Brief post-surgical electrical stimulation accelerates axon regeneration and muscle reinnervation without affecting the functional measures in carpal tunnel syndrome patients

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ABSTRACT

Electrical stimulation (ES) of injured peripheral nerves accelerates axonal regeneration in laboratory animals. However, clinical applicability of this intervention has never been investigated in human subjects. The aim of this pilot study was to determine the effect of ES on axonal regeneration after surgery in patients with median nerve compression in the carpal tunnel causing marked motor axonal loss. A randomized control trial was conducted to provide proof of principle for ES-induced acceleration of axon regeneration in human patients. Carpel tunnel release surgery (CTRS) was performed and in the stimulation group of patients, stainless steel electrode wires placed alongside the median nerve proximal to the surgical decompression site for immediate 1 h 20 Hz bipolar ES. Subjects were followed for a year at regular intervals. Axonal regeneration was quantified using motor unit number estimation (MUNE) and sensory and motor nerve conduction studies. Purdue Pegboard Test, Semmes Weinstein Monofilaments, and Levine's Self-Assessment Questionnaire were used to assess functional recovery. The stimulation group had significant axonal regeneration 6–8 months after the CTRS when the MUNE increased to 290±140 (mean±SD) motor units (MU) from 150±62 MU at baseline (p<0.05). In comparison, MUNE did not significantly improve in the control group (p>0.2). Terminal motor latency significantly accelerated in the stimulation group but not the control group (p>0.1). Sensory nerve conduction values significantly improved in the stimulation group earlier than the controls. Other outcome measures showed a significant improvement in both patient groups. We conclude that brief low frequency ES accelerates axonal regeneration and target reinnervation in humans.

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Introduction

It is well known that, in man, functional recovery after peripheral nerve injuries is frequently poor despite the regenerative capacity of injured neurons and the permissive growth environment provided by the Schwann cells in the distal nerve stumps (Fu and Gordon., 1997; Gordon et al., 2003, in press; Kline and Kim, 2008). Although poor functional recovery especially after proximal nerve injuries has generally been attributed to irreversible denervation atrophy of targets (Kline and Kim, 2008; Sunderland, 1978), progressive decline in the capacities of the neurons and Schwann cells to regenerate and support regeneration are now recognized for decline to ~5% in numbers of neurons that regenerate over time and distance (Fu and Gordon, 1995a,b; Sulaiman and Gordon, 2000; You et al., 1997).

Axon outgrowth is another factor that delays axon regeneration with a surprisingly long period of ~1 month required for all motoneurons to regenerate their axons across a surgical repair site in rats (Brushart et al., 2002). This “staggered axonal regeneration” with wandering of axons within the poorly organized extracellular matrix of the suture site into which Schwann cells migrate, culminates in many weeks for all neurons to regenerate their axons within the distal nerve stumps (Al Majed et al., 2000a; Witzel et al., 2005). Given the sluggish movement of Schwann cells in humans, the delayed outgrowth at the suture site may be even longer (Wood and Bunge, personal communication).

A 1-h period of low frequency electrical stimulation (ES) of surgically repaired rat hindlimb nerves accelerates axon outgrowth to promote regeneration of all motor axons within 3 rather than 8–10 weeks and with similar effect on sensory nerve regeneration (Al Majed et al., 2000b; Brushart et al., 2002, 2005; Geremia et al., 2007). Similar effects of ES in mice have been reported (Ahlborn et al., 2007; English et al., 2007). The important unanswered question that remains is whether this acceleration translates into earlier target reinnervation. More rapid recovery of muscle contractile force and evoked withdrawal reflexes after ES of crushed nerves are consistent with the accelerating ES effect on axon outgrowth but these outcome measures failed to distinguish whether ES promotes early axon regeneration of more neurons or it promotes sprouting and in turn,
earlier muscle force recovery through reinnervation (Nix and Hopf, 1983; Pockett and Gavin, 1985).

In this study, we asked whether ES accelerates nerve regeneration and target reinnervation in a human model of median nerve crush injury, carpal tunnel syndrome (CTS). In selected patients with clear evidence of at least 50% axonal loss, motor unit number estimation (MUNE) was used to evaluate how many axons reinnervated target thenar muscles over time, electrophysiological recordings to evaluate time course of regeneration of sensory as well as motor axons, and behavioral measures to evaluate functional outcomes. A randomized controlled pilot study was carried out to provide proof of principle that electrical stimulation accelerates axon regeneration and promotes more rapid reinnervation of denervated targets by the regenerating axons.

Materials and methods

This was a randomized controlled clinical trial that complied with the guidelines of and was approved by the Human Research Ethics Board at the University of Alberta. All subjects gave their informed consent.

