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“Supercharge Nerve Transfer to Enhance Motor Recovery, a Laboratory Study”

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Abstract

Purpose—To investigate the ability of a supercharge end-to-side (SETS) nerve transfer to augment the effect of regenerating native axons in an incomplete rodent sciatic nerve injury model.

Methods—Fifty-four Lewis rats were randomized to 3 groups. The first group was an incomplete recovery model (IRM) of the tibial nerve complemented with a SETS transfer from the peroneal nerve (SETS-IRM). The IRM consisted of tibial nerve transection and immediate repair using a 10mm fresh tibial isograft to provide some, but incomplete, nerve recovery. The 2 control groups were IRM alone and SETS alone.

Nerve histomorphometry, electron microscopy, retrograde labeling, and muscle force testing were performed.

Results—Histomorphometry of the distal tibial nerve showed significantly increased myelinated axonal counts in the SETS-IRM group compared to the IRM and SETS groups at 5 and 8 weeks. Retrograde labeling at 8 weeks confirmed increased motoneuron counts in the SETS-IRM group. Functional recovery at 8 weeks showed a significant increase in muscle specific force in the SETS-IRM group compared to the IRM group.

Conclusions—A SETS transfer enhanced recovery from an incomplete nerve injury as determined by histomorphometry, motoneuron labeling within the spinal cord, and muscle force measurements.

Clinical Relevance—A SETS distal nerve transfer may be useful in nerve injuries with incomplete regeneration such as proximal Sunderland II or III degree injuries, where long regeneration distance yields prolonged time to muscle reinnervation and suboptimal functional recovery.

Keywords

Nerve regeneration; nerve transfer; neurorrhaphy; peripheral nerve; supercharge end-to-side

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Introduction

The management of proximal second or third degree nerve injuries, or *incomplete* injuries, is challenging^{1,2} because partial regeneration is expected, but in a delayed fashion (~1mm a day) with the likelihood of incomplete recovery. The time necessary for muscle reinnervation and the number of regenerating axons that successfully reinnervate the muscle play a major role in motor recovery after nerve injury³⁻⁸. With extended denervation times, Schwann cells (SCs) in the distal nerve become less supportive of regenerating axons⁹⁻¹¹, the denervated muscle begins to atrophy resulting in profound muscle changes including apoptosis and fibrosis^{3,4,6-8,12-17}, and fewer motor neurons from the proximal nerve regenerate^{4,7,11,16,18,19}. Replacement of muscle fibers by adipose tissue has also been seen with chronic denervation^{16,20}. Interstitial fibrosis may prevent appropriate intramuscular axonal regeneration^{4,15}, leading to decreased numbers of motor endplates, and ultimately diminished motor recovery²¹. Therapeutic strategies that decrease the duration of end organ denervation may have a major clinical impact on motor recovery.

A “supercharge” end-to-side (SETS) nerve transfer moves an expendable, distal motor nerve donor to the side of an injured recipient nerve^{22,23}. This provides both earlier reinnervation of the target muscle as well as additional regenerating motor axons to more completely reinnervate neuromuscular junctions.

We have previously shown in a model of a *complete* nerve injury (Sunderland IV and V degree) that axonal regeneration indeed occurs across the SETS coaptation and that the regeneration is equivalent to that of an end-to-end nerve transfer²³. The purpose of this study was to evaluate the SETS effect in an *incomplete* recovery model^{3,24-26} using histomorphometric analysis, functional muscle force testing, and retrograde labeling.

Methods

Animals

Fifty-four adult male Lewis rats (200-250 grams; Charles River Lab, Wilmington, MA) were used. Rodents were housed in a central animal care facility. Food and water were provided *ad libitum*. Animals were monitored for appropriate postsurgical recovery and weight gain. All study procedures were approved by our institutional animal studies committee.

Experimental Design

Surgical Procedure—All surgical procedures were performed aseptically using an operating microscope. Animals were anesthetized by subcutaneous administration of a mixture of 75 mg/kg ketamine hydrochloride (Fort Dodge Animal Health, Fort Dodge, IA) and 0.5 mg/kg dexmedetomidine hydrochloride (Orion Corporation, Espoo, Finland). A gluteal muscle splitting incision was performed to expose the sciatic nerve and its trifurcation. Careful neurolysis was performed to isolate the peroneal and tibial nerves. The tibial nerve was sharply transected 8 mm distal to the sciatic trifurcation. Three experimental groups were studied (Figure 1). Six animals per group²³ and 2 endpoints were evaluated: 5 and 8 weeks.

