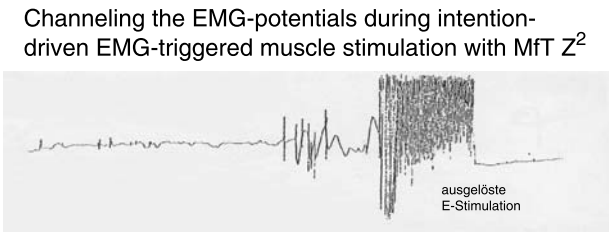

Fig. 2. Here the weak initial innervation pattern is clearly visible, followed by the result of imagining movement, which triggers the muscle stimulation

Repetitive training

The patients are inducted into the method at a therapy clinic to ensure that they have the necessary skills to use the device at home on their own. The frequency of the training sessions is vital for successful rehabilitation. Ideally the patient should practice several times a day with MfT Z^2.

Requirements for usage

The patient must have the mental ability to imagine a proper movement. A minimal nerve activity in the muscles has to be detectable in the affected muscle.

Indications

Peripheral nerve lesions.

Contraindications

Cardiac pacemaker, inflammation or thrombosis in the affected limb.

Myofeedback device MfT Z^2

MfT Z^2 is a medical, wireless device, comprising one separate surface-EMG, one EMG-amplifier and two independent stimulation generators. The surface-EMG provides information on the patient's channeling characteristics, the two-channeled stimulation gives immediate feedback on the results.

Parameter

EMG-sensitivity: 0–2000 µV
Muscle stimulation: 2-channelled
Modified square pulse: duration of 200 µsec
Frequency: 25–100 HZ
Duration of stimulation: 1–12 sec

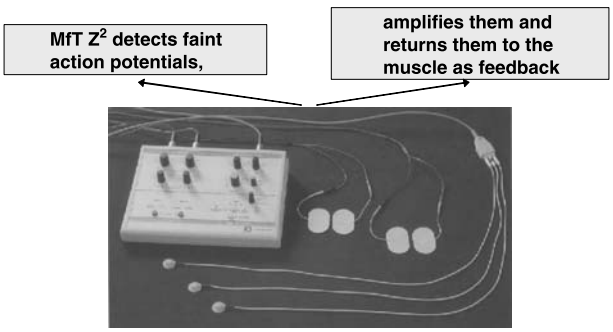

Fig. 3. The device MfT Z^2

Table 1. *Material*

Patient no.	Interval (yrs): accident → IMF® treatment
1	25.0
2	2.0
3	5.0
4	1.0
5	5.0
6	1.5
7	2.5
8	17.0
9	13.0
10	1.5
Ø	7.3 yrs

Results

Can plasticity be induced?
The answer: Yes, by modifying the level of activity [2]

Material

Treatment with IMF®: 32 patients → 10 patients with complete brachial plexus lesion between 26 and 57 yrs (36.1)

Duration of IMF® treatment

- Short treatment: 5 days: 1 patient
  2 weeks: 2 patients
- Extended treatment: 4 weeks: 2 patients
  6 weeks: 3 patients
  9 weeks: 2 patients

Assessments:
- Sensibility
  - 2-Point-Discrimination (2 PD)
  - Perception of vibration (25 Hz)
- Pain
  - Visual Analog Scale
- Muscle Power and Resisting Range of Motion according to Lovett

Table 2. *Muscle power/range of motion*

Grade		Motor deficit (%)
5	complete active range of motion against gravity with full resistance (normal)	0
4a	complete active range of motion against gravity with moderate resistance (good)	5–10
4b	complete active range of motion against gravity with some mild resistance (moderate)	15–25
3	complete active range of motion against gravity only, without resistance (fair)	30–50
2	complete active range of motion with gravity eliminated (poor)	60–80
1	evidence of slight contractility; no joint movement (trace)	90–95
0	no evidence of contractility (absent)	100

Results

Table 3. *Results of IMF®-Therapy*

Improvement of sensibility	No. of patients
– Short treatment	
Shoulder	2
Upper arm	2
Lower arm	0
	2/3 patients got a better sensibility
– Extended treatment	
Shoulder	7
Upper arm	7
Lower arm	6
	7/7 patients got a better sensibility
Pain pre-treatment VAS	Pain post-treatment VAS
– Short treatment	
0	0
8	5
8	5
	2/2 patients had reduced pain
– Extended treatment	
2	0
2	0
6	3
6	5
5	0
2	0
0	0
	4/6 patients had no pain at the end of the treatment; 2 patients had reduced pain
Improvement of muscle power max. 1 grade	No. of patients
– Short treatment	
1 unit of motion	2
– Extended of treatment	
2 units of motion	1
3 units of motion	1
5 units of motion	1
	5/10 patients with a reinnervation

Conclusion

IMF®-Therapy may be a promising additional rehabilitation tool in peripheral nerve lesion.

References

1. Adams JA (1971) A closed-loop theory of motor learning. J Motor Behav 3: 111–150, zitiert in Elsner B (2000) Der Erwerb kognitiver Handlungsrepräsentationen. Wissenschaftlicher Verlag, Berlin, p 116
2. Ebner *et al* (1997) Activity dependent plasticity in adult somatic sensory cortex. Sem Neurosci 9: 47–58
3. Elsner B (2000) Der Erwerb kognitiver Handlungsrepräsentationen. Wissenschaftlicher Verlag, Berlin
4. Hebb DO (1949) The organization of behavior. Wiley, New York
5. Hommel B (1996) The cognitive representation of action: Automatic integration of perceived action effects. Psychological Res 59: 176–186
6. Hommel B, Müsseler J, Aschersleben G, Prinz W (1999) The theory of event coding (TEC). A framework for perception and action (manuscript submitted for publication)
7. Hommel B (1997) Toward an action-concept model of stimulus-response compatibility. In: Hommel B, Prinz W (eds) Theoretical issues in stimulus-response compatibility. North-Holland, Amsterdam, pp 281–320
8. Hommel B (1998) Perceiving one's own action – and what it leads to. In: Jordan JS (ed) Systems theories and a priori aspects of perception. North-Holland, Amsterdam, pp 143–179
9. James W (1890) The principles of psychology. Dover Publications, New York
10. Jeannerod M (1994) The representing brain: neural correlates of motor intention and imagery. Behav Brain Sci 17: 187–245
11. Lotze RH (1852) Medicinische Psychologie oder die Physiologie der Seele. Weidmann'sche Buchhandlung, Leipzig
12. Prinz W (1992) Why don't we perceive our brain states? Eur J Cognitive Psychol 4: 1–20
13. Prinz W (1997) Perseption and action planning. Eur J Cognitive Psychol 9: 129–154
14. Rosenbaum DA, Krist H (1996) Antecedents of action. In: Heuer H, Keele SW (eds) Handbook of perception and action, vol. 2: motor skills. Academic Press, London
15. Rösler F (1997) Neuropsychologische Gedächtnisforschung. In: Lüer G, Lass U (Hrsg) Erinnern und Behalten: Wege zur Erforschung des menschlichen Gedächtnis. Vandenhoeck & Ruprecht, Göttingen pp 79–116
16. Sakata H, Taira M, Kusunoki M, Murata A, Tanaka Y (1997) The parietal association cortex in depth perception and visual control of & hand action. Trends Neurosci 20: 350–357
17. Sale DG, Digby G (1994) Neurale Adaptation im Verlaufe eines Krafttrainings. In: Komi PV (Hrsg) Kraft und Schnellkraft im Sport, dt. Übersetzung und Bearbeitung von G. Rost/R. Rost, Enzyklopädie der Sportmedizin, Bd. 3, dt. Ärzteverlag, Köln, pp 249–265
18. Shadmehr R, Holcomb HH (1999) Inhibitory control of competing motor memories. Exp Brain Res 235–251, quoted in Panzer, St.: Motorisches Lernen, Deutsche Zeitschrift für Sportmedizin 53(11) (2002): 313–316
19. Shadmer R, Brashers-Krug T (1997) Functional stages in the formation of human long-term motor memory. J Neurosci 17: 409–419, quoted in Panzer, St.: Motorisches Lernen, Deutsche Zeitschrift für Sportmedizin 53(11) (2002): 313–316

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In peripheral nerve regeneration environment enriched with activity stimulating factors improves functional recovery

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Summary

Enriched environment stimulates brain plasticity processes after brain lesion. Less is known about the influence of enriched environment with activity stimulating factors as determinants of functional outcome after peripheral nerve repair.

BDNF (brain-derived neurotrophic factor) plays a role in activity-dependent neuronal plasticity and changes in motor cortex in rats learning complex motor skills.

Our study aimed to elucidate if enriched environment influences functional results after peripheral nerve repair. The results in this rat sciatic nerve transection and repair model showed that environment enriched with activity stimulating factors can improve functional results.

Keywords: Enriched environment; BDNF; nerve regeneration; rehabilitation program.

Introduction

Developmental animal studies have shown that sensory experience leads to changes at different levels of the central nervous system. These developing concepts have presented new explanations for the variable and often disappointing results of peripheral nerve regeneration. Recent brain imaging techniques have demonstrated that functional synaptic reorganisation in brain cortex can commence within seconds and continue for a very long time. For instance, extensive use of the hand may result in enlargement of the corresponding projectional areas in the brain [14, 40]. Plasticity in the cerebral cortex refers to a change in properties of cortical neurons [13]. Though mechanisms involved in the plasticity process may vary, improved connectivity within individual neurons, modification of cortical representation areas, cortical maps as well as non-synaptic transmission may play a key role [10, 12, 18, 19, 21–23, 37, 38, 57].