Participants

Patients with carpal tunnel syndrome (CTS) were recruited from a university hospital electromyography clinic. The inclusion criterion used in this study was the presence of at least one of the following constellation of symptoms: (1) numbness and tingling in the median nerve distribution, (2) precipitation of these symptoms by repetitive hand activities and relieved by resting, rubbing, and shaking the hand, (3) nocturnal awakening by such sensory symptoms and (4) weakness of thumb abduction and thenar muscle atrophy. Subsequently, the presence of median nerve compression was confirmed by electrophysiological studies. Conventionally, confirmation of the diagnosis of CTS and classification of its severity are commonly based on nerve conduction results (Padua et al., 1997). Based on those criteria, patients with moderate and severe CTS who had not responded to conservative treatments were recruited for this study. Patients with electrophysiological evidence of conduction block across the carpal tunnel were excluded because in those cases, changes in MUNE cannot be meaningfully interpreted. Other exclusion criteria were presence of other neurological conditions and previous carpal tunnel release surgery (CTRS). We evaluated these factors through interviews and clinical examination. When indicated, further electrophysiological studies and investigations were carried out. The ulnar and superficial radial nerves were evaluated through clinical examination and electrophysiological studies to rule out other peripheral neuropathies which may have contributed to the hand symptoms.

Conventional nerve conduction study

All sensory and motor nerve conduction studies were performed on patients using a Viking Select EMG machine (Nicolet Biomedical, Minneapolis). Sensory and motor nerve conduction studies of the median nerve were done using standard techniques. (Dumitruc, 1995).

Median sensory nerve conduction study

The hand was cleansed with rubbing alcohol and the skin temperature was maintained at 30–34 °C with an infrared heatlamp. Disposable silver/silver chloride surface strip electrodes (Nicolet VIASYS Healthcare), measuring 1×2.5 cm, were used. The recording electrode was placed on the proximal interphalangeal joint (G1) and the reference electrode on the distal interphalangeal joint of the third digit (G2) (Fig. 1). A ground electrode (Gd) was placed on the dorsum of the hand. The median nerve was stimulated in mid palm and also just proximal to the distal wrist crease at Sw and Sp. The transcarpal sensory conduction velocity and the negative peak amplitude of the sensory nerve action potential (SNAP) were measured.

Median motor nerve conduction study

A disposable recording surface strip electrode was placed over the motor point on the thenar eminence muscles with a reference electrode placed over the dorsal aspect of the first metacarpophalangeal joint (Fig. 1). The median nerve was stimulated at supramaximal intensity at the wrist (Sw) (8 cm proximal to the recording electrode) and also at the elbow. The negative peak amplitude of the maximal compound muscle action potential, terminal motor latency and conduction velocity in the forearm were measured. The median nerve was also stimulated at the palm (Sp). Those patients with evidence of conduction block across the carpal tunnel were excluded from the study.

Motor unit number estimation (MUNE)

MUNE was performed on all patients using the multiple point stimulation technique to determine the number of motoneurons that regenerate their axons and innervate thenar muscles (Doherty et al., 1995). This was done using proprietary software on an Advantage EMG machine (Neurosoft, Virginia).

Recording

Disposable, self-adhesive surface electrodes over the thenar muscles of the thumb were used to detect the maximum compound muscle action potential (CMAP) and surface-detected motor unit action potential (S-MUAP) (Fig. 2). Placement of the electrodes was the same as that used for motor nerve conduction study. A 3×3 cm metal plate was positioned on the back of the hand as a ground. The bandpass filter was set at 5–2000 Hz.

Stimulation

Electrical stimulation of the nerve was performed with a handheld constant-current bipolar surface bar stimulator. The maximum CMAP of the median nerve was evoked by stimulating the median nerve at the wrist at 10% above the maximal intensity with a stimulus duration of 0.01 ms (Fig. 2B). The course of the median nerve was mapped from the elbow to the axilla by advancing the bar...
stimulator over the medial aspect of the arm at 1–2 cm intervals (Fig. 2A). Because the median and ulnar nerves are in close proximity in the upper arm, it was necessary to avoid co-stimulation of the ulnar nerve while mapping the course of the median nerve. Co-activation of the ulnar nerve was recognized by (1) an initial positive deflection of the CMAP, (2) abduction of the fifth digit, and (3) the radiation of an electrical sensation into the fourth and fifth digits. In earlier experiments, we also co-recorded from the hypothenar eminence and found that when the above conditions were avoided, there was no detectable action potential generated by the hypothenar muscles.

