Electrically stimulating nerve and muscle to enhance regeneration and reinnervation following peripheral nerve injury

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Abstract— **Recovery following peripheral nerve injuries is often incomplete. The gold standard treatment is surgical nerve repair performed immediately or shortly after injury. To date, there are no additional treatments that are used to enhance functional recovery. In this paper we outline two emerging applications of electrical stimulation to enhance nerve regeneration and functional recovery. The first is brief electrical nerve stimulation performed at the time of nerve repair that is used to accelerate nerve outgrowth across the injury site. The second is daily electrical muscle stimulation that reduces muscle atrophy and also accelerates muscle reinnervation.**

I. INTRODUCTION

Currently, clinical treatment of injured peripheral nerves is exclusively surgical, either releasing the source of nerve compression or reattaching the transected nerve directly or with grafting materials. Surgery encourages nerve regrowth by connecting nerve stumps but functional recovery remains inadequate [1]. Nerves in human patients regenerate slowly (~1mm/day) requiring long periods of time to reconnect with denervated target muscle or sensory end-organs. The more proximal the injury, the less likely a patient will fully recover. The window of opportunity for nerve regeneration is short with the regenerative capacity of the injured neurons and the regenerative support of the distal nerve stump declining with time and distance [2]–[4]. These factors together with the misdirection of regenerating nerves [5] account for the frequent poor recovery. As surgery is the only clinically acceptable treatment for nerve injuries, there exists a strong need to provide supplementary treatments to help patients fully recover. In our research we focus on two of the primary therapeutic targets of interest to enhance nerve regeneration and ultimately functional recovery: the regenerating axons within the proximal nerve stump and the denervated muscles.

II. ENHANCING NEURAL REGENERATION BY ELECTRICAL NERVE STIMULATION

The ability of electrical stimulation (ES) of the peripheral nerve proximal to the site of injury to accelerate nerve regeneration and target reinnervation was first established in 2000 for motoneurons and later for sensory neurons [6], [7]. Continuous ES for a period of 1-hour at 20 Hz, which has been adopted from animal studies, was also shown to be effective in accelerating target reinnervation in humans. Specifically, human nerve regeneration was accelerated following complete transection and immediate repair of the digital nerve [8] as well as following decompression of the carpal and cubital tunnels [9], [10]. The ES effect requires that action potentials are elicited in the antidromic direction (towards the neuronal cell body) [6], [7]. This in turn, upregulates neuronal cyclic adenosine monophosphate (cAMP) which leads to upregulation of brain-derived neurotrophic factor (BDNF) and ultimately facilitates the enhanced regenerative effect [11]. Injured axons regrow from

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the proximal stump and traverse the injury site to reach the distal stump and then later the denervated target organs. ES does not change the rate of regeneration but instead it accelerates nerve outgrowth across the injury site [12] which, for the re-growing axon, typically serves as the largest barrier to traverse.

While brief ES of the proximal nerve stump has shown to be effective, a number of questions and challenges remain to be overcome before this therapy is widely adopted. The first is to determine the optimal stimulation paradigm. While 20 Hz for one hour is effective in accelerating functional recovery, it is an open question as to the optimal paradigm for rats, let alone for humans. Second, is total recruitment of all axons by ES required for maximal effect? In previous studies in patients suffering from severe carpal tunnel or cubital tunnel syndrome, 20 Hz stimulation was performed continuously in the recovery room after the decompression surgery. The stimulus voltage was reduced so as to minimize discomfort that was experienced at higher voltages. Generally, voltages of approximately 4-6 V at a duration of 100-800 μs were used [9], [10]. At these levels of ES, all the remaining nerves with intact neuromuscular contacts were *not* stimulated as determined with electromyography (EMG). Whether or not maximum nerve recruitment by ES is required for full clinical efficacy remains an open question despite the evidence gained that the ES was effective in accelerating target reinnervation and functional recovery.

