Noninvasive activation of cervical spinal networks after severe paralysis

Parag Gad1, Sujin Lee2, Nicholas Terrafranca3, Hui Zhong1, Amanda Turner1, Yury Gerasimenko1,4,10 and V Reggie Edgerton1,5,6,7,8,9*

Affiliations:
1 Department of Integrative Biology and Physiology, University of California, Los Angeles, CA 90095 USA
2 Veterans Affair Healthcare System Spinal Cord Injury and Disorders Center, Long Beach, CA 90822
3 NeuroRecovery Technologies INC, San Juan Capistrano, CA 92675, USA
4 Pavlov Institute of Physiology, St. Petersburg 199034, Russia
5 Department of Neurobiology, University of California, Los Angeles, CA 90095 USA
6 Department of Neurosurgery, University of California, Los Angeles, CA 90095 USA
7 Brain Research Institute, University of California, Los Angeles, CA 90095 USA
8 Institut Guttmann. Hospital de Neurorehabilitació, Institut Universitari adscrit a la Universitat Autònoma de Barcelona, Barcelona, 08916 Badalona, Spain
9 The Centre for Neuroscience and Regenerative Medicine, Faculty of Science, University of Technology Sydney, Ultimo, 2007, NSW, Australia
10 Institute of Fundamental Medicine and Biology, Kazan Federal University, Kazan, Russia.

Parag Gad
Telephone: (310) 825-4780; Fax: (310) 206-9184; E-mail: paraggad@ucla.edu

Sujin Lee
Telephone: (949) 388-4430; Fax: (310) 206-9184; E-mail: sujinl3@uci.edu

Nicholas Terrafranca
Telephone: (949) 388-4430; Fax: (310) 206-9184; E-mail: nickt@neurorecoverytech.com

Hui Zhong
Telephone: (310) 825-4762; Fax: (310) 206-9184; E-mail: vzhong@ucla.edu

Amanda Turner
Telephone: (310) 825-4780; Fax: (310) 206-9184; E-mail: mandyturner@ucla.edu

Yury Gerasimenko
Telephone: (310) 825-1910; Fax: (310) 206-9184; E-mail: yuryg@ucla.edu
V Reggie Edgerton

Telephone: (310) 825-1910; Fax: (310) 206-9184; E-mail: vre@ucla.edu

* Corresponding Author

V. Reggie Edgerton, PhD

Department of Integrative Biology and Physiology

University of California, Los Angeles

Terasaki Life Sciences Building

610 Charles E. Young Drive East, Los Angeles, CA USA 90095-1527

Telephone: (310) 825-1910; Fax: (310) 206-9184; E-mail: vre@ucla.edu
Abstract

Paralysis of the upper extremities following cervical spinal cord injury (SCI) significantly impairs one’s ability to live independently. While regaining hand function or grasping ability is considered one of the most desired functions in tetraplegics, limited therapeutic development in this direction has been demonstrated to date in humans with a high, severe cervical injury. The underlying hypothesis is after severe cervical SCI, nonfunctional sensory-motor networks within the cervical spinal cord can be transcutaneously neuromodulated to physiological states which enables and amplifies voluntary control of the hand. Improved voluntary hand function occurred within a single session in every subject tested. After 8 sessions of noninvasive transcutaneous stimulation, combined with training over 4 weeks maximum voluntary hand grip forces increased by ~325% (in the presence of stimulation) and ~225% (when grip strength was tested without simultaneous stimulation) in chronic cervical SCI subjects (AIS B, n = 3. AIS C, n = 5; 1-21 years post injury). Maximum grip strength improved in both the left and right hands and the magnitude of increase was independent of hand dominance. We refer to the neuromodulatory method used as transcutaneous enabling motor control (tEmc) to emphasize that the stimulation parameters used are designed to avoid directly inducing muscular contractions, but to enable task performance according to the subject’s voluntary intent. In some subjects there were improvements in autonomic function, lower extremity motor function and sensation below the level of the lesion. Although a neuromodulation-training effect was observed in every subject tested, further controlled and blinded studies are needed to determine the responsiveness of a larger and broader population of subjects varying in the type, severity and years post injury. It appears rather convincing, however, that a “central pattern generation” phenomenon as generally perceived in the lumbosacral networks in controlling stepping neuromodulator is not a critical element of spinal neuromodulation to regain function among spinal networks.

Keywords:
Non-invasive spinal cord stimulation
Cervical spinal cord injury
Upper extremity rehabilitation
Tetraplegia
Introduction

Over one million people in the United States cannot walk and have minimal function in the upper limbs and hundreds of thousands have lost control of vital bodily functions due to spinal cord injury (SCI). While there has been considerable progress in rehabilitation efforts focusing on adapting the individual to the disability, improvements beyond the immediate spontaneous lost function has been limited. One approach has been to use brain-muscle interfaces to trigger muscle stimulation directly, thus bypassing the control that is intrinsic among the spinal networks. We have reported, that the cervical spinal networks can be modulated with epidurally implanted electrodes to achieve significant functional recovery of the upper limb in SCI patients as previously shown for regaining function of the lower limbs with lumbosacral epidural stimulation.