The first group was the incomplete recovery model (IRM) with SETS nerve transfer, termed SETS-IRM. To create the IRM, a 10 mm fresh tibial isograft was interposed between the 2 transected ends of the tibial nerve and coapted with 10-0 nylon suture. We have previously shown that this nerve graft model provides some, but incomplete, recovery^{3,24-26}. To create the SETS nerve transfer, the peroneal nerve was sharply transected approximately 15-20 mm distal to the sciatic nerve trifurcation. An epineurial and perineurial window was created in

the side of the tibial nerve approximately 15 mm distal to the proximal isograft coaptation. The peroneal nerve was then transferred in an end-to-side fashion using interrupted 10-0 nylon suture.

The second group represented the IRM alone. The tibial transection was performed and a tibial isograft was interposed in between the 2 cut ends as described above.

In the SETS control group, the tibial nerve was transected and both ends were cauterized and capped with silicone to prevent regeneration. The SETS nerve transfer from the peroneal to the tibial nerve was then performed as described above. The epineurial/perineurial window was created in the side of the tibial nerve approximately 5 mm distal to the capped end of the distal stump.

In all animals, muscle and skin were reapproximated, anesthesia was reversed and the animals were recovered on a warming pad and were monitored for postoperative complications.

At a 5 week endpoint, animals (n=6 per group) were re-anesthetized and the incision site was opened. Neurolysis was performed to expose the sciatic and tibial nerves. A 10mm section of the tibial nerve both proximal and distal to the SETS coaptation site was removed en-bloc. An additional group of animals (n=6 per group) underwent muscle force analysis performed at 8 weeks postoperatively²⁷. Once muscle force testing was complete, the nerves were sharply excised in the same manner as the 5 week groups for histomorphometric and electron microscopic analysis. The gastrocnemius muscle from the operated side and non-operated side of the 8 week animals were removed for mass analysis to calculate specific force. The animals were then killed. An additional cohort of animals divided into the same 3 groups, (n=6 per group) underwent retrograde labeling as described below.

Light Microscopy and Histomorphometry—Harvested tibial nerves from the Lewis rats were processed as previously described^{27,28}. Cross-sections, 1 μ m thick, were obtained from the distal region of the nerve and stained with toluidine blue. Images were examined using a digital image analysis system linked to morphometric software (Leco Instruments, St. Joseph, MI) and custom algorithms²⁹. Images taken at 1000X magnification with an oil-immersion lens were measured to determine fiber area and density of myelinated fibers. A single histologist (DAH) performed image analysis and was blinded to treatment group.

Retrograde Labeling—A separate cohort of animals were divided into the 3 experimental groups (n=6) and underwent retrograde labeling at 8 weeks with Fast Blue (Polysciences, Inc., Warrington, PA) as previously described^{30,31}. Retrograde labeling was performed to determine the origin of the distal regenerating nerve fibers—to differentiate discrete regenerating axons forming true, new motor connections from axonal sprouting as the source of increased distal fiber counts. Briefly, the distal tibial nerve was sharply transected 10 mm distal to the SETS or graft coaptation and the free end was dipped in Fast Blue for a period of 2 hours. Seven days later (9 weeks postoperatively), the animal was killed, and the spinal cord harvested for analysis of motoneuron counts at the L4-S2 levels by a blinded observer (DAH).

Dynamic Muscle Force Measurement—Postoperative motor recovery was assessed by measuring evoked force production in the gastrocnemius muscle as described previously²⁷. At 8 weeks postoperatively, animals were anesthetized (n=6 per group). The gastrocnemius muscle and distal tendon were exposed through a skin incision extending from the calcaneus of the foot to the popliteal fossa of the knee. The distal gastrocnemius tendon was then severed, freed from any attachments, and sutured to a stainless steel S-hook. The sciatic

nerve was exposed and lysed just distal to the point of trifurcation. The rat was then attached to the evoked muscle force apparatus. Measurement of evoked gastrocnemius muscle force from stimulation of the sciatic nerve was completed. Force measurements were standardized by adjusting the amplitude of applied electrical impulses and the length of the gastrocnemius muscle to produce maximum twitch force for each rat. The optimal stimulus amplitude and optimal muscle length were used for all subsequent measurements. Maximum isometric tetanic force was elicited by repeated electrical pulses (pulse width 0.2 msec, burst width 300 msec) of different frequencies (pulse frequency 30–400 Hz) to the sciatic nerve while resulting force production in the gastrocnemius muscle was recorded. Trials were separated by a 2-minute recovery period to reduce muscle fatigue. All animals were killed following muscle force analysis and tissue harvest.