At the Hospital for Sick Children in Toronto we are using the 20 Hz electrical stimulation paradigm in pediatric patients who sustained congenital or traumatic nerve injuries. We use a standard Grass SD9 or Digitimer DS7A stimulator and create bipolar hook electrodes from EMG needle electrodes (Fig. 1).
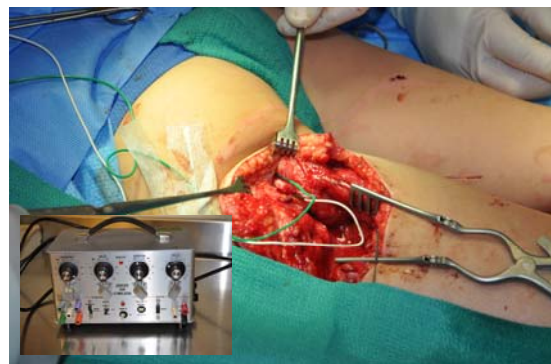

Figure 1 – Continuous 20Hz intraoperative electrical stimulation of a transected sciatic nerve was performed for one hour in a pediatric patient. The stimulator (Grass SD9) that was used to deliver the stimulation pulses is shown in the inset.

An advantage of our ES paradigm in patients is that we perform the ES of the injured nerve under general anesthesia. This permits the use of constant voltage or current bipolar electrical pulses throughout the treatment period. Anecdotal evidence of excellent functional recovery in a child nine months after sciatic nerve transection and repair via a sural

autograft with ES (Fig. 1), includes her ability to run freely with little evidence of any deficits [13]. Limitations to this approach however, include the one-hour window required with the current ES protocol and its concurrent expense of the prolonged operating time with limited operative facilities, and the difficulties inherent in the use of commercial stimulators. The surgeons and nurses are not familiar with the different stimulation parameters that must be adjusted on the stimulators. In addition, the 'set-up' of the ES hardware is far from ideal with the non-sterile stimulators needing to be placed at a distance from the sterile operative field. Additionally, the connections between the stimulator and the electrodes at the surgical site are long and the electrodes are presently difficult to secure on the nerve proximal to the site of surgical repair. Our laboratory is currently working to address these concerns.

III. ENHANCING REINNERVATION BY DAILY ELECTRICAL MUSCLE STIMULATION (EMS)

It is well known that electrical muscle stimulation following spinal cord injury can reduce disuse atrophy [14], a condition in which muscles remain innervated but inactive. Electrical stimulation of denervated muscles is difficult, the high capacitance of the muscle membranes requiring long duration electrical pulses to depolarize the membranes sufficiently to generate action potentials, and in turn, muscle contractions. When done effectively, the EMS reverses denervation atrophy, often completely [15]–[17]. In the case of partial nerve injuries, excessive daily exercise as well as daily continuous ES of intact nerves or denervated muscles was shown to be counterproductive for effective axonal sprouting in rat studies [18]–[21]. In the case of complete nerve transection or crush injuries, there are studies that advocate that EMS is effective in promoting functional recovery. The data is controversial however. For example, one animal study provided some evidence of small but significant negative effects on recovery of locomotion [22]. Another study showed detrimental effects of EMS with significant reduced numbers of reinnervated motor end-plates [23]. Particularly in light of the effective use of EMS in reducing atrophy of denervated muscle, we explored EMS paradigms to stimulate denervated muscles after nerve injuries and during the course of reinnervation. Classically, problems of EMS have suffered from the lack of rational choices of EMS paradigms, including the length of treatment, the number of contractions, pulse frequency and duration, and other stimulation parameters.

On the basis of a thorough consideration of the available paradigms used to date [24], we designed an EMS paradigm with moderate levels of daily muscle activation in a rat model that is readily translatable to the clinic. Notably, this EMS paradigm would not be a burden to patients. We settled on a 1-hour EMS paradigm that evoked 600 fused tetanic contractions and was repeated for five days per week in rats. We tested our EMS paradigm first on denervated gastrocnemius muscle [25]. Our continuation to a rat model of tibial nerve injury transection and immediate repair to mimic the clinical scenario of acute trauma, aimed to determine if daily EMS has a positive effect on muscle reinnervation, as suggested by the previous literature.