The BDNF (Brain-derived neurotrophic factor) system enhances the function and viability of select neuron populations, and its action appears to be crucial for maintaining molecular processes underlying cognitive function. BDNF promotes neuronal excitability [6, 29] and facilitates synaptic transmission [30, 34, 49, 52] and hippocampal BDNF seems necessary for the induction of LTP – long-term potentiation [33, 35, 39]. BDNF is synthezised predominantly by neurons located in the hippocampus [50] but mechanical stimulation of whiskers in mice upregulates BDNF mRNA expression in the somatosensory cortex [44]. In rats learning complex motor skills, BDNF and its receptor, TrkB protein, changes in the cerebellum and motor cortex [32].

Recently Johansson and Ohlsson [24] showed the importance of environment, social interaction and physical activity as determinants of functional outcome after cerebral lesion in the rat. Less is known about the influence of these parameters to functional recovery after peripheral nerve repair. An important question is to what extent environmental circumstances and physical activity influence peripheral nerve lesion-induced plasticity and improve functional restoration. Based on current data on brain plasticity in the intact and injured brain [21, 23, 26, 27, 37, 42, 45, 55–57] we have tested the

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hypothesis that in peripheral nerve regeneration enriched environment due to activity stimulating factors may stimulate brain/spinal plasticity and affect BDNF protein in the sensorimotor cortex, thus improving functional recovery. Functional results, electrophysiologic data and BDNF protein levels in the sensorimotor cortex are discussed.

Material and methods

Subjects were forty-two 6-month-old male Sprague-Dawley rats weighing 300–350 g. In each animal, the right sciatic nerve was dissected midthigh and transected by microsurgical scissors. In all animals, nerve ends were coapted immediately with 2 epineural sutures under microscopic magnification. The wound was closed in layers.

Groups

Enriched environment group

Social (four rats in each cage) housing of rats in enriched – activity stimulating environment (cage: 60 cm × 37 cm × 25 cm; running wheel, rat toys: dry chow filled balls) environment, $n = 21$.

Control group (non enriched environment)

Individually housed in standard cages without enriched environment, $n = 21$.

Baseline BDNF group

NON-operated control group, $n = 4$.

Functional studies

Sensorimotor performance was evaluated at the 3rd week after surgery and every week thereafter for 10 weeks.

1) BBB-Locomotor rating Scale [46]
2) Inclined Plane test [36, 58].
3) Toe spread test (modified) [15]
4) Placing tests.

Placing is the ability of an animal to place its hind limbs onto a surface for support in response to a proprioceptive impulse (joint bending) and to contact (light touch). Contact and proprioceptive placing can be distinguished visually by the joint displacement required to elicit the response.

– Proprioceptive placing test [16]
– Contact placing test [1, 3, 7, 8].

Cross-section area of the anterior tibial and peroneal muscles 3 months after surgery

The cross-section area of the anterior tibial muscle and of the peroneal muscles was used to assess innervation of the target organ. The area of the cross-sections was calculated with the maximum vertical and horizontal diameter.

Electrophysiologic analysis

All electrophysiologic studies were carried out after 3 months during re-operation described in a previous study [47]: 1) CNAP-(compound nerve action potential area), 2) peak action potential amplitude, 3) nerve conduction velocity, 4) latency.

BDNF-ELISA (sensorimotor cortex)

For baseline evaluation of BDNF protein levels within the sensorimotor cortex four animals were used without any treatment. BDNF protein levels were evaluated after 1 week, 3 weeks and 12 weeks after surgery. At the end of electrophysiologic assessment all animals were sacrificed. Brains were removed and cross-sections including the sensorimotor cortex according to Hall and Lindholm [17] were harvested and frozen immediately at −80 °C in liquid nitrogen until used for ELISA.

Statistic analysis

The statistic analysis was carried out with the statistics software Graph Pad Prism (Graph Pad Software Inc., San Diego, CA, USA). Normal distribution of data was tested with *Kolmogorov-Smirnov test*. Toe spread test, placing tests and electrophysiologic data were compared using *Mann–Whitney test*. *Unpaired t-test* was used for BBB Score, inclined plane test, cross-section area of tibial/peroneal muscle. All graphs in this study are shown as mean and standard deviation.

Results

This study showed that rats underwent immediate peripheral nerve repair postoperatively. Animals housed in environmental enrichment with activity stimulating factors had a significantly better functional outcome but did not show statistically significantly better electrophysiologic recovery. Moreover, an environment enriched with activity stimulating factors results in a steady state of BDNF protein within the sensorimotor cortex 1 week and 3 weeks after surgery compared to the BDNF protein levels of the surgically non-treated animals. From weeks three to twelve, however, the enriched environment group showed a significant increase of BDNF protein levels in the sensorimotor cortex within both sensorimotor cortices.

BBB locomotor scale (Table 1)

The results of hind limb function based on BBB score are summarized in Table 1. The enriched environment group showed statistically significantly superior results during the entire period of consideration, excluding weeks five and nine. Three months after surgery, complete functional recovery indicating that animals supported their weight, stepped consistently and demonstrated normal coordination of the fore limbs and hind limbs was not observed in any animal. Most animals in the enriched environment group maintained nearly normal paw position throughout the stance phase of gait, and did not

Table 1. *BBB locomotor rating scale (mean values and standard deviation) 3 to 12 weeks 12 after surgery*

BBB locomotor rating scale	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12
Enriched environment	14.7	15.0	14.9	16.0	16.2	16.7	16.7	17.6	17.7	18.3
SD	0.5	0.9	1.2	0.7	0.7	1.1	1.0	1.1	1.3	1.3
Control	12.6	13.1	13.3	13.4	13.7	14.9	15.7	15.6	16.3	16.6
SD	1.6	1.7	1.9	2.1	2.1	2.1	1.9	1.6	1.3	1.7
p	0.002	0.009	0.050	0.004	0.003	0.039		0.007	0.048	0.023
	**	**	ns	**	**	*	ns	**	*	*

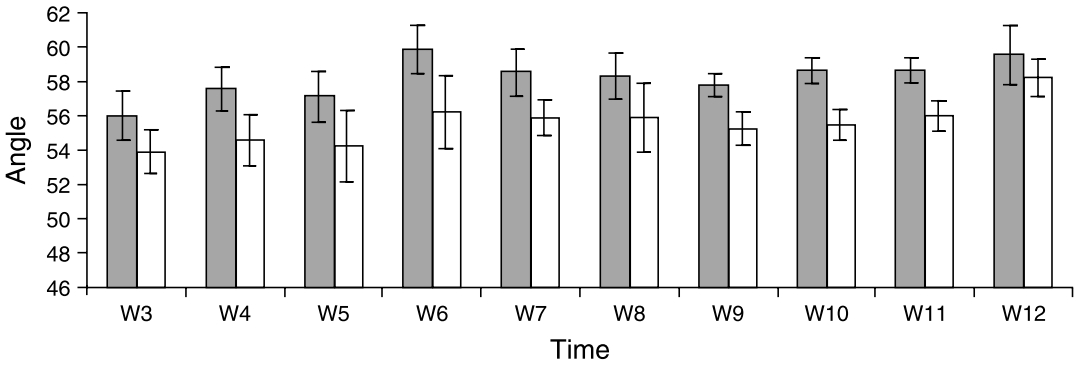

Fig. 1. Inclined plane test. ■ Enriched environment; □ control

display the external or internal rotation of the paw commonly observed in rats after lesion.

Inclined plane test (Fig. 1)

The results of hind limb function based on the inclined plane test are summarized in Table 2. The enriched environment group showed statistically significantly better results between the 3rd and 11th week after surgery.

Toe spread test

Between the 3rd and 6th week after surgery the difference between groups was not statistically significant. Between the 7th and 12th week after surgery the difference between groups was statistically highly significant (**$p = 0.0002$).

Contact placing test

Between the 7th and 11th week after surgery there was a trend towards better contact placing in the enriched

	Operated hind limb 3 months after surgery (mm^2)	**Non operated hind limb** 3 months after surgery (mm^2)
Enriched environment	**87.5**	**92.1**
SD	24.7%	15.7%
Control	**65.9**	**77.7**
SD	8.5	10.9
Normal control		**74.2**
SD		**10.9**

Fig. 2. Cross section area of the anterior tibial and peroneal muscles 12 weeks after surgery

environment group but the difference was not statistically significant.

Proprioceptive placing test

Since all animals in the enriched environment group showed proprioceptive placing three weeks after surgery, statistic evaluation was not possible.

Cross-section area of the anterior tibial and peroneal muscles (Fig. 2)

The results of the cross-section area of the anterior/tibialis muscles are summarized in Table 2. In the enriched environment group the cross-section area in the operated hind limb was 24.7% higher than in controls. This was statistically significant ($^{**}p = 0.002$). Within the enriched environment group there was no difference between the operated and non-operated hind limb. The cross-section area in the operated hind limb was 95% of the cross-section area in the non-operated hind limb.

In the non-operated hind limb the enriched environment group reached 118.5% of control ($^{*}p = 0.05$). The cross-section area in the operated hind limb within the control group was 84.8% of the non-affected side which was statistically significant ($^{*}p = 0.02$). The operated hind limb of the enriched environment group showed a 12.6% larger cross-section area than the non-operated hind limb of the control group.