Electron Microscopy—Nerve samples were prepared as described previously³². Electron microscopy was performed by a non-blinded operator (DAH) for descriptive analysis.

Statistical Analysis—Study groups were first evaluated for normality and equal variance. The datasets for all morphometry parameters, specific force, and motoneuron counts met both assumptions, and all groups were compared using analysis of variance followed by specific group comparisons by independent T-tests. For all comparisons, a *P* value <0.05 was considered statistically significant. Results are displayed as mean±STD.

Results

All animals survived in the SETS-IRM and SETS groups for retrograde labeling (n=6 per group). Two animals in the IRM group assigned to retrograde labeling died from anesthetic complications, giving an n=4. In the SETS group undergoing muscle force analysis, 1 animal died from anesthetic complications, giving an n=5. All animals in the other muscle force and histomorphometry groups survived, allowing for final outcome measures of n=6 per group.

Histomorphometry

Myelinated fiber counts in the SETS-IRM group at both 5 and 8 weeks were significantly higher than those of the IRM group and the SETS group (Figure 2). Nerve density, measured in nerves/mm², was significantly increased in the SETS-IRM group (24,581±10291) compared to the IRM group (15,200±4569). There was a trend towards increased nerve density in the SETS-IRM group versus the SETS group (19,717±3084), but this did not achieve statistical significance. Representative light microscopy cross sections of toluidine blue stained distal tibial nerve qualitatively revealed more robust regeneration in the SETS-IRM group (Figure 3).

Retrograde Labeling

The number of labeled motor neurons in the SETS-IRM group was significantly higher than the IRM group. There was a trend towards increased labeled motor neurons in the SETS-IRM group versus the SETS group, but this was not significant (Figure 4). Qualitatively, the intensity and density of Fast Blue labeling was increased in the SETS-IRM group compared to the IRM group (Figure 5).

Dynamic Muscle Force Measurements

When the sciatic nerve was stimulated, the gastrocnemius muscle specific force (N/cm²) was significantly higher in the SETS-IRM group compared to the IRM group. There was a trend toward increased muscle specific force compared to the SETS group; however, this was not significant (Figure 6). When the peroneal SETS nerve transfer was cut in the SETS-IRM

group, the peroneal contribution to gastrocnemius muscle force was removed, and stimulation of the sciatic nerve allowed for measurement of muscle force attributable to the *incomplete* reinnervation from tibial nerve only. With transaction of the peroneal SETS transfer, the specific force in the SETS-IRM group decreased to the point where significance was lost compared to the other groups (Figure 7).

Electron Microscopy

Cross sections of the nerve qualitatively revealed a larger amount of large myelinated fibers in the SETS-IRM group in comparison to the IRM and SETS groups alone (Figure 8).

Discussion

With proximal complete nerve injuries (Sunderland IV and V degree), the prolonged time to reinnervation of distal muscle results in atrophy and fibrosis, making the muscle unresponsive to reinnervation. For these injuries, end-to-end (ETE) nerve transfers have recently been popularized as an alternative to primary repair or grafting. However, in the situation of partial injuries (Sunderland II and III degree), an ETE transfer would preclude the possibility of recovery from the native, regenerating axons. For these in-continuity injuries with possibility of some recovery, the transfer of an expendable normal nerve to the side of the denervated nerve could potentially augment function.