Using the same recording electrodes, S-MUAPs with the lowest stimulus thresholds were elicited by stimulating the median nerve at multiple sites where the nerve is more superficially located at the wrist and between the elbow and the axilla (Fig. 2C). Stimulation was performed at 1 Hz with gradually increasing intensity until the first reproducible, “all-or-none” S-MUAP was evoked. To increase the yield, the next higher threshold S-MUAP could sometimes be obtained through template subtraction. By this means, up to a maximum of 3 S-MUAPs were acquired at each site and a total of 13 to 20 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-MUAPs were temporally aligned at the same onset latency before they were averaged. The motor unit number estimate was obtained using the following equation:

\[
\text{MUNE} = \frac{\text{CMAP}}{\text{Avg S-MUAP}}
\]

Behavioral measures

(1) Levine's Self-Assessment Questionnaire for CTS. To assess the subjective symptoms, patients were asked to complete the Levine's Self-Assessment Questionnaire for CTS symptom severity before and at intervals after the surgery (Levine et al., 1993). This questionnaire consisted of two parts: (1) symptom severity scale comprised of 11 items scored on a Likert scale inquiring about pain, paraesthesia, numbness, weakness, nocturnal symptoms and overall functional status, and (2) functional status scale comprised of 8 questions regarding activities of daily life commonly affected by CTS. The symptom severity scores ranged from 1 to 5 with 1 representing no symptoms and 5, very severe symptoms. The functional status scores also ranged from 1: no difficulty to do a task, to 5: inability to do a task. The patients were asked to rate the severity of the symptoms on the hand that was to be operated on.

(2) Semmes Weinstein Monofilaments (SWM). As sensory complaints are prominent in CTS, we used a test kit of 20 SWM (Sammons Preston Rolyan, Canada) to assess the impact of the CTS with and without electrical stimulation (ES) treatments on hand sensation. This tool, which examines the sensation threshold, has proven reliability (Bell-Krotoski and Tomancik, 1987) and is utilized in the clinical evaluation of peripheral nerve diseases such as diabetic neuropathy (Imai et al., 1989; Lee et al., 2003). Since pressure sensation involves primarily small, unmyelinated or poorly myelinated nerve fibers, this test complements the conventional sensory nerve conduction study well because the latter only evaluates large myelinated sensory nerve fibers.

Subjects were asked to place their hands over a table, and keep their eyes closed for the duration of this test. Each filament, starting with the smallest caliber, was tested over the pulp of digits. The filament was applied perpendicularly for 1 to 1.5 s in three trials. Subjects were asked to indicate the area where the filament was felt. We examined the sensory threshold of all fingers in random order. A positive response in at least 2 of the 3 trials marked the sensory threshold. We used the test results of the third digit where sensory nerve conduction study was also done, for data analysis.

(3) The Purdue Pegboard Test. We used the Purdue Pegboard Test (Model 32020, Lafayette Instrument Company, IN, USA) to monitor the impact of CTS with and without ES on manual dexterity. The Purdue Pegboard Test is a standardized hand function tool with proven validity and reliability in the functional evaluation of CTS patients. It consisted of 50 holes arranged in two parallel columns. Subjects were instructed to start the test on a verbal cue, while an examiner timed the test with a stopwatch. They had 30 s to fill the holes with pegs, initially with the hand that was intended for CTS surgery and then with the other hand. Each subset was repeated three times to obtain an average. Test scores equaled the number of filled holes.

Interventions

Subjects were randomized to the control or the stimulation group by using the random number generation function in a commercially available software program (Excel, Microsoft Inc.). The control group underwent open carpal tunnel release surgery (CTRS) only. The stimulation group underwent CTRS followed by 1 h of electrical stimulation of the median nerve.

Surgical technique

Operations were performed by a plastic surgeon (D.E.). The surgical procedure was the standard CTRS without epineurotomy or neurolysis of the median nerve (Rosenbaum and Ochoa, 1993). A tourniquet was inflated over the forearm. The surgeon drew a curvilinear mark over the palm to guide the incision. The transverse carpal ligament was divided along the ulnar side of the incision. No
further intervention was employed in the control group. For the stimulation group, two sterile 30 gauge stainless steel wires, insulated except 1 cm at the tip, were placed over the median nerve. One electrode was positioned at the proximal end and the other at the distal end of the incision. To record the CMAP during the post-surgical ES, the surgeon placed two surface electrodes (TECA, Oxford Instruments), one over the motor point on the thenar eminence muscles (G1) and the other over the dorsal aspect of the first metacarpophalangeal joint (G2) (see Fig. 1).