More recently, we have developed a prototype stimulation device that can non-invasively neuromodulate the lumbosacral spinal networks (transcutaneous enabling motor control, tEmc). When tEmc is combined with training, a rapid recovery of the ability to voluntarily generate bilateral rhythmic stepping-like movements in individuals that had been completely paralyzed for more than a year. The present study was designed to determine whether the same non-invasive tEmc approach would be effective in neuromodulating the cervical spinal networks to improve upper limb motor function. The objective of this study was to test the efficacy of using tEmc in combination with upper extremity exercises over the course of 4 weeks in recovering sensory-motor function. We hypothesized that the cervical spinal segments can be neuromodulated to physiological states that can enhance voluntary motor control when used in conjunction with motor training.

Methods

Experimental Design: This study was registered with ClinicalTrials.gov, number NCT01906424. Subjects were invited to participate in the study based on the following Inclusion and Exclusion criteria.

Inclusion Criteria: 1) Age > 18 years; 2) Spinal cord injury 1 or more years prior; 3) AIS A, B and C; 4) Non progressive traumatic SCI at C7 (vertebral level) or higher; 5) Ability to commit to 12 week participation; 6) Stable medical condition without cardiopulmonary...
disease or dysautonomia that would contraindicate participation in upper extremity rehabilitation or testing activities; 7) Not dependent on ventilation support; 8) No painful musculoskeletal dysfunction, unhealed fracture, pressure sore, or urinary tract infection that might interfere with upper extremity rehabilitation or testing activities; 9) No clinically significant depression or ongoing drug abuse; 10) Adequate social support network to be able to participate in weekly training and assessment sessions for the duration of the 12 week study period; 11) No current anti-spasticity regimen; 12) Must not have received Botox injections in the prior six months; 13) Limited use upper extremity for functional tasks.

**Exclusion Criteria:** 1) Pregnancy; 2) No functional segmental reflexes below the lesion.

From those that qualified, a select group were asked to complete a pre-screening questionnaire by phone interview; from those who qualified in the interview, 11 were selected to undergo a neurological and electrophysiological evaluation out of which 8 subjects (SCI AIS-B (n = 3) and AIS-C (n = 5)) qualified for the study. Neurological evaluations by the team physician included testing sensory (soft touch via Q-tip and pin prick) and motor scores. All subjects signed an informed consent form which was approved by the Institutional Review Board (IRB) at the University of California, Los Angeles (UCLA). Six of the 8 subjects completed the trial. 452495 discontinued due to a UTI and 259463 discontinued due to educational obligations after 4 sessions.

For each subject, 3 baseline sessions were conducted over a period of 10 days including:

1) Neurophysiological tests (spinally evoked potentials, 1Hz with a 1ms pulse width and monophasic waveform) to determine recruitment profiles of proximal and distal motor pools with increasing stimulation intensity ranging from 10mA to 200mA (or if noted to be uncomfortable)

2) Hand grip with Maximum Voluntary Contraction (MVC) without stimulation

3) Identifying appropriate stimulation parameters for each site of stimulation (30Hz, 1ms pulse width, biphasic waveform (AIS C) or monophasic waveform (AIS B))

4) Efficacy of one site vs a second site vs combined two-site stimulation in generating a MVC based on stimulation parameters identified in step # 3
At the end of the baseline sessions, the formal 4-week intervention program (2 sessions/week) using a proprietary multichannel transcutaneous stimulator to neuromodulate the spinal cord began. Each session consisted of 1-2hr/day including a series of voluntary hand grip tasks, beginning without tEmc, followed by series of 18 attempts to generate an MVC (for ~5 secs) for each hand with tEmc. At the end of the session, MVCs were repeated without tEmc.

At the end of the intervention, 6 subjects that completed the study underwent final neurological, functional and electrophysiological evaluation. Functional changes associated with “Quality of Life” were self-reported by all subjects before every session. Of special note, during and between all sessions, no adverse events were reported. The stimulation intensity used caused no discomfort of concern to the patients, did not negatively affect their breathing patterns, heart rate or blood pressure, cause any adverse skin reaction at the stimulation site nor result in any adverse effects on severity of self-reported spasticity.

tEmc stimulation protocol: The experiments were carried out with use of a proprietary non-invasive Transcutaneous Electrical Spinal Cord Stimulator (NeuroRecovery Technologies INC). During baseline and final evaluation, each subject was evaluated for functional responses to single versus two-site stimulation. In each case, it was noted that applying stimulation simultaneously at two sites was consistently more effective. This finding was consistent with previous studies of lower extremity function showing that multisite spinal cord stimulation using tEmc was more effective than single site stimulation for inducing involuntary stepping movements. With this determination, we delivered transcutaneous stimulation simultaneously at two sites along the midline between spinous processes C3-C4 and C6-C7 during every interventional session. The intensity of stimulation at each spinal level was adjusted sufficiently to enable maximal grip strength when applied in isolation, without causing discomfort (range: 10-250mA). Tonic EMG responses from proximal and distal muscles along with MVC forces were observed to optimize the stimulation intensity. Further, the stimulation parameters were also adjusted based on patient feedback. Stimulation was continuously delivered using 2.0 cm-diameter hydrogel adhesive electrodes (Axelgaard, ValuTrode® Cloth) as cathodes and two 5.0 x 10.0 cm² rectangular electrodes (Axelgaard, ValuTrode® Cloth) placed symmetrically on the
skin over the iliac crests as anodes. tEmc was delivered using biphasic or monophasic rectangular 1.0-ms pulses at a frequency of 30Hz, with each pulse filled with a carrier frequency of 10 kHz (Table 1). Note, since this was a proof of concept study, the patients were made aware when the stimulation was turned on to check for any adverse events.