Electromyography (EMG) three months after surgery (data not shown).

CNAP (Compound action potential area), Peak action potential amplitude, Nerve conduction velocity and Latency did not show statistically significant differences between groups.

ELISA BDNF (sensorimotor cortex)

The results of BDNF protein values within the left and right sensorimotor cortex are summarized in Table 2.

Table 2. *Mean values and standard deviation of the BDNF protein within the sensorimotor cortex 3 weeks and 3 months after surgery*

		Left (ng/g ww)	Right (ng/g ww)
3 months	enriched	85.7	86.7
	control	76.4	83.8
3 weeks	enriched	38.2	57.0
	control	65.3	73.3
1 week	enriched	36.1	50.5
	control	60.1	56.5
Non operated		39.1	56.9

Three weeks after surgery we found a statistically significant difference between the left and right sensorimotor cortex of both the enhanced environment and the control groups. Mean values of BDNF protein within the left sensorimotor cortex of the enhanced environment group was 41.5% lower than within the left sensorimotor cortex of the control group ($^{*}p = 0.05$). We found a trend of lower mean values between the left and right sensorimotor cortex in the enhanced environment group three weeks after surgery. There was no statistic difference between the enhanced environment and the control group three months after surgery.

Moreover, we did not find a difference of BDNF protein values within the sensorimotor cortex between the left and right hemisphere.

Discussion

This study in rats has demonstrated that in peripheral nerve repair, environment enriched with activity stimulating factors results in improved functional recovery and in enlarged anterior tibial/peroneal muscle cross-section area which may be a result of training-induced plasticity processes. Dynamic differences of BDNF protein levels in the sensorimotor cortex over a 3-month-period may reflect activity-dependent brain plasticity.

In our study we showed that environment enriched with activity stimulating factors leads to statistically significantly superior functional recovery in BBB Locomotor rating scale, toe spread test, inclined plane test, and even in the placing tests. We hypothesize that a crucial cause may be plasticity changes in the central nervous system and that regeneration at the peripheral nerve level may not be stimulated. Electrophysiologic data which did not show significant differences in groups may indicate non-affected peripheral nerve regeneration. Contact placing, a sensitive test for cortical motor function [16], did not show significant differences but did show trend-wise better results in enriched group from weeks 7 to 11. Proprioceptive placing appears to be an essentially spinal response [16]. All animals in the enriched environment group showed proprioceptive placing three weeks after surgery, suggesting improved spinal sensorimotor circuits. The inclined plane test in the activity stimulated rats showed significantly better results until the 11^{th} week. One possible explanation for the lack of difference after the 11^{th} week might be the time-dependent increase of hind limb muscle force. Due to its superior sensitivity, the BBB Locomotor rating scale showed a statistically significant difference during this period. Hence, experi-

ence-and activity-dependent signals may adapt the circuitry in the sensorimotor module to body anatomy and biomechanics enhanced by activity stimulating factors. Nevertheless, one question arose: why did we find an additional effect on muscle cross-section area in the operated hind limb of the enriched environment group? In non-operated extremity, our results showed the effect of training due to the difference in cross-sectional muscle area between groups. Is it possible that in the enriched environment group training-induced brain plasticity and spinal plasticity processes influenced the cross-section area of tibial/peroneal muscle in the operated hind limb? Our functional results strongly indicate an effect of central nervous system plasticity on peripheral target organs such as muscle. Additionally, changes in the activity of motor neurons and endplates may in turn change the properties of such reinnervated muscles which we did not assess in this study.

It is well established that environmental enrichment can induce chemical and morphologic changes in the brain of experimental animals [4, 20, 45]. It is known that physical activity increases expression of some neurotrophins. BDNF improves efficiency in cortical hippocampal circuits [31]. BDNF mRNA is regulated by sensory input in the visual cortex [11] and mechanical stimulation of whiskers in mice upregulates BDNF mRNA expression in the somatosensory cortex [44]. In our study, BDNF protein levels in the sensorimotor cortex in an environment enriched with activity stimulating factors surprisingly showed a steady state one week and three weeks after surgery compared to a non-operated control. Three months after surgery, however, BDNF protein increased rapidly within the sensorimotor cortex in the enriched environment group showing similar values as the control group. One can hypothesize that within the sensorimotor cortex of the rat the dynamic reaction of BDNF protein plays a key role in supporting plasticity processes. In contrast to our expectations, we did not find a difference between the right and the left hemisphere. Several hypotheses on the reason for these findings would be possible. At this point, however, our knowledge is not extensive enough to enable an explanation.

In a recent study [41] the effects of repetitive motor training on movement representations in adult monkeys were assessed. They concluded that repetitive motor activity does not produce functional reorganization of cortical maps. Instead, they propose that motor skill acquisition, or motor learning, is a prerequisite factor in driving representational plasticity in M1. In our experiment rats were not adapted to running wheel locomotion preoperatively. Hence, motor learning occurred postoperatively. Our results transferred to a human setting may indicate that after peripheral nerve repair a very early motor learning rehabilitation program might enhance functional recovery hypothetically due to superior functional cortical reorganization.

From the therapeutic point of view, rehabilitation following injuries in the peripheral nervous system may have interesting analogy with injuries in the central nervous system, such as stroke. In both situations, functional recovery is greatly dependent on brain and spinal plasticity, and on cortical/spinal functional reorganization.

Conclusion

In a rat sciatic nerve transection and repair model, activity stimulating factors enhance functional recovery. Our results suggest that in peripheral nerve repair very early training programs may be beneficial, augmenting peripheral nerve repair procedures and stimulating brain and spinal plasticity processes.

References

1. Amassian VE (1948) The use of contact placing in analytical and synthetic studies of the higher sensorimotor control system. In: Asanuma H, Wilson VJ (eds) Integration in the nervous system. Igakv-Shoin, Tokyo, pp 279–304
2. Basso DM, Beattie MS, Bresnahan JC (1995) A sensitive and reliable locomotor rating scale for open field testing in rats. J Neurotrauma 12: 1–21
3. Bard P (1933) Studies on the cerebral cortex. I. Localized control of placing and hopping reactions in the cat and their normal management by small cortical remnants. Arch Neurol Psychiatry 30: 40–74
4. Bennett EL, Diamond MC, Krech D, Rosenzweig MR (1996) Chemical and anatomical plasticity of brain. 1964. J Neuropsychiatry Clin Neurosci 8: 459–470
5. Biernaskie J, Corbett D (2001) Enriched rehabilitative training promotes improved forelimb motor function and enhanced dendritic growth after focal ischemic injury. J Neurosci 21: 5272–5280
6. Bolton MM, Lo DC, Sherwood NT (2000) Long-term regulation of excitatory and inhibitory synaptic transmission in hippocampal cultures by brain-derived neurotrophic factor. Prog Brain Res 128: 203–218
7. Bregman BS, Goldberger ME (1983) Infant lesion effect: I. Development of motor behavior following neonatal spinal cord damage in cats. Brain Res 285: 103–117
8. Bregman BS, Goldberger ME (1983) Infant lesion effect: II. Sparing and recovery of function after spinal cord damage in newborn and adult cats. Brain Res 285: 119–135
9. Brock TO, O'Callaghan JP (1987) Quantitative changes in the synaptic vesicle proteins synapsin I and p38 and the astrocyte-specific protein glial fibrillary acidic protein are associated with chemical-induced injury to the rat central nervous system. J Neurosci 7: 931–942
10. Buonomano DV, Merzenich MM (1998) Cortical plasticity: from synapses to maps. Annu Rev Neurosci 21: 149–186