Electrical stimulation

Subjects in the stimulation group were transferred to a neuro-physiology laboratory after CTRS. There was an approximately 30 min delay between termination of CTRS and initiation of electrical stimulation. With the patient in the lying position, the operated hand was stabilized in an elevated position. The stimulating electrodes were connected to a Grass (SD9) stimulator: the proximal wire electrode was connected to the cathode and the distal one to the anode. The surface electrodes on the thenar eminence were connected to an EMG machine (NeuroSoft Inc., Virginia). We gradually increased the stimulation intensity to the maximal tolerance limit (4–6 V, 0.1–0.8 ms duration) as a continuous 20 Hz train for 1 h. These intensities were sufficient to induce a fused tetanic contraction but low enough to not induce excessive discomfort. This protocol, including con-

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siderations 

Majed et al., 2000a,b, 2004; Brushart et al., 2002). The only exception was so chosen to approximate that used in our animal studies (Al Majed et al., 2000a,b, 2004; Brushart et al., 2002). The only exception was that supramaximal intensities used in animal studies was not feasible in human subjects. The CMAP of the thenar muscles were also monitored. After the termination of stimulation, electrodes were pulled out and discarded.

Outcomes

To compare the effectiveness of electrical stimulation with control CTRS, the subjects were evaluated twice before the operation (pre-op1 and pre-op2) to ensure reliability of the tests. Subjects were then followed for a year at 3 time points after the CTRS. The follow-up schedule was: (1) post-op1: 3rd month, (2) post-op2: 6th to 8th month, and (3) post-op3: 12th month. The 3rd month was selected for the first assessment based on the assumptions that the most optimal axonal growth rate is 1 mm/day and the distance between the compression site and the thenar muscles is ~70 to 80 mm, depending on the size of the hand. At each post-operative assessment, all the baseline measures were repeated.

Motor unit numbers and muscle function

To ascertain whether the motor unit number in CTS patients had an impact on their muscle function, we also measured muscle bulk and force generating capacity in a separate sample of 8 subjects. Cross sectional area of the mid belly of the median innervated thenar muscles (abductor pollicis brevis, opponent pollicis and superficial head of the flexor pollicis brevis) was measured using ultrasound imaging (Acuson Sequoia, Siemens, Germany). Mid belly of the muscles was located at the mid shaft of the 1st metacarpal bone. The medial border of the thenar muscles lies over the 2nd metacarpal bone while the lateral border is defined by the 1st metacarpal bone (see Fig. 3A). Superficial head of the flexor pollicis brevis, deepest of the three median innervated thenar muscles, is at the level of the flexor pollicis longus tendon. The tendon served as a useful landmark because it can be easily discerned by its shape and high echogenicity (see Fig. 3A). The perimeter of these muscles was traced and the cross sectional area calculated using the ultrasound machine bundled software.

Twitch force of the median nerve innervated thenar muscles was recorded using a LCCA-25 load cell (Omega Canada, Laval, Quebec) connected to the thumb via a loop placed around the base of the 1st proximal phalanx. Based on previous studies, the maximum resultant vector of the median innervated thenar muscles is at a 45° angle plane from the palm along which the load cell was aligned. After the hand and forearm were secured to the force platform by inelastic Velcro straps, the median nerve was stimulated supramaximally at the wrist and the twitch tension recorded (see Fig. 3C).

Statistical analysis

The statistical program SPSS 12.0 for Windows was used in this study. Sample size estimation was done based on published animal data with MUNE being the primary outcome measure (Al Majed et al., 2000b). Assuming a difference in treatment effect size of 26%, standard deviation of 30%, with α = 0.05 and β = 0.20, to have sufficient power to detect a significant difference, 20 subjects would be required for this study. Test–retest reliability of MUNE, a major outcome measure used in this study, was tested using linear regression analysis. Using Shapiro–Wilk Test of normality, we found that nerve conduction studies, and SWM results were signi
cantly (p < 0.05). Additionally, Mauchley's test of sphericity was statistically significant (p < 0.05) for the Levine’s Self-Assessment Questionnaire and Purdue Pegboard...
Test scores rendering parametric tests unsuitable. To be conservative and to reduce the chances of type I error, we decided to use non-parametric statistical methods for their analysis. We used Kendall’s W and Wilcoxon Signed Ranks test to compare those outcome measures within each group of subjects. To examine whether the preoperative outcome measure values were significantly different between the two groups, the Kruskal–Wallis test was employed. In contrast, the MUNE results were normally distributed and the variance was homogeneous between the treatment and control groups, changes within each group following intervention were therefore analyzed using paired Student’s t tests. In addition, comparisons of changes between the two groups following intervention were analyzed using univariate general linear model statistics. Linear regression analysis between the MUNE, cross sectional area and twitch force of the thenar muscles was done. The statistical significance was set at $p<0.05$.