As early as 1873, Letiéviant's textbook described with a series of drawings ETE, end-to-side (ETS), and reverse end-to-side (RETS) or SETS transfer (Figure 9)³³. In 1929, Foerster illustrated the same nerve transfers in his publication^{34,35}. Balance in 1903 reported the transfer of the distal stump of the injured facial nerve to the side of the normal accessory nerve for facial reanimation. This surgery was performed in 1895^{36,37}. Lurje described an ETS case in 1948³⁸ by Viterbo et al in 1992^{37,39}. The ETS involves the transfer of an injured nerve to the side of an intact normal nerve. For a more detailed history of the ETS transfer, multiple reviews are available^{37,40-43}. What has been demonstrated through experimental study is that *sensory* axons will collaterally sprout spontaneously from a normal nerve to a denervated nerve, and we routinely use this technique clinically to recover some sensation in non-critical sensory territories including nerve graft donor sites⁴⁴. Motor axons, however, require a deliberate injury (i.e., a crush or neurotomy) to the donor nerve to generate sprouting^{45,46}.

Transfer of an intact motor nerve into the side of an injured nerve has been termed RETS^{23,47,48}. To simplify this RETS nomenclature, we prefer the term supercharge end-to-side (SETS)⁴⁹, in a way similar to the vascular description⁵⁰. In 1988, Terzis described this in a clinical report and termed it the babysitter procedure. It involved taking a normal motor fascicle from the hypoglossal nerve and transferring it to the side of an injured, in-continuity facial nerve to gain some reinnervation of the facial musculature while awaiting cross facial nerve grafting⁵¹. Millesi also reported clinical experience with this technique⁵².

In a rat model, Isaacs et al compared primary repair of a transected peroneal nerve to RETS transfer of the tibial nerve to the side of the peroneal nerve, both with and without peroneal nerve transection. Muscle force testing of the extensor digitorum longus (peroneal innervated) demonstrated statistically similar outcomes between the peroneal primary repair group and the RETS tibial to peroneal nerve transfer group where the peroneal nerve had been transected (RETS only). No muscle force could be attributed to the tibial nerve transfer when the peroneal nerve had not been injured. The authors concluded that a RETS nerve transfer could innervate a denervated muscle⁴⁷. In a second set of experiments, Isaacs et al compared muscle force in the gastrocnemius muscle between 2 groups—one that had transection/primary repair of the tibial nerve and one that had transection/primary repair as

well as SETS transfer of peroneal nerve to the tibial nerve. Proximal stimulation was performed, followed by stimulation after elimination of a potential regenerative pathway. Both axonal pathways—direct repair and SETS transfer—achieved noteworthy reinnervation⁴⁸.

Isaacs et al were not able to “find the origin of the axons that successfully achieved motor reinnervation”⁴⁸. To more clearly determine the source of these nerve fibers, we used Thy1-green fluorescent protein transgenic Sprague Dawley rats, whose neural tissues express green fluorescent protein^{23,53,54}. These animals provided actual visual confirmation that donor axons could be transferred from a donor nerve into the side of a recipient nerve after epineurotomy and that the axons would regenerate distally toward a new muscle target (Figure 10). This study additionally showed that SETS transfer was as effective at donating axons to an injured nerve as a traditional ETE transfer as evidenced by equivalent axonal counts on histomorphometry.

In the current study, we evaluated the efficacy of the SETS transfer in an incomplete recovery model and used retrograde labeling to evaluate motoneuron contribution versus axonal sprouting. The intensity and quantity of ventral horn motoneuron labeling was significantly higher in the SETS-IRM group compared to the IRM alone or SETS alone groups (Figures 4 and 5). Because the labeling was performed distal to the coaptation of the SETS transfer, this increase in the SETS-IRM group was attributable to axonal contribution from both nerves: the incompletely injured native nerve (tibial) and the SETS transfer (peroneal). The fact that higher motoneuron counts at the spinal cord level correlated with higher myelinated fiber counts in the distal tibial nerve within the SETS-IRM group rules out sprouting alone as an explanation for higher fiber counts. The SETS transfer provides *additional* motoneurons to the target muscle. Furthermore, myelinated nerve fiber counts were significantly higher per histomorphometry in the SETS-IRM group, which correlated with greater function, as muscle specific force was significantly higher as well. Electron microscopic analysis supported the histomorphometry results.