At short intervals after surgical nerve transection and repair, we recorded evoked muscle potentials in response to stimulation of transected nerve, proximal to the repair site. Thereby we determined whether more nerves regenerate and reinnervate the denervated muscle after daily EMS. As shown in Fig. 2, daily EMS significantly increased the number of regenerated tibial nerves that reinnervated the muscle as early

as two weeks post injury and repair [26]. When the EMS was performed over the longer period of three months, the early accelerated muscle reinnervation was evident throughout the period of continued nerve regeneration [27].
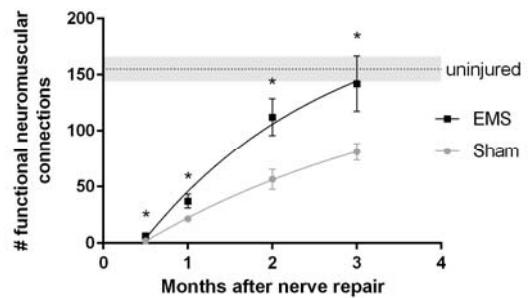

Figure 2 – Following tibial nerve transection and immediate repair, the number of regenerated tibial nerves that make functional neuromuscular connections was significantly higher at every time point (p<0.05, unpaired t-test) after daily EMS. The number was determined by recording evoked electromyographic signals from the rat gastrocnemius muscle in response to tibial nerve stimulation at 1 Hz.

Moreover, this effect translated into significantly improved skilled locomotion. In these latter studies, we examined the running of rats along a tapered beam as illustrated in Fig. 3A. The experimental rats in which the denervated muscles were electrically stimulated for three months, demonstrated significantly improved skilled locomotion as early as four weeks, their feet slipping over the beam significantly fewer times than the feet of the control rats whose denervated muscles were not stimulated (Fig. 3B). Overall, daily EMS significantly improved functional recovery and reinnervation in rats following peripheral nerve injury and repair.
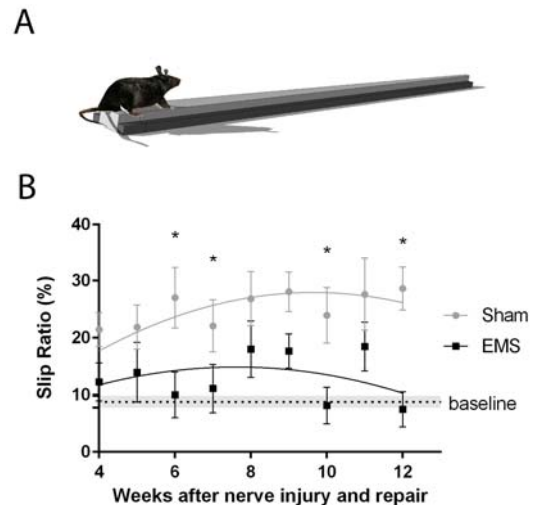

Figure 3 – Skilled locomotion assessment of rats was evaluated using a tapered beam test (A). Rats run across a narrowing beam and the number of slips are counted and expressed a percentage of total steps taken. In muscles that received daily EMS the slip ratio was significantly lower than in sham stimulated muscles (p < 0.05, repeated measures ANOVA) as early as 6 weeks post tibial nerve injury and repair (B). Rats with non-stimulated muscles continued to have impaired skilled locomotion 12 weeks post tibial nerve injury and repair.

What is the mechanism of this EMS effect? Using the same tibial nerve injury and surgical repair model in rats we examined mRNA levels of the two most potent neurotrophic factors for motoneurons: brain-derived neurotrophic factor (BDNF) and glial cell-derived neurotrophic factor (GDNF),