**EMG Recording:** Muscle activity was recorded from select proximal (Bicep Brachia) and distal (Flexor Digitoriun and Extensor Digitoriun) muscles via surface EMG electrodes (LabChart and PowerLab, ADInstruments). Data were recorded and sampled at a rate of 10 kHz and were analyzed using LabChart software. EMG data were filtered using a 60-Hz notch filter and a Butterworth bandpass filter of 10 to 1000 Hz. Peak-to-Peak analysis were conducted using the LabChart software to analyze spinally evoked EMG responses. The EMG data were filtered, rectified to analyze area under the curve during MVC tasks to calculate the integrated EMG (iEMG).

**Hand Grip Function:** Over the course of the 1-2 hr. training session, the subjects performed 2 tasks, 1) Maximum voluntary contraction (isometric) to assess and train for grip strength; 2) Voluntary rhythmic efforts of submaximal contraction (isometric) to evaluate and train for opening and closing one’s hand, equivalent to squeezing / grasping and releasing objects (voluntary movement). Each voluntary rhythmic effort maneuver was performed for 10-30 seconds (one every one to two seconds) and was repeated 18 times with each hand (left and right) over a 1-2 hr. period. The size and shape of the transducer easily accommodated the variety of hand sizes. Due to the isometric nature of the contraction, the transducer allowed subjects to train for gripping, grasping, and squeezing using primarily their forearm muscles. We found that the isometric device made it was more feasible to minimize forces that could be contributed by the subject’s shoulder and upper arm muscles compared to when pulling a spring-loaded grip device \(^3\). \(^9\). In addition, the subjects were instructed and closely monitored to assure that a neutral wrist position was maintained while performing the maximum voluntary efforts and voluntary rhythmic efforts to avoid any compensatory mechanisms such as tenodesis. The possibility of the transfer of forces emanating from movements of the more proximal arm, shoulder
and trunk muscles was also monitored for each effort, followed by examining video recordings. (Supplementary Fig 2).

**Statistical analyses:** All data are reported as mean ± SEM. Two-way repeated measures analysis of variance (ANOVA) was used to determine overall differences across time. Wilcoxon matched-paired signed rank test was used as a post-hoc test to identify difference between “tEmc Off” and “tEmc On” on a day-to-day basis. Mann Whitney U test was used to determine individual differences between “tEmc On” vs “tEmc Off” at pre-intervention and post intervention and between “tEmc Off” and “tEmc On” at pre-intervention vs. post-intervention. Coefficient of variation (cv) was calculated to determine consistency of responses at baseline. The criterion for statistical difference was set at $P < 0.05$ for all comparisons.

**Results**

All subjects demonstrated some detectable level of grip strength, ranging from ~0.1N to ~5N (mean cv = 8.78%, n = 6 subjects), without stimulation during the 3 baseline tests over 10 days (Supplementary Figure 1). We also observed that spinal networks controlling multiple primary upper extremity muscles can be activated via non-invasive spinal stimulation (single pulses) over a range of intensities (stimulation at C6-C7, 1ms PW, 10mA-200mA) (Fig. 1A). At submaximal levels of stimulation, the responses were further amplified when combined with voluntary effort to make a fist (similar to a maximum voluntary contraction, MVC). Evoked responses (middle (MR, ~17-20ms) and long (LR, 20-100ms) latency responses) at 1Hz spinal stim were amplified during MVC suggesting a highly integrated and synergistic interface between the spinal and voluntarily controlled supraspinal networks (Fig. 1B). The increase in MR amplitude from control (no voluntary effort) to MVC was highest in the Biceps while the increase in the LRs were higher in the forearm Flexors and Extensors, suggesting the activation of a larger interneuronal network projecting to the more distal motor pools (Fig. 1C-D).

During the first treatment session, all subjects were capable of generating greater grip force with compared to without tEmc. Further, the levels of activation of distal (forearm) muscles increased while the activation in proximal muscles decreased when
exposed to multisite stimulation (Fig. 2A). This is consistent with previous findings when stimulating the lumbosacral spinal cord in our studies of the lower extremity showing a more favorable effect of multisite stimulation compared to single site stimulation to control locomotor activity\textsuperscript{10} and other motor functions\textsuperscript{11, 12}. Based on the increased force voluntarily generated by the subjects, we elected to use multisite stimulation over the course of the 4 weeks of intervention. The subjects (n=5 both hands, n = 1 (491863) one hand) consistently generated higher forces both with and without stimulation at the end of an intervention session compared to pre-intervention (Fig. 3 & 4). Along with an increase in force, all subjects demonstrated a reduced reliance on proximal upper arm muscles and an increased activation of distal forearm muscles consistent with the need to stabilize the wrist during MVC\textsuperscript{13}, suggesting some functional reorganization of brain-to-spinal network connectivity projecting to different motor pools (Fig. 2C). Consistent with this functional reorganization, we observed that the spinal cord evoked responses were larger for distal muscles at the end of the intervention compared to before intervention while the amplitudes of evoked responses were lower for the biceps at post-intervention compared to pre-intervention (Fig. 4). Significant intersubject variability was observed both during baseline (Supplementary Figure 1) as well as during the day to day performances (Fig 4A). Furthermore, over the course of multiple treatments all subjects demonstrated a progressive increase in grip strength in both hands without any stimulation at the start of every session compared to before the intervention demonstrating a residual and therefore a cumulative impact. That is, a significant level of the elevated performance compared to baseline persisted (2-5 days) until the next training session occurred (Fig. 4B).