Results

Subjects with carpal tunnel syndrome

Twenty-five eligible CTS subjects participated in the study, all of whom had surgical decompression of the carpal tunnel under local anesthesia (1% lidocaine). However, 4 subjects (2 males and 2 females) withdrew from the study because of development of other medical conditions or occupational commitments which prevented them to return for follow up. Two of these patients belonged to the control and 2 to the stimulation group. Therefore, the results are from 21 subjects: 8 males and 13 females. The patients were 20 to 86 years old, with a mean (±SD) age of 56±17 years. The breakdown of age and sex and the physical attributes in both groups are shown in Table 1. The subjects were housewives, nurses, medical and laboratory technicians, cooks, manual laborers, and retirees. All had had progressive symptoms for at least 2 years.

Eighteen subjects were right hand dominant. Although they all had bilateral symptoms, it was more severe in their dominant hand that was subsequently operated. Ten patients were assigned to the control group (no electrical stimulation, ES) and 11 patients to the stimulation group (1 h 20 Hz ES). All subjects attended the first post-operative follow-up, whereas 19 of them were available for the second and third post-operative evaluations. Two of the subjects who missed appointments belonged to the control and one to the stimulation group.

Muscle denervation in severe carpal tunnel syndrome

We stimulated the median nerve proximal (Sw) and distal (Sp) to the site of compression at the carpal tunnel and recorded the evoked compound muscle action potential (CMAP) with the recording electrode (G1) placed over the muscles at the thenar eminence (Fig. 1). As shown in Fig. 4, we discriminated and excluded patients with early CTS on the basis of conduction block where CMAPs evoked at the wrist and palm were equally reduced, and (B) another patient with conduction block of the median nerve at the carpal tunnel where the amplitude of the CMAP was reduced at the site proximal to the carpal tunnel as compared to the normal CMAP amplitude evoked by stimulation below the carpal tunnel. The latter patients were excluded from the study.

Using the multiple point stimulation technique for motor unit number estimation (MUNE), Doherty and Brown reported 288±23 (mean±SE) motor units (MU) in the thenar muscles of healthy subjects (Doherty and Brown, 1993). The preoperative MUNE in the selected patients with severe CTS was reduced by ~50% as compared to that in the healthy subjects (Fig. 5A). The estimated number of innervated MUs was the same in 2 preoperative recording sessions and the numbers were also not different in the control and stimulated groups of patients.

The underlying axonal injury seen in these CTS patients was likely long-standing as reflected by the substantially larger single surface recorded motor unit action potential (S-MUAP) amplitudes recorded in the 2 preoperative recording sessions as compared to healthy subjects (Fig. 5B). Amplitudes of the S-MUAP in healthy subjects reported by Doherty and Brown were 0.91±0.11 of the maximum CMAP (Doherty and Brown, 1997). In contrast, for the CTS subjects in

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Mean (±SD) of age, gender and handedness of the human patient subjects in control (no electrical stimulation) and stimulation (1 h 20 Hz electrical stimulation) groups.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Age</td>
<td>61±16 years.</td>
</tr>
<tr>
<td>Gender</td>
<td>7 females: 3 males</td>
</tr>
<tr>
<td>Handedness</td>
<td>2 left: 8 right</td>
</tr>
<tr>
<td></td>
<td>Stimulated</td>
</tr>
<tr>
<td>Age</td>
<td>53±18 years.</td>
</tr>
<tr>
<td>Gender</td>
<td>6 females: 5 males</td>
</tr>
<tr>
<td>Handedness</td>
<td>1 left: 10 right</td>
</tr>
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this study, amplitude of the S-MUAPs was $1.92\pm0.44\%$ of maximum CMAP (range 0.06–61%; median±95%CI = 0.64±1.06%). Although amplitudes of the S-MUAPs in the control group was substantially larger than the stimulation group, the difference was not statistically significant ($p=0.22$). This shift of the distribution to larger values indicated that the fewer surviving MUs had had adequate time to sprout and enlarge by reinnervation of denervated muscle fibers.

