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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

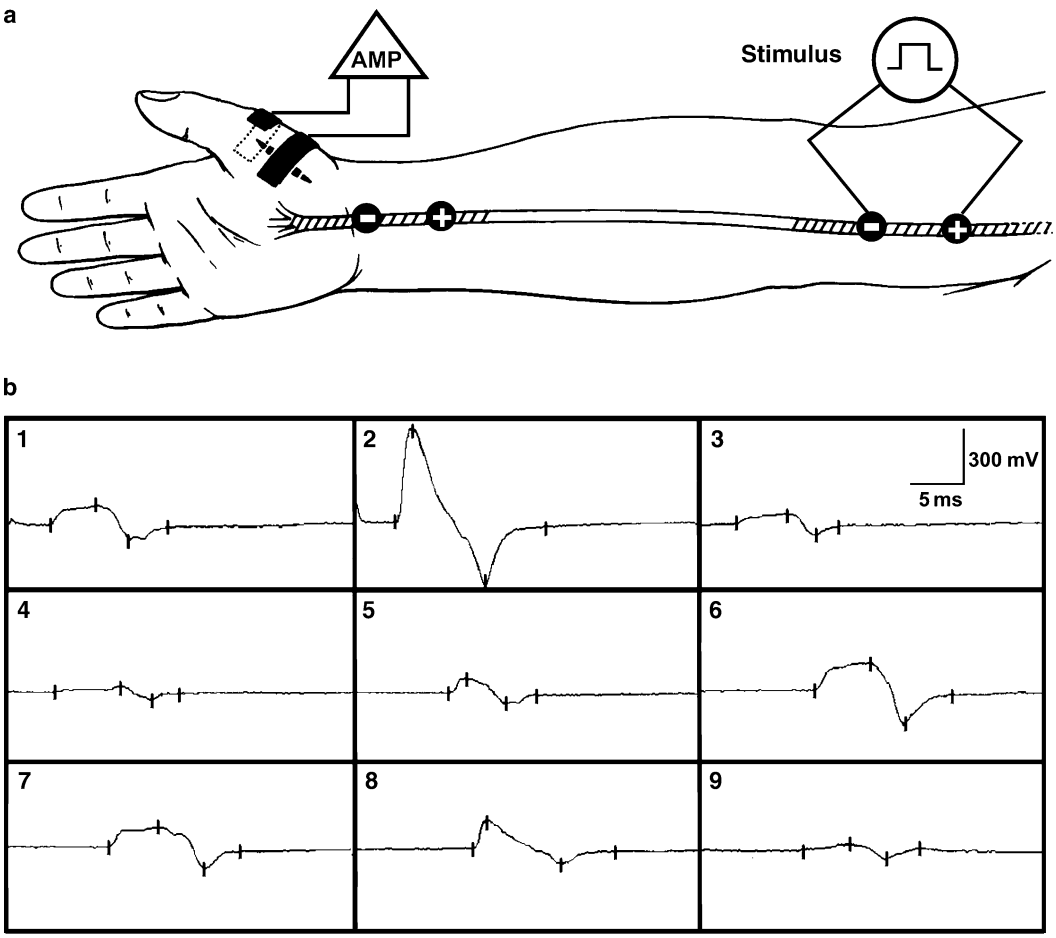

Fig. 1. (a) Figurative illustration of the electromyographic recording from the thenar muscles of the median eminence in response to stimulation of the median nerve at progressively more proximal sites along the nerve in the forearm. (b) Examples of the electromyographic signals elicited in an all-or-none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