in the distal stump (Fig. 4A) and the muscle (Fig. 4B), two weeks after nerve injury and repair. We found that the mRNA levels of both of these trophic factors were significantly upregulated in the muscles but *not* the distal nerve stumps following daily EMS.
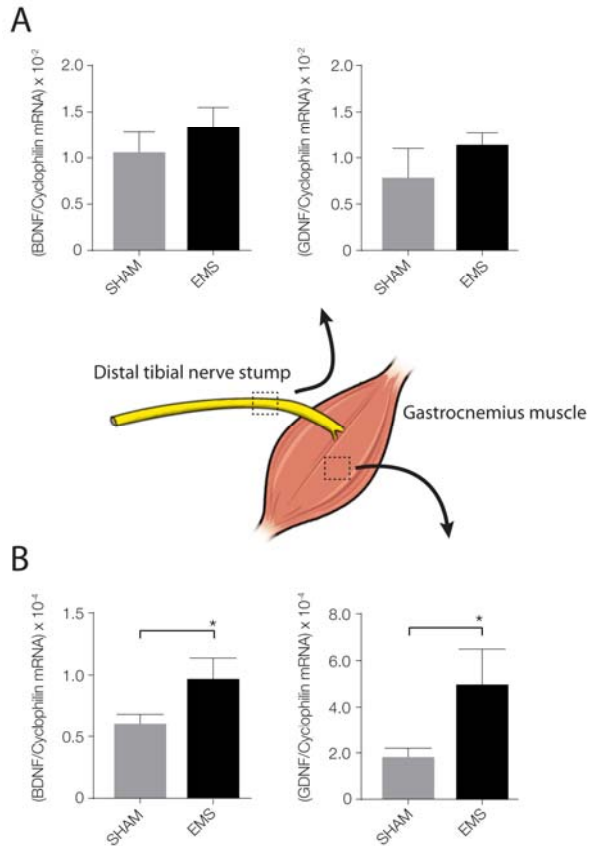

Figure 4 – Levels of mRNA of brain-derived neurotrophic factor (BDNF) or glial cell-derived neurotrophic factor (GDNF) were not significantly elevated in (A) the distal nerve stump after 2 weeks of EMS following tibial nerve transection and repair. Intramuscular levels of BDNF and GDNF *were* significantly ($p < 0.05$, t-test) elevated in the gastrocnemius muscle (B) that received daily EMS in comparison to sham stimulated muscles.

We discounted the possibility that the large electrical currents used to stimulate denervated muscles may depolarize axons within the proximal nerve stump to mimic the ES effect of promoting axon outgrowth and accelerated muscle reinnervation. We ascertained that, despite the conduction of antidromic action potentials to the proximal nerve stump immediately after nerve transection and repair, our initiation of daily EMS two days following surgery was sufficient for complete failure of electrical propagation of retrograde action potentials. These findings provide strong evidence that the EMS effect is localized to the muscle.

The upregulation of intramuscular mRNA levels of GDNF may accelerate functional connection with denervated neuromuscular junctions by allowing axons to branch extensively, a phenomenon that was observed in mice that genetically over-expressed intramuscular GDNF [28]. Another explanation may be that increased intramuscular mRNA levels of BDNF and GDNF lead to increased synthesis of the trophic factors. The factors may diffuse proximally and act on the advancing growth cone to enhance nerve outgrowth.

IV. CONCLUSIONS

Our findings demonstrate that both electrical nerve stimulation (ES) and electrical muscle stimulation (EMS) promote nerve regeneration and accelerated muscle reinnervation, the effects mediated via neurotrophic factor upregulation in the neuron and the muscle, respectively. Whether the two therapies produce a synergistic effect is an important question that remains to be addressed.

The clinical application would be a novel and effective adjunct to surgical repair of peripheral nerve injuries. The therapies would be the first treatment, other than surgery itself, to promote nerve regeneration, and in turn, functional recovery. Thereby, the adverse effects of time and distance on nerve regeneration and target reinnervation could be ameliorated [2], [3]. Presently, clinicians use off the shelf commercial equipment to stimulate nerves proximally for 1-hour during the course of a surgery or post-operatively [9], [10] (Fig.1). Commercial stimulators for EMS of denervated muscle are not widely available with one exception, the Stimulette Den2X that is available only in Europe. Development of small portable stimulators that can be used for different denervated muscle groups is necessary to facilitate the adoption of EMS in patients with nerve injuries. Once these issues are addressed, we anticipate that the adoption of these two therapies of electrical stimulation of nerve and denervated muscle to complement nerve surgery will occur in the near future.

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