**Brief electrical stimulation accelerates reinnervation of denervated muscle in severe carpal tunnel syndrome**

Brief (1 h) ES of surgically repaired rat peripheral nerve accelerated axon regeneration across the repair site without affecting the rate of axon regeneration within the distal nerve stump (Al Majed et al., 2000b; Brushart et al., 2002). We used the same stimulation regime, in this study of CTS patients. MUNE was used to evaluate whether ES immediately after CTRS in severe CTS patients, promotes and/or accelerates reinnervation of denervated thenar muscles. In the control (non-stimulated) group of patients, the numbers of innervated MUs were determined by MUNE at regular intervals up to 12 months after CTRS. There was no significant change in the MUNE even up to a year after surgery, even though there was a trend for the number to increase between 6 and 12 months (Fig. 6A). In contrast, there was already a trend for the MUNE to increase within 3 months in the patients after CTRS and ES (Fig. 6B). By 6–8 months, the number of MUs in the thenar muscles had increased significantly, comparable to the numbers reported in healthy subjects. Hence, in striking contrast to the failure of regenerating nerves after CTRS alone, all the regenerating motor axons in the stimulation group had made functional connections with denervated muscle fibers by 6–8 months.

The mean amplitude of the S-MUAPs 3 months after CTRS without ES did not change significantly compared to preoperative baseline (Fig. 6C). In contrast, 3 months after CTRS with ES, there was a significant reduction in the mean S-MUAP amplitude as compared to preoperative values (from 1.35% maximum CMAP to 0.89% maximum CMAP, Mann–Whitney U test) (Fig. 6D). The early reduction in the ES group indicated the presence of newly regenerated motor axons had innervated some muscle fibers while they had not in the control non-stimulated group.

Small, newly regenerated MUs contribute little to the amplitude of the maximum CMAP so that the lack of significant change in the CMAP amplitude following CTRS with and without ES was not surprising (Fig. 7). Since the maximum CMAP amplitudes were not normally distributed, they are shown as box and whisker plots where the upper and lower limits of the box represent the 75th and 25th percentiles, respectively, while the upper and lower limits of the whiskers represent the 90th and 10th percentiles, respectively. The transverse line within the box represents the median. Not surprisingly, the maximum CMAP amplitudes recorded preoperatively were smaller than in the healthy controls and they remained so throughout the postoperative follow-up period in both the control and stimulated groups of CTS patients.

**Brief electrical stimulation promotes improvements in axonal conduction speeds in severe carpal tunnel syndrome**

Demyelination is one of the most common sequelae of compressive neuropathy (O’Brien et al., 1987). In order to determine whether ES also affects remyelination, we measured the conduction speeds of both motor and sensory nerve fibers across the carpal tunnel. The...
motor terminal latency in the severe CTS patients was similarly slower than normal in both the control and stimulation groups at baseline (Fig. 8). The latency did not change significantly in the control group in the post-operative period, while, in contrast, significant acceleration in the motor latency occurred early in the stimulation group at 3 months (Fig. 8B). Motor terminal latencies from 3 months onward were the same as that for healthy subjects (Fig. 8B). Similarly, earlier recovery of conduction velocity of the sensory nerve fibers was found in the stimulation group at 3 months when amplitude of the SNAP had not yet changed significantly from pre-operative values (Figs. 9B, D). In contrast, recovery was delayed in the control patient group (Figs. 9A, C). Together, these data buttress the possibility that ES may enhance remyelination in addition to accelerating axon regeneration and muscle reinnervation.

Rapid improvements in behavioral outcomes following CTRS

In order to evaluate the functional outcome of motor and sensory target reinnervation, we chose the Purdue Pegboard Test to gauge hand performance, Semmes Weinstein Monofilaments for pressure sensation and the Levine’s CTS Questionnaire for subjective symptoms. Rapid improvements in all these functional outcomes were found regardless of whether the subjects were in the control or stimulation groups (Figs. 10A, B and 11A, B). We compared the test results with those in the opposite untreated hands in order to determine whether the improvements seen in the Purdue Pegboard Test were due to practice. Since there was no change of performance in the non-treated hand, practice effect is an unlikely explanation for the improvements of hand function after CTRS in both the control and stimulation groups (Figs. 10C, D). Rather, a more likely explanation for better hand performance on the Purdue Pegboard Test was rapid recovery in pressure sensitivity at the digit tips after CTRS (Fig. 11A and B). Since smaller, poorly myelinated sensory nerve fibers are less vulnerable to degeneration due to compression (Nishimura et al., 2004), transmission along those nerve fibers can be more readily restored once the compression is alleviated. Lastly, with subjective interpretation of symptom severity on the Levine’s CTS Questionnaire, the subjects reported rapid resolution of symptoms with or without stimulation (Figs. 11C, D). Given that pain and tingling sensations experienced by CTS patients are positive symptoms of sensory nerve fiber irritation, ready resolution of these symptoms is commonly observed in patients following conventional CTRS.