The electrophysiological evidence from spinal cord evoked potentials suggests that an increased level of spinal network excitability occurred with tonic tEmc and training. This higher excitability, in turn, enabled greater voluntarily generated forces of networks projecting to the distal forearm motor pools, reflecting more motor units exceeding their motor threshold and/or activated at a higher frequency (Fig. 5).

One subject (739144) classified as motor complete (AIS B) generated a barely detectable force and evidence of some minimal level of flexor and extensor EMG during the first day of testing without any tEmc (Fig. 6). When tEmc accompanied a voluntary
effort on the first day of testing, the subject generated a larger voluntary force along with increased tonic EMG activity in distal muscles. The oscillating force of < 0.1N in the presence of tEmc as shown in Figure 6 occurred although the effort was intended to be tonic. Along with an increase in maximal grip strength, all subjects demonstrated an increased capability to generate submaximal rhythmic voluntary contractions as well (Fig. 7). This suggests an improvement in not just the ability to squeeze the force transducer but also demonstrated better rhythmic control in opening and closing their hands.

Given that the stimulation electrodes were placed at the midline of the spinal column and both of the upperlimbs were trained similarly, we asked whether the improved function was dependent on hand dominance before the injury or to the upperlimb with the higher level of function remaining post injury? The dominant hand (before SCI) did not determine the stronger hand (after SCI). In this study, all 8 participants were right hand dominant (before SCI), however, 3 out of the 8 were stronger on the right side and 5 were stronger on the left side (at pre and post intervention). The strength of each hand and rate of recovery of grip strength varied based on the severity of the injury to that hand (Fig. 8). Smaller increases in grip forces were reported in subjects with the lower initial motor scores and lower initial grip forces, while at higher initial motor scores and higher initial grip forces, the increased grip strength were exponentially higher (Fig. 9). However, one subject (491863) was markedly different wherein the subject’s left arm did not improve even though the subject was one of the strongest at pre-intervention. Clinically, most of the subjects also showed an increase in their sensory and/or motor scores in International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI) examination with an overall mean increase of 4.4±3.3 points (n = 6 subjects) in the motor score (P>0.05) and a mean increase of 8.4±2.9 points (n = 6 subjects) in the sensory scores (significant at P <0.05) (Fig. 10).

Along with improved hand strength, some patients subjectively self-reported an improvement in performing tasks typically associated with activities of daily living. For example, improved trunk control made it more feasible to sit upright on the edge of their bed without back support and the ability to maintain an upright posture while standing in a standing frame. All subjects reported improvements in finger and hand dexterity. For
example, the ability to successfully pinch and withdraw a debit card from the ATM machine; pinch a clothes hanger clip to release the garment from the hanger; and to open their hand to hold a cup single handed, use a cell phone, rotate a door knob; turn a key lock and open a water bottle with a twist-off cap were reported by at least one subject (Table 2, Supplementary Figure 3). Along with changes in upper extremity, some of the patients reported an improvement in lower extremity function including the ability to march in place between parallel bars and the ability to voluntarily flex specific joints of the lower limbs (without stimulation). These motor function changes were accompanied by improvement in one or more autonomic functions, including improved bowel function, increased perspiration below the level of injury, increased duration of reflex erections in males and increased sensation of bladder fullness (Table 2). Patients classified as AIS B with barely detectable voluntary movement of their fingers at the start of the intervention were capable of voluntarily moving their fingers both in a gross fashion to make a fist as well as selective activation of specific fingers on command (Supplementary video 1). Anecdotally, all subjects routinely reported the maintenance of the improved function in subsequent treatment session (2-5 days between treatments on Tues and Thurs). Further, two subjects were capable of maintaining their grip strength when tested after 60 days of no stimulation (data not shown). It seems likely that the persistence of function without continuing tEmc interventions results from the persistence of the newly enabled and/or enlarged networks which can now be used in a plethora of voluntary movements to complete a range of motor task as desired throughout the normal day’s activities. It appears that this enabling phenomenon is an important feature which can improve the quality of daily life of these individuals, It also seems likely that this persistence can lead to further recovery enabling them to regain more fine motor skills in carrying out a wider range of activities of daily living such as grasping a cup of water to drink or holding a utensil to eat (details of improvements in specific subjects are summarized in Table 2).