11. Castren E, Zafra F, Thoenen H, Lindholm D (1992) Light regulates expression of brain-derived neurotrophic factor mRNA in rat visual cortex. Proc Natl Acad Sci USA 89: 9444–9448
12. Chen R, Cohen LG, Hallett M (2002) Nervous system reorganization following injury. Neuroscience 111: 761–773
13. Ebner FF, Rema V, Sachdev R, Symons FJ (1997) Activity-dependent palsticity in adult somatic sensory cortex. Semin Neurosci 9: 47–58
14. Elbert T, Pantev C, Wienbruch C, Rockstroh B, Taub E (1995) Increased cortical representation of the fingers of the left hand in string players. Science 270: 305–307
15. Gale K, Kerasidis H, Wrathall JR (1985) Spinal cord contusion in the rat: behavioral analysis of functional neurologic impairment. Exp Neurol 88: 123–134
16. Goldberger ME, Bregman BS, Vierck CJ Jr, Brown M (1990) Criteria for assessing recovery of function after spinal cord injury: behavioral methods. Exp Neurol 107: 113–117
17. Hall RD, Lindholm EP (1974) Organization of motor and somatosensory neocortex in the albino rat. Brain Res 66: 23–38
18. Hallett M (2001) Plasticity of the human motor cortex and recovery from stroke. Brain Res Rev 36: 169–174
19. Hickmott PW, Merzenich MM (2002) Local circuit properties underlying cortical reorganization. J Neurophysiol 88: 1288–1301
20. Holloway RL Jr (1966) Dendritic branching: some preliminary results of training and complexity in rat visual cortex. Brain Res 2: 393–396
21. Jenkins WM, Merzenich MM (1987) Reorganization of neocortical representations after brain injury: a neurophysiological model of the bases of recovery from stroke. Prog Brain Res 71: 249–266
22. Johansson BB (2000) Brain plasticity and stroke rehabilitation. The Willis lecture. Stroke 31: 223–230
23. Johansson BB, Grabowski M (1994) Functional recovery after brain infarction: plasticity and neural transplantation. Brain Pathol 4: 85–95
24. Johansson BB, Ohlsson AL (1996) Environment, social interaction, and physical activity as determinants of functional outcome after cerebral infarction in the rat. Exp Neurol 139: 322–327
25. Johansson BB, Belichenko PV (2001) Environmental influence on neuronal and dendritic spine plasticity after permanent focal brain ischemia. In: Ito U, Bazan NG, Ito U, Marcheselli VL, Kuroiwa T, Klatzo I (eds) Maturation phenomenon in cerebral ischemia IV. Springer, Berlin Heidelberg, pp 77–83
26. Jones TA, Schallert T (1994) Use-dependent growth of pyramidal neurons after neocortical damage. J Neurosci 14: 2140–2152
27. Jones TA, Kleim JA, Greenough WT (1996) Synaptogenesis and dendritic growth in the cortex opposite unilateral sensorimotor cortex damage in adult rats: a quantitative electron microscopic examination. Brain Res 733: 142–148
28. Jovanovic JN, Benfenati F, Siow YL, Sihra TS, Sanghera JS, Pelech SL, Greengard P, Czernik AJ (1996) Neurotrophins stimulate phosphorylation of synapsin I by MAP kinase and regulate synapsin I-actin interactions. Proc Natl Acad Sci USA 93: 3679–3683
29. Kafitz KW, Rose CR, Thoenen H, Konnerth A (1999) Neurotrophin-evoked rapid excitation through TrkB receptors. Nature 401: 918–921
30. Kang H, Schuman EM (1996) A requirement for local protein synthesis in neurotrophin-induced hippocampal synaptic plasticity. Science 273: 1402–1406
31. Kang H, Schuman EM (1995) Long-lasting neurotrophin-induced enhancement of synaptic transmission in the adult hippocampus. Science 267: 1658–1662
32. Klintsova AY, Dickson E, Yoshida R, Greenough WT (2004) Altered expression of BDNF and its high-affinity receptor TrkB in response to complex motor learning and moderate exercise. Brain Res 1028: 92–104
33. Korte M, Carroll P, Wolf E, Brem G, Thoenen H, Bonhoeffer T (1995) Hippocampal long-term potentiation is impaired in mice lacking brain-derived neurotrophic factor. Proc Natl Acad Sci USA 92: 8856–8860
34. Levine ES, Crozier RA, Black IB, Plummer MR (1998) Brain-derived neurotrophic factor modulates hippocampal synaptic transmission by increasing N-methyl-D-aspartic acid receptor activity. Proc Natl Acad Sci USA 95: 10235–10239
35. Linnarsson S, Bjorklund A, Ernfors P (1997) Learning deficit in BDNF mutant mice. Eur J Neurosci 9: 2581–2587
36. Murphy MP, Rick JT, Milgram NW, Ivy GO (1995) A simple and rapid test of sensorimotor function in the aged rat. Neurobiol Learn Mem 64: 181–186
37. Nudo RJ, Wise BM, SiFuentes F, Milliken GW (1996) Neural substrates for the effects of rehabilitative training on motor recovery after ischemic infarct. Science 272: 1791–1794
38. Nudo RJ (1999) Recovery after damage to motor cortical areas. Curr Opin Neurobiol 9: 740–747
39. Patterson SL, Abel T, Deuel TA, Martin KC, Rose JC, Kandel ER (1996) Recombinant BDNF rescues deficits in basal synaptic transmission and hippocampal LTP in BDNF knockout mice. Neuron 16: 1137–1145
40. Pascual-Leone A, Torres F (1993) Plasticity of the sensorimotor cortex representation of the reading finger in Braille readers. Brain 116 (Pt 1): 39–52
41. Plautz EJ, Milliken GW, Nudo RJ (2000) Effects of repetitive motor training on movement representations in adult squirrel monkeys: role of use versus learning. Neurobiol Learn Mem 74: 27–55
42. Pons TP, Garraghty PE, Mishkin M (1988) Lesion-induced plasticity in the second somatosensory cortex of adult macaques. Proc Natl Acad Sci USA 85: 5279–5281
43. Puurunen K, Jolkkonen J, Sirvio J, Haapalinna A, Sivenius J (2001) Selegiline combined with enriched-environment housing attenuates spatial learning deficits following focal cerebral ischemia in rats. Exp Neurol 167: 348–355
44. Rocamora N, Welker E, Pascual M, Soriano E (1996) Upregulation of BDNF mRNA expression in the barrel cortex of adult mice after sensory stimulation. J Neurosci 16: 4411–4419
45. Rosenzweig MR (1984) Experience, memory, and the brain. Am Psychol 39: 365–376
46. Schiaveto DS, da Silva CA, Del Bel EA (2004) Methodological evaluation to analyze functional recovery after sciatic nerve injury. J Neurotrauma 21: 627–635
47. Schmidhammer R, Zandieh S, Hopf R, Mizner I, Pelinka LE, Kroepfl A, Redl H (2004) Alleviated tension at the repair site enhances functional regeneration: the effect of full range of motion mobilization on the regeneration of peripheral nerves – histologic, electrophysiologic, and functional results in a rat model. J Trauma 56: 571–584
48. Schouenborg J (2004) Learning in sensorimotor circuits. Curr Opin Neurobiol 14: 693–697
49. Sherwood NT, Lo DC (1999) Long-term enhancement of central synaptic transmission by chronic brain-derived neurotrophic factor treatment. J Neurosci 19: 7025–7036
50. Sugaya K, Chouinard M, Greene R, Robbins M, Personett D, Kent C, Gallagher M, McKinney M (1996) Molecular indices of neuronal and glial plasticity in the hippocampal formation in a rodent model of age-induced spatial learning impairment. J Neurosci 16: 3427–3443
51. Svoboda K, Denk W, Kleinfeld D, Tank DW (1997) In vivo dendritic calcium dynamics in neocortical pyramidal neurons. Nature 385: 161–165

52. Tyler WJ, Pozzo-Miller LD (2001) BDNF enhances quantal neurotransmitter release and increases the number of docked vesicles at the active zones of hippocampal excitatory synapses. J Neurosci 21: 4249–4258
53. Volkmar FR, Greenough WT (1972) Rearing complexity affects branching of dendrites in the visual cortex of the rat. Science 176: 1445–1447
54. Wang T, Xie K, Lu B (1995) Neurotrophins promote maturation of developing neuromuscular synapses. J Neurosci 15: 4796–4805
55. Weiller C, Ramsay SC, Wise RJ, Friston KJ, Frackowiak RS (1993) Individual patterns of functional reorganization in the human cerebral cortex after capsular infarction. Ann Neurol 33: 181–189
56. Weiller C (1998) Imaging recovery from stroke. Exp Brain Res 123: 13–17
57. Xerri C, Merzenich MM, Peterson BE, Jenkins W (1998) Plasticity of primary somatosensory cortex paralleling sensorimotor skill recovery from stroke in adult monkeys. J Neurophysiol 79: 2119–2148
58. Yonemori F, Yamaguchi T, Yamada H, Tamura A (1998) Evaluation of a motor deficit after chronic focal cerebral ischemia in rats. J Cereb Blood Flow Metab 18: 1099–1106

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Cognitive re-education and early functional mobilisation in hand therapy after bilateral hand transplantation and heterotopic hand replantation – two case reports

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Summary

The main challenge for a successful hand therapy after heterotopic hand replantation is the reeducation of patients' sensory and motor perception. The case of a 28-year-old patient is described. After resection of a tumour and amputation of the elbow, tendons of the hand had to be joined to only three muscles of the upper arms. Elbow extension and flexion had to be trained to control the wrist, fingers, and thumb movements.

In a similar way, the main focus after ortotopic hand transplantation lies on retraining the wrist, finger, and thumb functions. This is illustrated by a second case of a patient who had lived for 5 years with myoelectric protheses on both lower arms and had forgotten these functions.

The final aim in both cases was regaining of daily living and working skills. The therapy was started with fitting supporting thermoplastic splints. Early motioned passive and passive-assistive active mobilisation prevented tendons adherences and initiated hand-functions. An intense sensory remaining programme and cognitive therapeutic exercises ensured the sensory and motoric activation of the referring cortical hand areals. At conclusion of therapy it can be said that both patients have fully taken up their professional duties again and that they are able to manage successfully their activities of daily living on their own.

Keywords: Cognitive re-education; functional mobilisation; hand transplantation; cortex.

Introduction

In 1994, a 47-year-old policeman lost both his hands by a letter bomb explosion. He wore myoelectric prostheses until he underwent a bilateral hand transplantation 5 years later in March 2000 at the Medical University Innsbruck (Surgical and allied health care teams involved: Department of Plastic and Reconstructive Surgery: H. Piza-Katzer/H. Hussl/M. Ninkovic; Department of Trauma Surgery: S. Pechlaner/M. Gabl; Department of Anaesthesia: R. Kornberger/M. Hollenstein-Zacke/A. Kuen/B. Leitner; Department of Transplantation Surgery: R. Margreiter/St. Schneeberger, Department of Rehabilitation: M. Ninkovic/C. Kaiser/ M. Barbach; Occupational therapy: D. Estermann).