The peroneal nerve contains approximately half the number of motor neurons of the tibial nerve⁵⁵. Studies have shown that if the muscle is reinnervated in a timely fashion, full recovery can be obtained with less than half of the original number of innervating motor neurons¹¹. This is accomplished via compensation from increased motor unit size within the muscle (i.e. fewer motor neurons innervate more muscle fibers). We believe that the SETS transfer provided motor axons at an earlier time point due to its proximity to the denervated muscle. The number of motor neurons that successfully connected with the denervated muscle via the SETS was sufficient to recover force that was not statistically different from the SETS-IRM. However, the force generated by the SETS group was not statistically different from the IRM group alone. In contrast, the SETS-IRM was statistically different from the IRM. While the innervating motor neurons from the SETS alone could garner force that was indistinguishable from the SETS-IRM, it was not large enough to provide statistical benefit over the IRM. Thus it is likely that fewer motor neurons in the SETS group were innervating a higher ratio of motor fibers in the muscle to provide compensation in muscle force. In support of this hypothesis, while not significant, there were a greater number of motor neurons in the SETS-IRM group in comparison to that of the SETS alone.

The specific muscle force of the SETS-IRM group did not drop to the same level as the IRM group after transection of the SETS nerve transfer (Figure 7). We believe this was due to earlier muscle reinnervation, similar to that of babysitting²². With this effect, even if the same number of native tibial nerve axons regenerated to the muscle in the IRM and SETS-IRM groups, the muscle performed better in the SETS-IRM group because it had been protected from atrophy. For this reason, although not directly proven in our analysis, we

believe that the SETS transfer provided a protective effect on muscle function beyond simply delivering more motor neurons. Further studies will address this hypothesis.

There are currently no proven surgical options for incomplete nerve injuries (Sunderland II and III degree). Although the SETS concept is not new and has been used clinically since the 1980's, we believe that this study adds further support for the SETS transfer as a treatment option for this challenging nerve injury.

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1. Experimental groups: Incomplete recovery model (IRM), "Supercharge" end-to-side (SETS) transfer to incomplete recovery model (SETS-IRM), and SETS alone. Red line indicates area where histomorphometry and retrograde labeling was performed.

2.

Myelinated fiber counts at 5 and 8 weeks for each of the 3 experimental groups: There is a significant increase in the amount of myelinated fibers in the distal tibial nerves at both 5 and 8 weeks in the SETS transfer to incomplete recovery model (SETS-IRM) group in comparison to the other 2 groups.

3. Toluidine blue staining of cross sections of the distal tibial nerve for each of the 3 experimental groups at 8 weeks: Larger images correspond to 400x magnification, while images in lower left corner are 1000x magnification. Robust axonal regeneration can be seen in the SETS transfer to incomplete recovery model (SETS-IRM) group in comparison to the other 2 groups: IRM and SETS. White line = 20 μ m.

4. Motor Neuron Counts: After retrograde labeling with Fast Blue, motor neuron counts demonstrate a significant increase in labeling in the SETS transfer to incomplete SETS-IRM group as compared to that of the IRM group.

5.

Retrograde labeling: Qualitative and quantitative increase in intensity and density of Fast Blue staining of the motor neuron pool of the SETS-IRM group in comparison to that of the IRM group.

6. Measured specific force (N/cm²) in the 3 experimental groups: There is a significant increase in the amount of force generated in the SETS-IRM group in comparison to that of the IRM group.

7.

Measured specific force (N/cm²) in the 3 experimental groups after the SETS contribution of SETS-IRM group was cut: After the SETS contribution was divided, the specific force did not decrease to the level of the IRM group alone. Dotted line indicates specific force generated in the SETS-IRM prior to the division of the SETS contribution.

8.
Electron Microscopy: Cross sections of the distal tibial nerve at 2500X. (A) SETS-IRM, (B) IRM, (C) SETS.

Adapted from Létiévant's *Traité Des Sections Nerveuses*. 1873.

9.

Illustrations from Letiévant's textbook in 1873 depicting end-to-end transfers (III), an end-to-side transfer (I), and a supercharge end-to-side transfer (II).

Adapted from Kale SS, Glaus SW, Yee A, et al. Reverse end-to-side nerve transfer: from animal model to clinical use. *J Hand Surg Am.* Oct 2011;36(10):1631-1639 e1632.

10.

Confocal imaging of a transgenic Thy1-GFP rat demonstrating timeline and direction of axonal regeneration through the SETS coaptation and down the denervated tibial nerve.