Discussion

The present study was designed as a proof of concept and feasibility to use tEmc to neuromodulate the cervical spinal segments to improve upperlimb function. Using a completely noninvasive intervention, we have modulated the functional potential of
cervical spinal networks of severely injured human subjects to physiological states that enabled greater voluntary controlled sensory-motor function of the upper limbs in individuals that had been paralyzed up to 20+ years. We show the following effects of noninvasive neuromodulation of cervical spinal networks: 1. Recovery of increased hand grip function within one session and a mean increase of ~325% (with tEmc), from baseline measurements after 8 treatment sessions over 4 weeks. 2. Direct physiological evidence of reorganization of supraspinal-spinal networks, e.g., changes in amplitude of spinally evoked motor responses after training with tEmc and a significant increase of hand grip strength without tEmc after only 8 treatment sessions. The present data demonstrate that the levels of connectivity of descending-ascending networks between the brain and spinal cord that initially were not detectable in the absence of tEmc can be transformed with tEmc and training to a significantly greater level of function, including strength and control of grip forces. 3. Improvement in the functional state of autonomic control systems (in some individuals) emerged in response to neuromodulatory-training interventions. These improvements parallel the changes reported using epidural stimulation and locomotor training. We are not aware of any studies reporting improvement in autonomic function in individuals with chronic SCI. 4. All subjects’ self-reported improvement in one or more measures of quality of life parameters including increased sensation below the level of lesion, improved trunk control, improved bladder and bowel control (Table 2).

Limitations and immediate questions to address

The present data suggest that the novel transcutaneous modulation intervention used facilitated the recovery of sensory-motor function in individuals with a severe cervical spinal injury. A number of issues need to be addressed, however, before it can and should be available for general clinical use. This study was designed to carefully and systematically explore the relative responsiveness of individuals with severe, chronic paralysis after a cervical spinal injury to an array of experimental neuromodulatory parameters. The present results call for further critical tests of the effectiveness of the neuromodulatory intervention, one being a fully blinded control design and including other clinically relevant tests such as GRASSP and SCIM. A broader, even more heterogeneous and larger number of subjects, e.g., severity, spinal level of injury and years post injury, will also provide a
clearer perspective on the potential impact of this intervention on upperlimb function. Additionally, the correlation of the frequency and duration of the intervention to the improvement in sensori-motor function is still unknown, considering that patients implanted with epidural spinal electrodes several years ago\textsuperscript{4, 15} are still improving their lower extremity function\textsuperscript{16}.

“How do the spinal neuromodulatory procedures noted above compare with other methodologies?” For example, surgical tendon transfers\textsuperscript{17}, implantable functional electrical stimulation systems\textsuperscript{18} and bionic gloves\textsuperscript{19} have been reported to improve grip strength in tetraplegics. The improved grip strength of 225\% observed in the present study after 8 sessions (without tEmc) was significantly higher and occurred more rapidly than has been observed with surgical interventions\textsuperscript{17}, bionic gloves\textsuperscript{19} or implantable muscle stimulators. We are unaware, of an interventional device that has been applied to subjects with injuries as severe and as chronic as studied here, that has recovered similar magnitudes of sensory, motor and autonomic functions as rapidly as observed in the present study. Further, these data do not provide a direct comparison of efficacy with many other strategies that have shown some facilitation of motor function. Obviously, head-to-head comparisons of the potential of tEmc intervention with other interventions, will be in order so that comparisons of the cost-to-benefit ratios with respect to magnitude of effects, technical costs, time commitment for the subject, etc. can be weighed. It is important also to recognize, however, that we have no evidence that the intervention as used here has been developed to its optimal potential.

**Comparisons of the present non-invasive neuromodulatory strategy with other interventions designed to recover functions post paralysis**

Common questions of high relevance in the area of neuromodulation of the cervical as well as the other spinal segments are, what are the advantages of “epidural spinal stimulation over noninvasive transcutaneous spinal stimulation. Though some answers are becoming clear, much uncertainty remains and several observations reflect important advantages of both noninvasive and invasive modulation\textsuperscript{20}. Multiple functions can be treated with the transcutaneous approach in the same individual simply by moving the electrodes to stimulate at different spinal levels at the most efficacious site for a given
function along the entire length of the spine. Some patients with less severe injuries may need the stimulation only a brief time. For growing children or for older patients there will be advantages to avoid the surgical procedure. The economic consequences are likely to be >10-fold compared to an epidural implant. A disadvantage of the tEmc approach is the inconvenience of the subject being able to readily don and doff the electrodes over the spine. In some cases there may be significant advantages of selecting a more focused network that could be achieved with an implant, but in other cases a broader network neuromodulation can improve motor functions involving large groups of motor pools as needed for standing, stepping and upperbody functions. For example, the success in executing most hand movements depends on positioning the more proximal muscles of the arm, the shoulder and even the position of the trunk. In our opinion, however, at this early stage in realizing the potential of improving multiple upper extremity functions with neuromodulatory strategies and the fact that we still have only a modest understanding of the systemic, network, cellular and synaptic mechanisms involved, multiple neuromodulatory technologies should continue to be developed. Thus, we predict that it will be an advantage to have the option to use spinal epidural or transcutaneous approaches to neuromodulate the cervical, thoracic and/or lumbosacral spinal cord to facilitate sensory-motor function as well as autonomic functions such as for cardiovascular, bladder, bowel and sexual function.