For 2 years the patient had received 6 h of physio- and occupational therapy five days a week at the Medical University Innsbruck, which was continued thereafter in his home town, but less intensively. The initial step in the hand therapy rehabilitation programme was splinting of both hands, which was followed by passive and active mobilisation, electrical stimulation, sensory reeducation and proprioperceptional training according to Perfetti. Adaptive devices to facilitate activities of daily living were provided and adjusted to his special needs. Strength, sensitivity and functional developments in the hands were measured at regular intervals by a dynamometer, pinchmeter, goniometer, two-point--discriminator and sensimapping.

To prevent oedema as well as contractures in the wrist and finger joints, the first splint placement was in ''intrinsic plus position'' (90° flexion in MCPs and 0° extension in PIP and DIP joints). These splints were removed during passive mobilisation as well as early functional active mobilisation. After six weeks the patient was fitted with a wrist splint placing the thumbs in opposition, which allowed active finger movements of digits II–V and permitted active tip-to-tip grip of the index and middle finger with the thumb.

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A documentation of sensimapping shows the progress of sensibility in both hands which progressed to half way up the palm in the 12th postoperative week, advancing to the middle joints in the 24th week and to the finger tips in the 39th week.

To facilitate activities of daily living, initially a softball with an elastic ribbon was attached to a fork to enable the patient to eat by himself until active flexion of his index finger enabled him to hold cutlery without grip adaptations. As the thumb opposition had not been strong enough to turn the key in the lock of his house for two years, the patient received a special interdigital key tool which enabled him to lock and unlock doors by himself.

For writing by hand with prostheses (with which the patient had initially been outfitted with) he had to lift up the forearm from the table as the protheses had only a flexion and extension function with the 1–3 finger. After his ''new right hand'' was trained with a writing programme, he was able to hold a pen and write from the 6th postoperative month on. After five postoperative years, he was able to hold a pen and write in a physically correct way, fluently, with forearm supination, wrist extension and opposition of the thumb, holding the pen between the thumb and the tip of the index finger.

The patient has now a desk job in a police department, for which he underwent training in handling a telephone as well as working with a computer. He can use the keyboard and the mouse of the computer with an isolated index finger movement (Fig. 1). He has been taking piano lessons for the past two years.

Five years after bilateral transplantation, the patient can actively make a complete fist with his right hand, whereas in the left hand there is a tip-to-palm distance of 2 cm. This is because the patient had two fractures of the left forearm – which happened when he had been riding a motorbike through South America. The patient started to ride again his motorbike one and a half years after the transplantation.

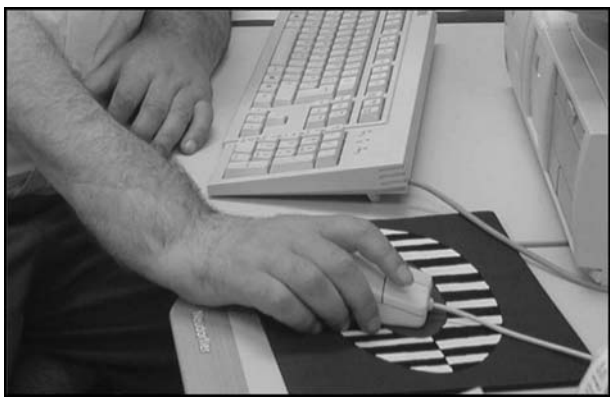

Fig. 1. 52-year-old man 5 years after transplantation of both hands. He can use the keyboard and the mouse of a computer and works in a police department

The grip strength measured with a dynamometer is 17.2 kg in his right hand vs. 8 kg in his left hand. The pinch grip of his right thumb shows 1.2 kg and the left thumb 2.1 kg. The sensitivity controlled with a two-point discriminator is between 10–15 mm in all finger nerves. Temperature discrimination between warm and cold and tactile discrimination of materials with different surfaces is fully present.

The advantages of the bilateral hand transplantation are that the patient is independent in activities of daily living, the sense of touch has been restored and he can gesture with his hands when he is speaking. However, there are risks such as the danger of tumour development associated with life-long needed intake of immunosuppressant medications to prevent rejection of the transplanted hands.

The second case report is on a 28-year-old farmer who developed an epithelioid sarcoma in his left forearm that was diagnosed too late. The patient refused the options of either an upperarm amputation or irradiation of the forearm and chemotherapy. Professor H. Piza-Katzer offered this patient the possibility of resecting the tumour together with the forearm and elbow and of replanting his left hand onto his left upper arm, as she had performed this kind of operation once already 16 years ago in a very similar epithelioid sarcoma case. The patient gave his consent to this surgical intervention.

The heterotopic replantation took place in August 2004. After resection of the tumour and amputation of the elbow and tumor region, twenty tendons of the hand had to be joined to only three muscles of the upper arm (m. biceps, m. triceps and m. brachialis). Therefore, it was necessary to mark each nerve and tendon of the distal forearm precisely. The m. biceps and m. brachialis were split in 11 parts and all flexor tendons were interwoven into m. biceps and brachialis. All extensor tendons were interwoven into m. triceps. The ostheosyntheses of the humerus with radius and ulna was accomplished by a plate.

We believe that there are three main factors that contributed to the functional success of this operation: first of all, the excellent motivation and cooperation of the patient was of great help. Secondly, the short anoxemia time, the innovative surgical technique involving careful splitting of the muscles of the upper arm with which the tendons of the forearm muscles were interwoven, careful

coaptation of nerves of different diameters and osteosyntheses of bones with plates represent the surgical factors towards functional gains. Finally, intensive postoperative therapy twice daily, being fitted initially with thermoplastic intrinsic-plus and later on with opponens splints, very early functional mobilisation (which started already on the 3rd postoperative day), regular sensory re-educational training, electrical stimulation (which the patient carried on by himself at home) and bilateral performance of daily living activities are the postoperative care and training programmes that no doubt helped the patient to successfully employ his heterotopically replanted hand.

Before the operation we had no certainty how the cortical reorganisation would develop. Would it be possible to gain a fluency in finger movements? Would an isolated finger function be possible? How would the sensibility and strength develop? What would happen after the amputation with the elbow area in the cortex which had been active for 28 years?

Therapy was started on the 3rd postoperative day in a bilateral way: the patient flexed his right elbow by using his m. biceps and in his imagination also flexed his left elbow – which had been resected – to gain a feeling of how to flex the fingers of his left hand. The same process was repeated with the m. triceps for extending his fingers of the left hand. When the patient was familiar with this exercise, we concentrated on the left hand alone. After three weeks of intensive therapy, the patient reported that he could not remember what it was like to have had an elbow in his left arm.

Part of the occupational therapy was also training in activities of daily living. As the patient has to drive his car a lot, we trained him to hold and steer a steering wheel which was fixed to the therapy table. A very intensive sensory re-educational training was performed with different materials such as brushes and pieces of cloth with different textures. For the functional key and tip-to-tip grip training, we used many functional games (like card and dice games, etc.).

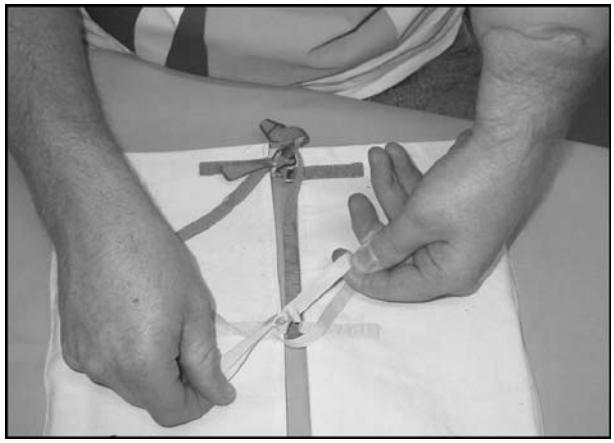

Fig. 2. 28-year-old man 15 months after a heterotopic replantation of the left distal lower arm and hand to the upperarm. He was trained in activities of daily living

Fifteen months after the operation the patient's left hand is strong enough to hold the milking machine, climb a ladder, carry his suitcase and manage all activities of daily living by himself. He can once again take up his favourite hobby of cross-country skiing – he just needs a longer left ski pole because of his shortened left arm.

The strength measured with a dynamometer is 12.5 kg in the left hand vs. 52.5 kg in his right hand. The key grip measured with a pinchmeter shows 3.4 kg in his left thumb vs. 12.8 kg in his right thumb. The patient is able to discriminate between different surfaces as well as heat and cold with all fingers in the replanted hand. Two-point discrimination is between 9 and 14 mm, and there is protective sensitivity in finger nerve 6.

In the replanted hand, active finger flexion to form a complete and firm fist is fluent; finger extension is possible up to 0°. The left wrist can be flexed and extended in an isolated fashion. With the well regenerated opponens function, the patient is able to perform a correct tip-to-tip grip with his left thumb and index finger (Fig. 2), which enables him for instance to unbutton the small right sleeve buttons.

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Functional, morphological and biomolecular assessment of posttraumatic neuro-muscular recovery in the rat forelimb model

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Summary

Over the last five years, we have used the rat forelimb model for investigating neuromuscular recovery after microsurgical nerve reconstruction of median and ulnar nerves by end-to-side neurorrhaphy and muscle-vein-combined tubulization (using both straight and Y-shaped guides). The outcome of nerve repair at different postoperative times was assessed by functional, morphological and biomolecular analysis. Results showed that both end-to-side and tubulization repair of rat median and ulnar nerves led to successful axonal regeneration along the severed nerve trunk as well as to a partial recovery of the lost function as assessed by grasping test. Biomolecular analysis by means of reverse transcription polymerase chain reaction (RT-PCR) demonstrated early overexpression during nerve regeneration of the gliotrophic factor NRG1 and two of its receptors: erbB2 and erbB3. Finally, our experience also suggests that the rat forelimb experimental model is particularly appropriate for the study of microsurgical reconstruction of major mixed nerve trunks. Furthermore, since the forelimb model is less compromising for the animal, it should be preferred to the hindlimb model for many research purposes.