Motor unit number does not affect cross sectional area and twitch tension of the thenar muscles in CTS

Because damage to the median nerve in the vast majority of CTS patients is a chronic process, the remaining surviving motor axons usually have ample time to sprout and to reinnervate the muscle fibers. Indeed, previous studies on chronic axonal injury did not find significant decline in muscle force until the motor unit number fell below 90% of the control subjects (McComas et al., 1971). To test that...
hypothesis in CTS patients, we measured the cross sectional area and twitch tension of the median innervated thenar muscles in a separate sample of 8 CTS patients. Although they had motor unit numbers ranged from 50 to 267, no significant correlations between the motor unit number, cross sectional area and twitch tension were found (see Fig. 3C). These results support our contention that in a chronic axonal loss injury process such as CTS, the extent of motor axonal loss except in the most severe cases does not affect the muscle force and mass.

Discussion

This pilot study serves as proof of principle for positive outcomes of brief post-surgical ES and demonstrated that indeed, brief 1 h low frequency ES immediately following decompression surgery in patients suffering from severe CTS resulted in early and complete reinnervation of the thenar muscles. Surgery alone was not sufficient to cause significant improvement in muscle reinnervation even 1 year after the operation.

The findings are not simply due to resolution of conduction block following carpal tunnel decompression because all patients were screened for conduction block preoperatively and excluded from the study if block was evident. The findings demonstrated for the first time that the brief continuous ES at 20 Hz that accelerates axon outgrowth in animal models of nerve injury in rats (Al Majed et al., 2000b; Brushart et al., 2002, 2005; Geremia et al., 2007) and mice (Ahborn et al., 2007; English et al., 2007) is also effective in accelerating target reinnervation in the human. Moreover these findings provide direct evidence of accelerated muscle reinnervation to account for the earlier functional recovery of muscle force and reflex discharge demonstrated in rats by Nix and Hopf (1983) and Pockett and Gavin (1985). Importantly the single bout of ES immediately after surgery, by accelerating axon outgrowth, was sufficient to result in target reinnervation 6 months later.

Since the compressive axonal injury that occurs in CTS is a chronic one, the ability for the affected motor axons to grow and regenerate is likely compromised by the delay. Diminished capacity for axonal regeneration was demonstrated in a chronic axotomy animal model where the extent of axon regeneration was reduced by up to 66% when a chronically axotomized tibial nerve was cross-sutured and regrew into a freshly cut common peroneal nerve in the rat hindlimb (Boyd and Gordon, 2002; Fu and Gordon, 1995a). With consideration of the diminished regenerative capacity of chronically injured neurons, our findings of no significant thenar muscle reinnervation even a year after carpal tunnel release without ES is perhaps not surprising. In that light, the striking regenerative capacity of axotomized median motoneurons that were electrically stimulated for 1 h after CTR surgery, was remarkable: the axon regeneration in the group of patients whose median nerve was electrical stimulated, was not only more rapid but all the axotomized motoneurons regenerated their axons and the MU number in the reinnervated thenar muscles was the same as that in normal healthy individuals (Fig. 6). These data imply that even chronically injured motor axons retain the ability to regenerate if they are electrically stimulated for just 1 h after surgical repair, a conclusion that is readily testable in an animal model. Death of motoneurons after prolonged axotomy at distal sites is unlikely because motoneurons can survive a year or more after chronic axotomy (Gordon et al., 1991; Vanden Noven et al., 1993).

Animal studies demonstrated that 1 h 20 Hz ES accelerates axonal outgrowth at the site of injury but not the rate of axonal regeneration in distal nerve stumps (Brushart et al., 2002; Udina et al., 2008). Given the distance of 70–80 mm from the site of compression in the carpal tunnel to the thenar muscle endplates in CTS patients, and assuming an approximate regeneration rate of 1 mm/day (Sunderland, 1947), the regenerating axons in the distal nerve stumps would be expected to reach the intramuscular nerve sheaths of the partially denervated thenar muscles 6 months later.

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thenar muscles within 3–4 months. Our finding that the MUNE did not increase significantly even as late as 12 months after CTRS without ES indicates that the progress of axon outgrowth across the site of compression is very slow. In this context, the acceleration of axon outgrowth by ES to achieve the significant muscle reinnervation by 6 months and complete muscle reinnervation by 12 months is considerable.

The motor nerve conduction studies further confirmed the positive impact of stimulations on axon regeneration: the significant improvement in the terminal motor latency of the median nerve occurred earlier in the stimulation group and was detectable as early as 3 months post-CTRS and stimulation (Fig. 8). The fact that the CMAP amplitude did not change in the stimulated group of patients following CTRS and ES is not surprising even though significant axonal regeneration had occurred, the reason being that CMAP amplitude is affected by changes in the amplitude and configuration of the constituent MUAPs (Rashidipour and Chan, 2008). In early reinnervation, MUAPs are usually small, polyphasic and contribute little to increase the CMAP amplitude. That was one of the major reasons that led to the introduction of more direct methods of estimating MU numbers (McComas, 1995).