A key advantage of spinal cord stimulation, either via spinal epidural or transcutaneous stimulation, is to engage rather than bypass this exquisite control system by directly stimulating the skeletal muscles. In addition, to capitalizing on the intrinsic control of a wide range of movements, as the supraspinal networks reorganize in concert with the spinal networks, spinal cord stimulation results in recruitment of motor units in a more normal order than occurs with direct muscle stimulation. This recruitment order of motor unit phenotypes provides a greater resistance to neuromuscular fatigue. In addition, the modulation of the physiological state of spinal interneurons plays a key role in enabling a continuum of the coordinated activation of more motor pools to enable and/or facilitate successful execution of a wide range of intended motor events.
Improvement in hand function facilitates the integration of multiple physiological systems

Complex muscle synergies can be engaged by activating the relevant spinal networks\(^2\). For example, neuromodulation of spinal networks at segments C3-C4 facilitated the control of upper and lower extremity movements. These observations are consistent with the idea that propriospinal neurons from the cervical pre-enlargement project to more caudal as well as rostral spinal networks, thus engaging a broad spectrum of motor pools, approximately as they might occur normally to accommodate the presumed support system needed to execute upperlimb functions\(^2\), \(^3\), \(^4\), \(^5\), \(^6\).

Other evidence of the integration of cervical and the more caudal spinal segments in spinal injury models have been demonstrated. For example, quadrupedal stepping of rats on a treadmill with an incomplete thoracic spinal cord injury activates cervical spinal networks that enable locomotor activity of the hind limbs\(^7\). Similarly, passive movement of the forelimbs in a rhythmic stepping pattern in a decerebrated cat can induce stepping in the hindlimbs even after hindlimb stepping has been blocked with a serotonergic antagonist\(^8\). It appears that the extensive remodeling capacity of spinal networks\(^9\) and its robust capability to respond to therapeutic interventions\(^10\), \(^11\), \(^12\), \(^13\) after spinal cord injury can result in a persistent synergistic presence of cervical, thoracic and lumbosacral spinal networks. Upper extremity rehabilitation, which integrates hand, arm and trunk function is also important from other perspectives. For example small improvements in upper extremity function can amplify the quality of life of subjects with paralysis\(^14\). The major point here is that theoretically the networks along the sensory motor axis will be most effective if the reorganization among networks at all levels of this axis is functionally highly synergistic. The evidence to date suggest that this synergism among networks can occur and probably be facilitated via activity dependent mechanisms following a spinal injury.

Need for Neuromodulation during training:

Although there could be some question of how much of the improvements in function observed in the present study could be attributed to training alone, i.e., without the motivation of receiving tEMc during training, this seems remote for the following reasons. During the first 3 baseline sessions of the present study, all subjects showed very
stable responses without stimulation (~0.1N – 5N, cv = 8.78%, Supplementary Fig. 1). Further, in a study of 17 subjects performing a similar MVC task once per week for 20 weeks an insignificant increase in MVC (3.78 N to 6.14 N) of subjects with AIS scores ranging from A-D (A (12), B (1), C (2), and D (2)) were reported⁹. Neither were there any changes in the SCIM scores. The very severely injured subjects (AIS A, B and some C’s) cannot train if they have no or only minimal voluntary movement. In another previous study²⁶, we used tEmc to modulate rhythmic stepping movements in AIS A and B subjects, when initially, no movement and therefore no training could occur without the neuromodulation. In subsequent treatment sessions of tEmc combined with voluntary effort, however, all of the subjects eventually recovered rhythmic stepping without tEmc. Some subjects recovered to the point that their voluntary performance was better without than with simultaneous stimulation. It is important to recognize however that it was the neuromodulation-training paradigm that enabled this transformation, from no voluntary movement, to a bilateral coordinated stepping-like movement in less than 18 treatment sessions. By that stage in the treatment, significant function had emerged even when no stimulation was provided²⁶.

The criticalness of combining the neuromodulation with an activity-dependent mechanism in the recovery of multiple sensory-motor and autonomic functions already was clearly evident in our initial paralyzed subjects that we implanted for epidural stimulation of the lumbosacral spinal networks, when no improved motor functions emerged with 80 sessions of training without stimulation. Only when they were combined, did the recovered functions emerge⁴. We have reported a similar interdependence of enabling and activity-dependent mechanisms phenomenon in adult rats with a complete mid-thoracic spinal cord transection³⁷ in which spontaneous cage activity increased 5-fold when a tonic subthreshold stimulus strength (80% of motor threshold) was applied, compared to when all conditions were identical, but no stimulation was provided. These examples of modulation by either implanted or transcutaneous electrodes show that the stimulation can enable one to move, which in turn enables training. Thus, there seems to be a clear interdependence of these two variables as they are used in improving multiple physiological systems⁴. The interactive effects of the two interventions are highly dynamic and for all practical purposes, spinal neuromodulation and training are functionally
inseparable. What remains unclear and important to define, however are some basic principles that can provide a strategy for optimizing the dose of tEmc and of training at any given functional deficit state in a given subject at any given point post-injury and in a given state of ‘wellness”. For very practical reasons (how often and how much training or stimulation), these questions are and will remain a challenge as we gain an increasingly clearer understanding of the mechanisms involved.