Keywords: Peripheral nerve regeneration; Schwann cells; neuregulin; erbB receptors; muscle denervation; functional recovery; rat.

Introduction

Although in the last few years much progress in the microsurgical repair of peripheral nerves has been made, research in this field is still very active and new approaches for nerve reconstruction are currently being investigated in many laboratories worldwide [9, 10, 22]. In particular, recent scientific advancements have focused on two surgical strategies for peripheral nerve repair: end-to-side neurorrhaphy [6, 11] and tubulization [2, 19].

While both surgical approaches have been known since the nineteen century [for historical review see [6 and 17], submitted for publication to Microsurgery], the interest in their clinical employment have increased over the last years [2, 6] because of the advancements in the surgical materials and techniques, as well as the contextual progress in the scientific knowledge on the basic mechanisms underlying peripheral nerve fiber regeneration. However, in spite of the many studies published on the employment of these techniques, the results are still conflicting and thus more experimental research is required for elucidating several unanswered biological questions that still exist regarding both end-to-side and tubulization nerve repair and, eventually, to optimize the future clinical employment of these two surgical strategies.

Over the last five years, we have used the rat forelimb model to investigate several aspects of nerve regeneration after microsurgical reconstruction of median and ulnar nerves. Both end-to-side neurorrhaphy and muscle-vein-combined tubulization (using both straight and Y-shaped guides) surgical techniques were employed for nerve reconstruction. The outcome of nerve regeneration at different postoperative times was assessed by functional, morphological and biomolecular analysis. Finally, careful animal surveillance was adopted in order to assess the impact of forelimb experimental nerve surgery on animal well-being.

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Methods and materials

Seventy-two Wistar adult female rats weighting 250–300 g were used in this study. The surgical procedures for nerve reconstruction were carried out in part at the Laboratory of Microsurgery of the Ecole de Chirurgie de Paris and in part at the Department of Animal and Human Biology of the University of Torino. Approval for these experiments was obtained from the local Institution's Animal Care and Ethics Committee.

Under deep anaesthesia, the median nerve of the left forelimb was approached from the axillary region and, under operative microscope, carefully exposed and transected removing a 1-cm long nerve segment. The severed median nerve was then repaired with different techniques after having twisted its proximal stump and sutured it to the pectoral muscle to prevent spontaneous axonal regeneration.

In the first group of experiments, end-to-side neurorrhaphy on the intact ulnar nerve was carried out after opening a perineurial window.

In a second group of experiments, tubulization nerve repair by straight muscle-vein-combined nerve guides was compared to nerve repair by autologous nerve grafting using either the removed median nerve segment turned of 180° or 2–3 segments of sural nerve withdrawn from the left hindlimb.

In a third group of experiments Y-shaped muscle-vein-combined guides were used to repair both median and ulnar nerves using only one of the two nerves as the proximal source of axons.

Postoperative quantitative functional evaluation of median nerve recovery was made by means of the grasping test [14] that assesses the action of the extrinsic flexor digitorum muscles (m. *flexor digitorum sublimis* and m. *flexor digitorum profundus*) that, in the rat, are innervated by the median nerve only.

Animals were sacrificed at different post-operative times and the repaired nerves were withdrawn. The *m. flexor digitorum sublimis* and the *m. flexor digitorum profundus* (that, in the rat, are innervated by the median nerve only) were also withdrawn and then immediately weighted to assess muscle trophism.

For histological analysis, nerve samples were processed with routine techniques for light, confocal and electron microscopy [18].

Finally, for reverse transcription polymerase chain reaction (RT-PCR) analysis, the samples were processed as described before [12, 18]. Oligonucleotide primers were designed in order to recognize mRNA coding for GFAP (glial fibrillar acid protein), S100, erbB2, erbB3 and NRG1 α2-β1.

Statistical analysis was performed using a one-way analysis of variance (ANOVA) test and statistical significance was established as $p < 0.05$. All statistical tests were performed using the software "Statistica per discipline bio-mediche" (McGraw-Hill, Milano, Italia).

Finally, since the assessment of animal welfare was one of our objectives in order to comparatively assess the forelimb with the hindlimb experimental model, a careful postoperative animal surveillance was adopted to check for passive and active movements, auto-mutilation, skin ulcers, and joint contracture, especially during early post-operative days. In addition, the distress caused by carrying out the grasping test was carefully evaluated during testing sessions.

Results

End-to-side nerve repair

Light, confocal and electron microscope analysis showed that nerve fiber regeneration along the repaired median nerve occurred in all operated animals. Postoperative

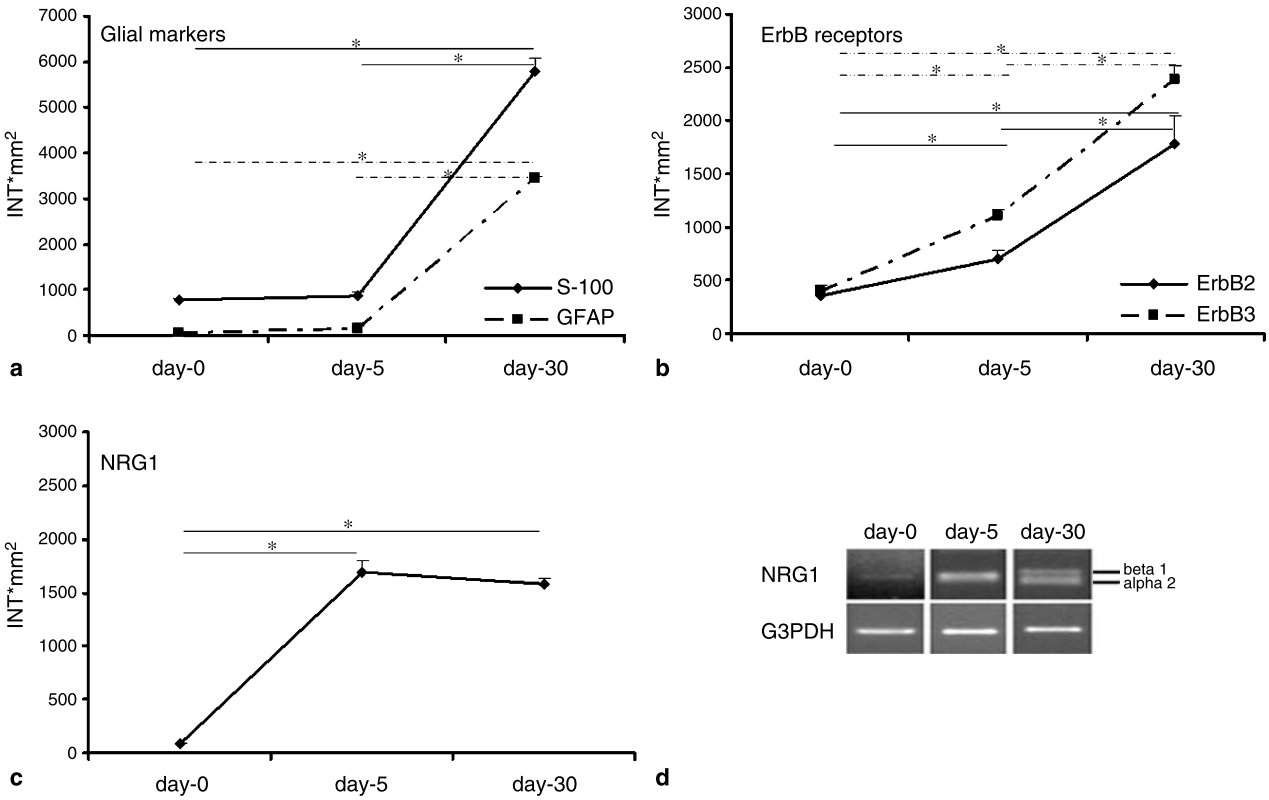

Fig. 1. Time course RT-PCR analysis of glial markers (a) erbB2, erbB3 (b) and NRG1 (c) transcripts from RNA extracts of fresh muscle-vein-combined nerve guides. Statistical significances are indicated by asterisks ($^*p < 0.05$). NRG1 mRNA, at day 5 postoperative shows only the α2 isoform while at day 30 both α2 and β1 isoforms are detected (d)

functional assessment showed that voluntary motor control of the extrinsic digitorum muscles innervated by the median nerve dropped to zero after transection and begun to recover 5 months after surgery and, after 6 months, reached a value corresponding to $22.8 \pm 15.4\%$ of the pre-operative performance. Muscle mass, which dropped to $52.6 \pm 4.3\%$ of its normal weight in chronically denervated muscles, in the nerve repair group was on average $84.8 \pm 12.3\%$ of the normal at time of withdrawal.

Muscle-vein-combined nerve repair

Light, confocal and electron microscope analysis showed that nerve fiber regeneration along the repaired median nerve occurred in all operated animals. Functional testing by the grasping test showed that recovery of median nerve function had already begun 2 months after surgery and reached, on average, $55.2 \pm 9.5\%$ of the normal by month 6 post-operatively. Statistical analysis showed that recovery of voluntary motor function was significantly ($p < 0.05$) higher than with end-to-side neurorrhaphy ($22.8 \pm 15.4\%$), but significantly ($p < 0.05$) lower than with autograft nerve repair by reversed median nerve segment ($75.1 \pm 13.8\%$) and with autologous sural nerve segments ($76.8 \pm 11.2\%$).