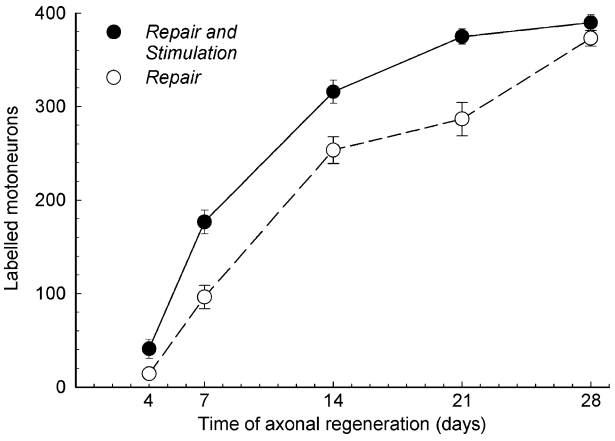

Fig. 2. The mean number (±S.E.) of axotomized femoral motoneurons that regenerated their axons 1.5 mm into the distal nerve stump across the suture line of the transected and surgically repaired nerve. The number was significantly elevated at all times measured from 4 to 21 days after the surgical repair 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

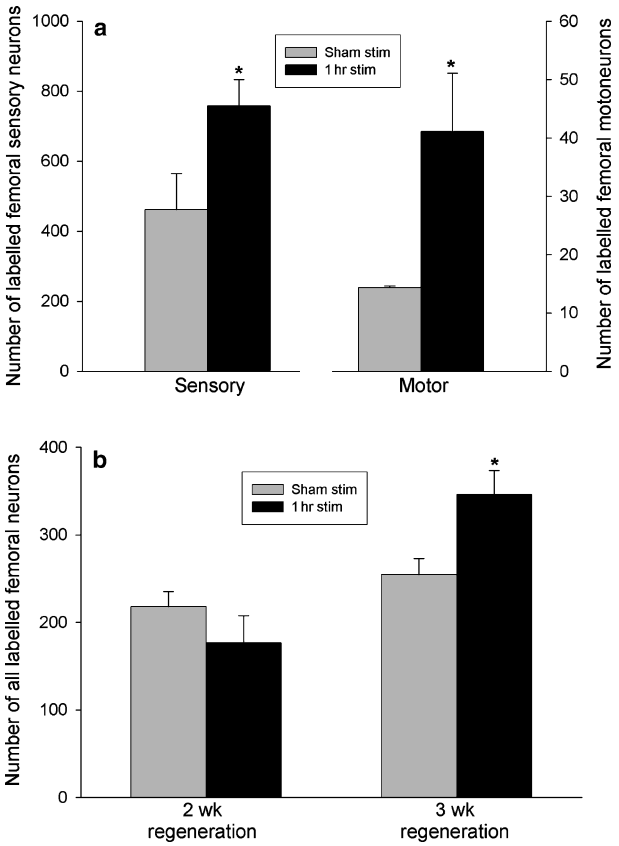

Fig. 3. A 1 h period of 20 Hz stimulation of the nerve immediately after surgical repair of the transected and repaired femoral nerve significantly elevated the number of (a) sensory and motor neurons that regenerated their axons 1.5 mm into the distal nerve stump across the surgical repair site in 4 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

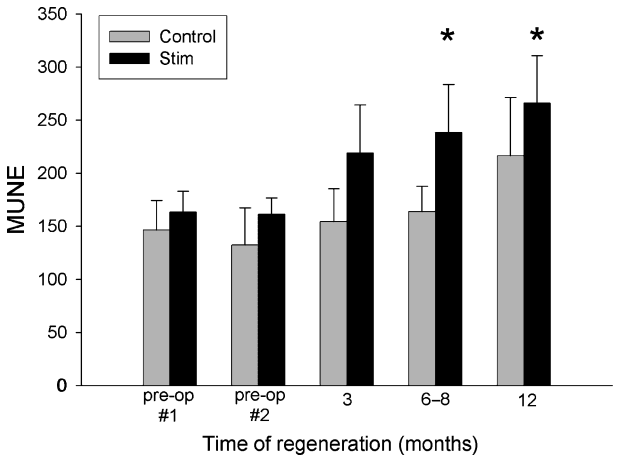

Fig. 4. A 1 h period of 20 Hz stimulation of the median nerve immediately after carpal tunnel release surgery in human patients with moderate and severe carpal tunnel syndrome effectively increased the number of motoneurons that regenerated and reinnervated the muscles of the thenar eminence. The motor 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.

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