Summary

Our working hypothesis is that there are spinal neuronal networks above, within, and below a spinal lesion in a significant number of individuals with chronic, severe paralysis that can be neuromodulated into an elevated functional state. This can occur with specific modes of repetitive spinal stimulation which facilitates an emergence of supraspinal-spinal connectivity when simultaneously receiving highly coordinated and predictable proprioceptive and cutaneous input. A consistent concept that is evolving is that the improved functions observed in previous and the present study is that it is possible to engage surviving, but non-functional spinal networks to ones with greater intrinsic automaticity in generating coordinated motor tasks. This result reflects the presence of functional supraspinal-spinal connectivity that can mediate a conscious effort to generate a maximum grip force.

This is a feature of these spinal and supraspinal networks that, to date, has been largely overlooked in efforts to regain upper and lower sensori-motor as well as autonomic function after paralysis. We propose, therefore, that the present data provide compelling reasons to further define the critical variables and parameters to optimize functional recovery. The increasing number of examples of regained/improved supraspinal control after “complete” paralysis suggest that the basic biology of a spinal lesion that is presently clinically defined to be motor complete must, at least, be challenged.

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**Author Disclosure Statement**

V.R.E, Y.P.G, N.T. and PG, researchers on the study team hold shareholder interest in NeuroRecovery Technologies and hold certain inventorship rights on intellectual property licensed by The Regents of the University of California to NeuroRecovery Technologies and its subsidiaries.
References


Table 1: Table listing the stimulation parameters used for the 6 individuals that completed the study.

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<tr>
<th>Sub ID</th>
<th>Mode</th>
<th>Frequency</th>
<th>PW</th>
<th>Site</th>
<th>Amp</th>
</tr>
</thead>
<tbody>
<tr>
<td>511282</td>
<td>Biphasic</td>
<td>30Hz</td>
<td>1ms</td>
<td>C3-4/C6-7</td>
<td>70/70mA</td>
</tr>
<tr>
<td>793944</td>
<td>Monophasic</td>
<td>30Hz</td>
<td>1ms</td>
<td>C3-4/C6-7</td>
<td>180/210mA</td>
</tr>
<tr>
<td>773762</td>
<td>Monophasic</td>
<td>30Hz</td>
<td>1ms</td>
<td>C3-4/C6-7</td>
<td>90/140mA</td>
</tr>
<tr>
<td>491863</td>
<td>Biphasic</td>
<td>30Hz</td>
<td>1ms</td>
<td>C3-4/C6-7</td>
<td>90/100mA</td>
</tr>
<tr>
<td>265582</td>
<td>Biphasic</td>
<td>30Hz</td>
<td>1ms</td>
<td>C3-4/C6-7</td>
<td>110/140mA</td>
</tr>
<tr>
<td>511282</td>
<td>Biphasic</td>
<td>30Hz</td>
<td>1ms</td>
<td>C3-4/C6-7</td>
<td>120/120mA</td>
</tr>
</tbody>
</table>
Table 2: Table demonstrating the quality of life changes self-reported by the 8 subjects involved in the study.

<table>
<thead>
<tr>
<th>SUB ID</th>
<th>Years post SCI</th>
<th>AIS</th>
<th>Upper Extremity</th>
<th>Trunk</th>
<th>Lower Extremity</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>265582</td>
<td>21</td>
<td>C</td>
<td>Regained ability to pinch-withdraw debit card from ATM machine</td>
<td></td>
<td></td>
<td>Improved sensation in arms and trunk</td>
</tr>
<tr>
<td>452495</td>
<td>11</td>
<td>C</td>
<td>More fluid hand and finger movement</td>
<td></td>
<td></td>
<td>Significantly lower spasms in trunk and legs</td>
</tr>
<tr>
<td>491863</td>
<td>9</td>
<td>C</td>
<td>Improved ability to type on a computer</td>
<td>Improved trunk stability while sitting</td>
<td>Regained ability to flex hips and ankles and step in place</td>
<td>Improved sensation; regained ability to perspire below level of injury</td>
</tr>
<tr>
<td>058613</td>
<td>4</td>
<td>C</td>
<td>Regained ability to hold a spoon &amp; fork and handle a cell phone</td>
<td>Able to sit upright</td>
<td>Regained ability to flex ankles</td>
<td></td>
</tr>
<tr>
<td>739144</td>
<td>13m</td>
<td>B</td>
<td>Regained ability to move fingers on command</td>
<td>Improved control of posture during sitting</td>
<td></td>
<td></td>
</tr>
<tr>
<td>773762</td>
<td>13</td>
<td>B</td>
<td>Regained ability to move fingers on command</td>
<td>Improved ability to sit upright</td>
<td></td>
<td>Regained ability to perspire; regained sexual function</td>
</tr>
<tr>
<td>ID</td>
<td>Age</td>
<td>Gender</td>
<td>Characteristics</td>
<td></td>
<td></td>
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<tr>
<td>--------</td>
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<td>----------------------------------------------------------------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>511282</td>
<td>13</td>
<td>C</td>
<td>Improved ability to grasp, ping and hold a utensil, handle a door knob, hold a cup of water and open a sealed water bottle.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Improved trunk control while sitting and while standing in a stand chair.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ability to march in place.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Improved bowel control.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>259463</td>
<td>18m</td>
<td>B</td>
<td>Regained voluntary control of hand and fingers.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Improved trunk control and awareness of core.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Improved sensation in lower extremities.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure Legends