Biomolecular analysis by RT-PCR (Fig. 1) showed that glial markers (GFAP and S100), NRG1 and its receptors erbB2 and erbB3 were significantly up-regulated over the first post-operative month after muscle-vein-combined nerve repair. Interestingly, while at postoperative day 5 only the $\alpha 2$ isoform of NRG1 was detectable, at day 30 also the presence of the $\beta 1$ isoform of NRG1 was clearly detectable (Fig. 1d).

Y-shaped muscle-vein-combined nerve repair

Histological analysis (Fig. 2) carried out ten months after surgery showed that nerve fiber regeneration occurred in both median and ulnar nerves repaired by Y-shaped muscle-vein-combined conduits, irrespectively of the use of the median (Fig. 2a,b) or ulnar (Fig. 2c,d) nerve as proximal ''donor'' nerve stump.

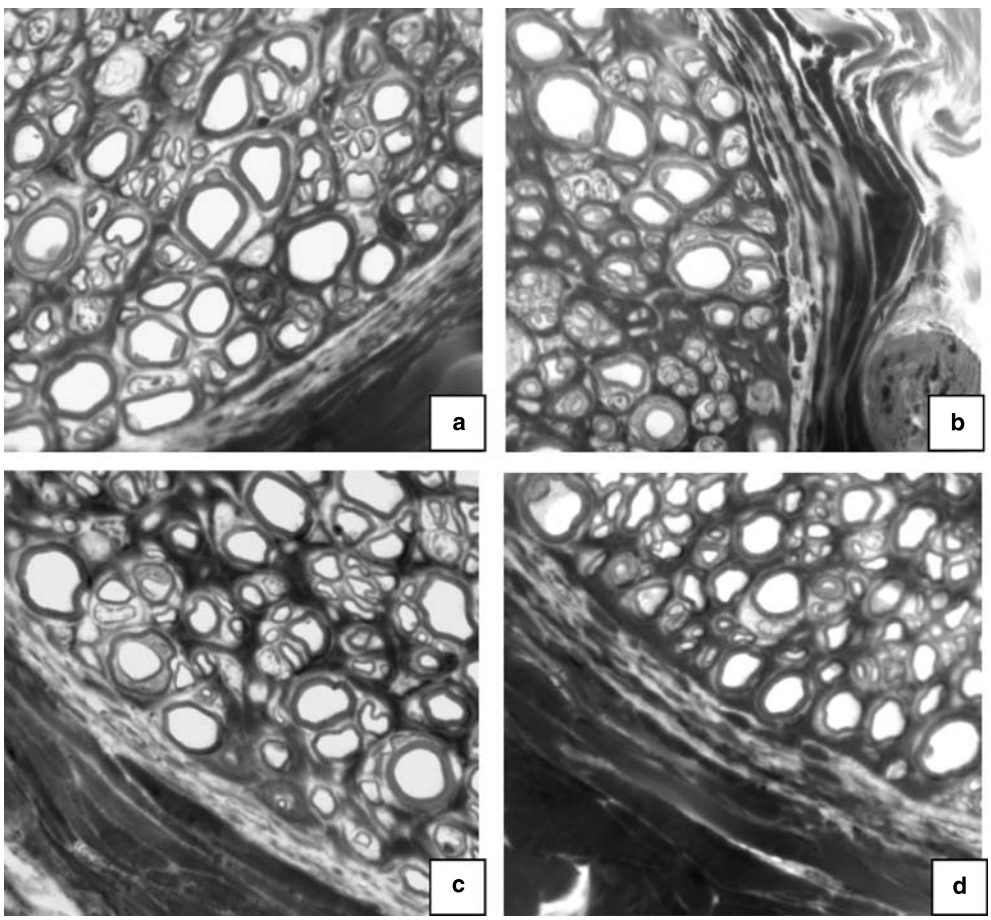

Fig. 2. Photomicrographs of transverse semi-thin sections of median and ulnar nerves repaired by Y-shaped muscle-vein-combined conduits and withdrawn at month-10 postoperative. (a, b) The proximal stump of the median nerve was used as the ''donor'' nerve stump. (c, d) The proximal stump of the ulnar nerve was used as the ''donor'' nerve stump. Toluidine blue staining. Magnification = 1,000 ×

Quantitative evaluation of the recovery of both muscle trophism (assessed by measuring muscle mass) and function (measured by the grasping test), at month-10 post-operative, showed that both parameters were significantly ($p < 0.05$) lower when the ulnar nerve was used as the proximal nerve stump (70.8 ± 4.1 and $52.7 \pm 6.3\%$ of normal values respectively) in comparison to the experimental groups in which the median nerve stump was sutured to the proximal limb of the Y-shaped conduit (85.2 ± 4.9 and $67.7 \pm 10.2\%$ of normal values respectively).

Assessment of animal well-being

Observations of post-operative consequences of forelimb's nerve lesions on animal well-being demonstrated that contemporary median and ulnar nerve transection did not cause any detectable distress to the rats, even in the earlier postoperative stages. In addition, the employment of the forepaws to lean on the ground during walking produced a continuous movement of the joints that avoided the occurrence of retractions. Finally, auto-mutilation was never observed in our experiments.

Discussion

Experimental research on neuromuscular recovery has grown significantly over the last few years and, in particular, increased attention has been given to the study of nerve regeneration and motor recovery after microsurgical nerve reconstruction because of the high incidence of nerve injuries [4, 13]. In a recent series of experiments, we have used the rat forelimb model to investigate neuromuscular recovery after nerve reconstruction by means of two different microsurgical techniques: end-to-side neurorrhaphy [6] and muscle-vein-combined tubulization [2].

The investigation of end-to-side nerve repair, performed between the transected median nerve and the epineurium of the ulnar ''donor'' nerve, showed that this surgical strategy not only leads to axonal regeneration and muscle reinnervation but also permits the partial restoration of voluntary control of the motor function lost after nerve damage [15]. Interestingly, when end-to-side nerve repair of the median nerve on the ulnar ''donor'' nerve was followed by low-level laser-therapy [1, 8], a faster and higher recovery of the voluntary control of motor function was observed [7].

Results of nerve repair by means of muscle-vein-combined guides showed that also this particular type of tubulization permits the partial restoration of voluntary control of the motor function lost after nerve damage, although slower in comparison to autograft nerve repair. In addition, results of the biomolecular investigation suggested that the early activation of the NRG1/ErbB system [12, 18] could partially explain the good results obtained with the muscle-vein-combined nerve repair technique [2].

Particularly interesting are the results of Y-shaped muscle-vein-combined conduits [21], which showed that it is possible, in the rat, to contemporarily repair the distal nerve stumps of two severed nerves using only one single proximal stump and that this surgical strategy will lead to the partial recovery of voluntary control of the motor function even when the proximal ''donor'' stump is not the same nerve trunk.

Finally, from a methodological point of view, our experience suggests that the rat forelimb experimental model is particularly suitable for the study of microsurgical reconstruction of major mixed nerve trunks [3, 14, 16, 20]. Since this experimental procedure is less invalidating for the animal and the results obtained are more likely to be translated to the clinical practice, therefore the rat forelimb model should be preferred to the hindlimb model for many research purposes in the experimental study of posttraumatic neuromuscular recovery.

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References

1. Anders JJ, Geuna S, Rochkind S (2004) Phototherapy promotes regeneration and functional recovery of injured peripheral nerve. Neurol Res 26: 233–239
2. Battiston B, Geuna S, Ferrero M, Tos P (2005) Nerve repair by means of tubulization: literature review and personal clinical experience comparing biological and synthetic conduits for sensory nerve repair. Microsurg 25: 258–267
3. Bontioti E, Kanje M, Lundborg G, Dahlin LB (2005) End-to-side nerve repair in the upper extremity of rat. J Periph Nerv Syst 10: 58–68
4. Evans GR (2001) Peripheral nerve injury: a review and approach to tissue engineered constructs. Anat Rec 263: 396–404
5. Fields RD, Le Beau JM, Longo FM, Ellisman MH (1989) Nerve regeneration through artificial tubular implants. Prog Neurobiol 33: 87–134