Similar trends were also found in the sensory nerve conduction studies designed to evaluate regeneration of the large myelinated nerve fibers. Recovery of the conduction velocity and SNAP amplitude occurred earlier, by the 3rd month post-intervention in the stimulated group as opposed to the 6th month in the control group. However, these physiological differences are not likely to be functionally important as large myelinated fibers subserving vibration and proprioception sensations do not play a critical role in most daily activities (Videler et al., 2008). Rather, the ability to perceive touch and pressure subserved by small thinly myelinated nerve fibers is more important for hand function. Since those nerve fibers are less vulnerable to compressive injury, it is not surprising that there was no significant difference in the patterns of recovery in sensory symptom and hand function assessed by the Levine's Questionnaire and Purdue Pegboard Test between the control and stimulated groups of patients. Both groups reported marked symptom improvement within 3 months of surgery. Symptoms including tingling, burning and aching pain due to mechanical nerve irritation and micro-ischemia can all be readily alleviated by CTRS.

The lack of blinding in this human study was unlikely to affect the quantitative tests of axon regeneration and muscle reinnervation. The validity of the MUNE technique used, the multiple point stimulation method, has been established (Doherty and Brown, 1993; Rashidipour and Chan, 2008) and its test–retest reliability is high (Felice, 1995; Porter et al., 2008). Therefore, despite the relatively small number of subjects and baseline inter-subject variability, the sample size was sufficient to detect the remarkable reinnervation of the denervated thenar muscles by all the injured (axotomized) motoneurons. The outcome measure tools of nerve conduction studies and the MUNE are objective measures that cannot be easily influenced by the subject or the examiner. The Levine's Self-Assessment Questionnaire for CTS was the only subjective outcome measure that had the potential to be biased by patients' knowledge about the treatment. However, in this study both the stimulation and control groups showed a significant improvement in their symptom severity by 3 months.

The molecular basis for the effects seen with post-surgical ES has been investigated in a number of recent studies (Al Majed et al., 2004;...
Fig. 11. The Semmes–Weinstein Monofilament Test (A, B) and the Levine’s Self-Assessment Questionnaire (C, D) improved equally quickly after CTRS in both the control and stimulated groups of patients. The upper and lower limits of the box represent the 75th and 25th percentiles, respectively while the upper and lower limits of the whiskers represent the 90th and 10th percentiles, respectively. The median is represented by the line within the box and the outliers are represented by the dots that lie beyond the whiskers.

Fig. 12. Molecular mechanisms of enhanced nerve regeneration (modified from Hannila and Filbin, 2008). Electrical stimulation has been shown to upregulate cAMP that promotes nerve regeneration by activating cAMP response element binding (CREB) while inhibiting Rho via protein kinase A (PKA). See text for a detailed description.
**Sharma et al., in press; Udina et al., 2008.** A key player that emerges is cyclic AMP (cAMP). Through activation of protein kinase A (PKA) and cAMP response element binding (CREB) protein, transcription of cytoskeletal proteins including actin and tubulin, required to accelerate nerve regeneration is triggered. In addition, cAMP is also instrumental in up-regulating a number of cytokines including IL6 while inhibiting Rho, a key protein in the Ngr/p75NTR pathway that prevents cytoskeletal assembly. Electrical stimulation brings about these changes by upregulating brain derived neurotrophic factor (BDNF) and its trkB receptor at the soma. A summary of these molecular pathways is shown schematically in **Fig. 12**.

Employing a constellation of outcome measure tools in this study, we have provided proof of principle that, in this randomized control pilot study, 1 h ES of the median nerve after surgical decompression accelerates axonal regeneration to promote complete muscle reinnervation. The procedure of ES immediately after surgery was proven feasible in the clinical setting. Additionally, patients did not develop any complications due to surgery or the stimulation. The present study sets the stage for future human trials of ES in promoting axon regeneration and muscle reinnervation. The increase in the speed of axon regeneration may be particularly crucial for functional recovery after nerve injuries such as cubital tunnel syndrome with entrapment of the ulnar nerve at the elbow or within the Arcade of Struthers at the upper arm where the axotomized neurons must regenerate their axons over much longer distances. Lastly, post-surgical ES may also benefit transected nerve injuries. Lundborg et al. reported motor and sensory recovery in transected median nerve after the proximal and distal nerve stumps were approximated by a silicone chamber but the recovery took 3 years (Lundborg et al., 1954). It is our hope that brief low frequency ES of the proximal nerve stump immediately after surgical repair will expedite the functional outcome of these procedures.

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