Figure 1: A) A series of spinally evoked responses (mean of 5 responses at each intensity) from proximal and distal muscles from one subject 491863 at rest with increasing intensities of stimulation at Pre-intervention. In B the thick black line represents the average control response when stimulated at 110mA, while the red trace was generated during a maximal voluntary effort to generate a grip force. C) Mean±SD (n = 5 response) spinally evoked middle responses (latency ~15ms) in B. D) Area under the curve of the rectified long latency EMG (latency 30ms-100ms) in B.
**Figure 2:** A) Representative EMG and force during a MVC during the first treatment session in an AIS C subject without stimulation; then with one site stimulation at different locations; and then with two site simultaneous stimulation. B) Representative EMG and force during a MVC in the same subject as in A) before (Pre-intervention) and after (post-intervention) stimulation.
mean±SE EMG amplitude (n = 6 subjects) during the MVC at pre and post intervention with and without 2-site stimulation shown in A. Biceps: Biceps brachii, Extensors: Extensor Digitorium, Flexors: Flexor Digitorium.

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Figure 3: A) Six individual subjects’ MVC force for left and right hands at the start and end of intervention with (red) and without (black) stimulation.  B) Normalized mean ± SE (n = 6 subjects) at Day 1 (pre-intervention) and Day 8 (Post-intervention) during tEmc ON and tEmc Off. * - tEmc On significantly different from tEmc off, † - Day 8 significantly different from Day 1.
Figure 4: A) Six individual subjects’ MVC force for left and right hands during the 8 treatment sessions without stimulation B) Normalized mean ± SE (n = 8 subjects, day 1-4, n = 6, day 5-8) forces generated without tEmc for the stronger hand. * - Significantly different from Day 1. The dotted line represents a 4th order curve fitted to the data.
**Figure 5:** A) An example of mean (n = 5 responses) evoked potential at pre-intervention (black) and post intervention (green). B) An example of mean evoked potential recruitment curve for proximal and distal muscles recorded at pre-intervention (black) and post intervention (green). C) Normalized change in maximum evoked responses (n = 6 subjects, both hands) shown in C. * significantly different from 0 at P<0.05
Figure 6: Representative EMG and force during maximum voluntary contraction with and without tEmc in an AIS B subject (739144) on the first treatment session.
Figure 7: Representative EMG and force during rhythmic submaximal voluntary efforts in an AIS B subject (739144) before (pre-intervention) and after (post intervention) without tEmc.
Figure 8: Cumulative maximal voluntary contraction (MVC) force and numerical motor score for the left and right hands (all right dominant at pre-injury) for 6 individuals over the course of the 8 treatment sessions. Note the blue and orange lines are plotted based on the first and second days of intervention, thus those data points that fall above the line represent greater response compared to the responses seen in the first to the second intervention.
Figure 9: A) Individual MVC forces at the Day 1 (hollow) and Day 8 (solid) of intervention relative to the start and end UE motor scores for left and right hands. Note no change in motor score of subject 511282 but with one of greater improvement in grip force. Right
hand of subject 739144 did not change in UE motor score and had minimal increase in grip force. Also note subject 491863’s left hand did not improve even though the subject had a strong initial MVC and UE motor score. B) Increased MVC force relative to initial UE motor scores for the subjects shown in A. The 12 dots represent the left and right hands of 6 individuals listed in A., $r^2$ (linear) = 0.259, $r^2$ (exponential) = 0.452. C) Increased MVC force relative to initial grip force for the subjects shown in A. 491863’s left data point (non-responder) is not included in C. Black line represents an exponential curve fitted to the data points. Note the marginal increase in grip force at lower initial motor scores and lower initial grip forces, compared to higher increased grip strength at higher initial motor scores and higher initial grip forces.
Figure 10: A) Subject characteristics (n = 6) including motor and sensory scores before (yellow) and after the intervention (orange). Level of SCI, neurological level based on ISNCSCI exam. B) Examples of dermatomes for motor and sensory scores before and after the intervention for 2 subjects. Note: subject 511282 had suffered an injury to the C7 vertebrae qualifying the subject for the study, however based on the AIS exam (motor and sensory scores) the subject level of injury was classified as a C8 AIS C.
**Supplementary Figure 1:** Maximum voluntary contraction (MVC) forces for each individual for their stronger hand across the 3 baseline days. Note, the 3 baseline days were spread over a 10 day period.
Supplementary Figure 2: Image of the custom built setup including the isometric force transducer.
Supplementary Figure 3: A) Patient demonstrating handling a fork before and after the intervention. B) Same patient demonstrating the ability to hold a cup before with two hands and after the intervention with a single hand. C) Series of pictures demonstrating the ability to successfully open a bottle of water post intervention.
Supplementary Video: A representative video demonstrating a patient (AIS B) attempting to make a fist voluntarily with and without tEmsc.