6. Geuna S, Papalia I, Tos P (2006) End-to-side (terminolateral) nerve regeneration: a challenge for neuroscientists coming from an intriguing nerve repair concept. Brain Res Rev 52: 381–388
7. Gigo-Benato D, Geuna S, de Castro Rodrigues A, Tos P, Fornaro M, Boux E, Battiston B, Giacobini-Robecchi MG (2004) Low-power laser biostimulation enhances nerve repair after end-to-side neurorrhaphy: a double-blind randomized study in the rat median nerve model. Lasers Med Sci 19: 57–65
8. Gigo-Benato D, Geuna S, Rochkind S (2005) Phototherapy for enhancing peripheral nerve repair: a review of the literature. Muscle Nerve 31: 694–701
9. Hall S (2005) The response to injury in the peripheral nervous system. J Bone Joint Surg 87B: 1309–1319
10. Lundborg G (2005) Nerve injury and repair. Churchill Livingstone, Edinburgh
11. Millesi H, Tsolakidis S (2005) End-to-side coaptation: An important tool in peripheral nerve surgery. Eur Surg 37: 228–233
12. Nicolino S, Raimondo S, Tos P, Battiston B, Fornaro M, Geuna S, Perroteau I (2003) Expression of alpha2a-2b neuregulin-1 is associated with early peripheral nerve repair along muscle-enriched tubes. Neuroreport 14: 1541–1545
13. Noble J, Munro CA, Prasad VS, Midha R (1998) Analysis of upper and lower extremity peripheral nerve injuries in a population of patients with multiple injuries. J Trauma 45: 116–122
14. Papalia I, Tos P, Stagno d Alcontres F, Battiston B, Geuna S (2003) On the use of the grasping test in the rat median nerve model: a reappraisal of its efficacy for quantitative assessment of motor function recovery. J Neurosci Methods 127: 43–47
15. Papalia I, Geuna S, Tos P, Boux E, Battiston B, Stagno D'Alcontres F (2003) Morphologic and functional study of rat median nerve repair by terminolateral neurorrhaphy of the ulnar nerve. J Reconstr Microsurg 19: 257–264
16. Papalia I, Tos P, Scevola A, Raimondo S, Geuna S (2006) The ulnar test: A method for the quantitative functional assessment of posttraumatic ulnar nerve recovery in the rat. J Neurosci Meth 154: 198–203
17. Papalia I, Geuna S, D'Alcontres FS, Tos P (2007) Origin and history of end-to-side neurorrhaphy. Microsurgery 27: 56–61
18. Raimondo S, Nicolino S, Tos P, Battiston B, Giacobini-Robecchi MG, Perroteau I, Geuna S (2005) Schwann cell behavior after nerve repair by means of tissue-engineered muscle-vein combined guides. J Comp Neurol 489: 249–459
19. Schmidt CE, Leach JB (2003) Neural tissue engineering: strategies for repair and regeneration. Annu Rev Biomed Eng 5: 293–347
20. Sinis N, Schaller HE, Becker ST, Lanaras T, Schulte-Eversum C, Muller HW, Vonthein R, Rosner H, Haerle M (2006) Cross-chest median nerve transfer: a new model for the evaluation of nerve regeneration across a 40 mm gap in the rat. J Neurosci Meth 156: 166–172
21. Tos P, Calcagni M, Gigo-Benato D, Boux E, Geuna S, Battiston B (2004) Use of muscle-vein-combined Y-chambers for repair of multiple nerve lesions: experimental results. Microsurg 24: 459–464
22. Weber RV, Mackinnon SE (2005) Bridging the neural gap. Clin Plast Surg 32: 605–616

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Surgery on muscles in consequence of peripheral nerve lesions

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Summary

In peripheral nerve surgery main attention is frequently paid to surgery on the nerves, and possibilities to increase the chances of useful recovery by surgery on the muscles are often overlooked. Overstretching of paralyzed muscles reduces the chances of useful recovery even if the nerve regenerates well. The same is true if the paralyzed muscles have to work against an overwhelming force of antagonists. The weak force of a regenerated muscle can significantly be improved by synergistic muscle transfer. Different possibilities in this regard are demonstrated by typical cases.

Keywords: Muscle regeneration; nerve regeneration; function; muscle transfer.

Introduction

Palliative surgery to replace the lost function in case of an irreparable nerve lesion is performed because peripheral nerve lesions are treated surgically. A great number of methods have been developed; some of them have become classical. The characteristic feature is that muscles of a normal nerve are transposed on tendons of paralyzed muscles to transfer the power of the normal muscle to the tendon of the paralyzed muscle in order to imitate its function. Classical examples are tendon transfer in radial nerve palsy, different techniques to deal with claw hand deformity in ulnar nerve lesions, and restoration of opposition in median nerve lesions. These techniques needed special rehabilitation to teach the patient to innervate another muscle to do the function as was done with other muscles before the injury. This process was facilitated by the fact that muscles with synergistic functions were used to restore the lacking ones. A typical example is the transfer of wrist extensors for finger flexion: normally when the patient flexes fingers to grasp an object, he usually does this with extended wrist and simultaneous contraction of the wrist extensors. A simultaneous nerve repair with palliative tendon transfer is contraindicated. If the paralyzed muscles recover, a total imbalance of forces will occur, leading to deterioration of function.

This is however not the subject of this discussion.

In the late sixties I frequently had to treat peroneus nerve lesions and I made the same experience as many other surgeons: often the results were poor. Peroneus nerve lesions had a bad reputation as far as prognosis after repair was concerned, and several studies had been published to investigate why prognosis was so poor. Techniques of palliative surgery were developed, all of them combined with arthrodesis of the lower ankle joint.

A grading system was established to compare the results (Table 1).

In our first series of major peroneus nerve lesions treated by nerve grafting, all five cases were classified as poor (Table 2).

In the next series of 12 cases we had also poor results in the majority of cases (Table 3 left).

In spite of the fact that the originally paralyzed muscle showed signs of muscle regeneration an analysis of the reasons for the poor results came to the following conclusions:

1) The peroneus nerve innervated muscles of the ventral compartment are much weaker as their opponents. The unfavourable balance of forces might be the reason for poor recovery.

   We all know that even without having problems with the peroneus nerve, a patient that has to stay

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in bed for some weeks will develop a drop foot if no physiotherapy is performed.

2) Contracture of the Achilles tendon develops rather quickly. Again, the weak force of the muscles will not be able to lengthen the Achilles tendon.

What may be done:

Transfer of the tibialis posterior muscle to support the muscles in regeneration. The regenerating muscles are protected against overstretch.

Table 1. *Grading system for evaluation of results after peroneus nerve lesion based on a follow-up study of 1972*

Good	ability of dorsiflexion + dorsal extension of toes patient can walk and run
Satisfactory	ability of dorsiflexion to 0 dorsal extension of toes patient can walk
Fair	ability of dorsiflexion but less than 0 degrees patient needs splint
Poor No regeneration	muscles innervated: no effect

Table 2. *Peroneus nerve lesion*

Good	0
Satisfactory	0
Fair	0
Poor	5
No regeneration	0

First series: nerve grafting procedure was performed only, number of patients treated $n = 5$ (1972).

Table 3. *Peroneus nerve lesion*

	After nerve grafting	After tibialis post. muscle transfer and lengthening of the Achilles tendon
Good	1	9
Satisfactory	1	2
Fair	0	1
Poor	8	0
No regeneration	2	0

Second series: nerve grafting procedure was performed followed by tibialis posterior muscle transfer and lengthening of Achilles tendon. Number of patients treated $n = 12$ (1972).

Table 4. *Peroneus nerve lesion*

Good	7
Satisfactory	6
Fair	0
Poor	0
No regeneration	0

Third series: nerve grafting procedure and tibialis posterior muscle transfer as well as Achilles tendon lengthening were performed simultaneously. Number of patients treated $n = 13$ (1972).

Lengthening of the Achilles tendon should be performed.

We applied these two procedures in 12 patients. Thereafter results improved impressively.

In our next series of cases we performed peroneus nerve grafting and tibialis posterior muscle transfer as well as Achilles tendon lengthening, either simultaneously or a short time thereafter (Table 4).

It has to be underlined that after the tibialis posterior tendon transfer not only dorsiflexion in the upper ankle joint improved, but also extension of the toes and stabilisation. In no case arthrodesis of the lower ankle joint was necessary. In many cases results were so good that the transferred tendon could be returned.

Favorable muscle balance seems to be an important factor for restoration of function after peripheral nerve lesions. This experience was published for the first time in 1975 [2] and 1987 [3]. Our results were confirmed by Ferraresi *et al.* [1].

Situations in which surgery may enhance regeneration to restore function (restorative surgery in contrast to palliative surgery that replaces lost function) can be summarized as follows:

– *Unfavourable muscle balance*:

Example: Peroneus nerve innervated muscles against tibialis innervated muscles of the leg:

Treatment: Tibialis posterior tendon transfer to improve the muscle balance.

– *Overstretching of muscles*:

Examples:

Radialis nerve innervated muscles of the forearm:

Treatment: Pronator teres transfer.

Ulnar nerve innervated interossei muscles:

Treatment: Capsulodesis of Zancolli

Peroneus nerve innervated muscles of the leg:

Treatment: Tibialis posterior tendon transfer.

Co-contractures:

Example: Triceps and biceps:

Treatment: Botox, Lengthening of triceps tendon.

Unfavourable mechanical conditions:

Example: Luxation of the head of humerus in brachial plexus lesions:

Treatment: Suspension by fascia lata sling to provide better conditions for abduction.

Augmentation of force:

Example: Weak recovery of deltoid muscle.

Treatment: Reinforcement by trapezius muscle.

– *Improvement of soft tissue conditions* by plastic surgery.

Summary

The peripheral nerve surgeon should not only pay attention to the repair of injured nerves but also feel responsible for restoration of function.

References

1. Ferraresi S, Garozzo D, Bufatti P (2003) Common peroneal nerve injuries: results with one stage nerve repair and tendon transfer. Neurosurg Rev 26: 175–179
2. Millesi H (1975) Unfallschäden peripherer Nerven. In: Zenker R, Demler F, Schink W (eds) Chirurgie der Gegenwart, 4th ed. Urban & Schwarzenberg, München, pp 1–75
3. Millesi H (1987) Lower extremity nerve lesions. In: Terzis JK (ed) Microreconstruction of nerve injuries. Saunders, Philadelphia, pp 243–249

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