Short-term peripheral nerve stimulation ameliorates axonal dysfunction after spinal cord injury

Running title: Peripheral stimulation improves axonal function after spinal cord injury

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ABSTRACT

There is accumulating evidence that peripheral motor axons deteriorate following spinal cord injury (SCI). Secondary axonal dysfunction can exacerbate muscle atrophy and contribute to peripheral neuropathies and neuropathic pain and lead to further functional impairment. In an attempt to ameliorate the adverse downstream effects that developed following SCI, we investigated the effects of a short-term peripheral nerve stimulation (PNS) program on motor axonal excitability in 22 SCI patients. Axonal excitability studies were undertaken in the median and common peroneal nerves (CPN) bilaterally before and after a 6-week unilateral PNS program. PNS was delivered percutaneously over the median nerve at the wrist and CPN around the fibular head and the compound muscle action potential (CMAP) from the abductor pollicis brevis and tibialis anterior recorded. Stimulus intensity was above motor threshold and pulses (450 μsec) were delivered at 100 Hz with a 2 second on/off cycle for 30 minutes, 5 days per week. SCI patients had consistently high thresholds with a reduced CMAP consistent with axonal loss; in some patients the peripheral nerves were completely inexcitable. Nerve excitability studies revealed profound changes in membrane potential,
with a “fanned-in” appearance in threshold electrotonus, consistent with membrane depolarisation, and significantly reduced superexcitability during the recovery cycle. These membrane dysfunctions were ameliorated after 6 weeks of PNS, which produced a significant hyperpolarising effect. The contralateral, non-stimulated nerves remained depolarised. Short-term PNS reversed axonal dysfunction following SCI and may provide an opportunity to prevent chronic changes in axonal and muscular function and may improve rehabilitation outcomes.

**Keywords:** nerve excitability; peripheral nerve stimulation; rehabilitation; SCI.

**Abbreviations:** APB = abductor pollicis brevis; ASIA = American Spinal Injury Association; CMAP = compound motor action potential; CPN = common peroneal nerve; EMG = electromyography; MN = median nerve; SCI = spinal cord injury; TA = tibialis anterior
INTRODUCTION

There is increasing evidence to suggest that following SCI, function of peripheral motor axons caudal to the lesion is compromised. This is reflected in reduced CMAP amplitudes (Kirshblum et al. 2001; Lin et al. 2007; Nogajski et al. 2006; Rutz et al. 2000; Van De Meent et al. 2010), slowing of conduction velocity (Nogajski et al. 2006), increased excitability thresholds (Lin et al. 2007) and altered H-reflexes (Hiersemenzel et al. 2000; Leis et al. 1996; Nakazawa et al. 2006; Schindler-Ivens and Shields 2000) after SCI. More recently, studies utilising novel threshold tracking nerve excitability techniques have further identified complex changes in biophysical properties of peripheral motor axons in patients with acute (Boland et al. 2009; Boland et al. 2011), subacute and chronic SCI (Lin et al. 2007). Nerve excitability is a non-invasive electrophysiological technique which provides information regarding the activity of various ion channels, energy-dependent pumps and ion exchange processes activated during impulse conduction in peripheral axons (Burke et al. 2001; Kiernan et al. 2000; Krishnan et al. 2009). This technique has been used extensively to study the biophysical properties of human peripheral nerves in-vivo and have provided important mechanistic insight into axonal ion channel dysfunction in wide range of neurological disorders including toxic, metabolic, acquired and inherited demyelinating neuropathies (Burke et al. 2001; Krishnan et al. 2008), amyotrophic lateral sclerosis (Vucic and Kiernan 2006) and SCI (Boland et al. 2011; Lin et al. 2007). More specifically, axonal depolarisation was consistently observed in both upper and lower limb nerves following SCI (Boland et al. 2011; Lin et al. 2007). Furthermore, abnormal axonal excitability could be detected as early as 6 days post spinal injury (Boland et al. 2009), and the period of acute excitability changes coincided with the development of hyper-reflexia during the later stage of spinal shock (Boland et al. 2011). The results from the aforementioned studies suggest that SCI has a profound downstream effect on the somatic motor nervous system below the neurological level of injury. The pathophysiology underlying SCI-induced axonal dysfunction is likely to be multifactorial and must involve complex interactions between decentralisation, ischemia (acute and chronic), subsequent inactivity, and disuse atrophy. Irrespective of the precise mechanism, secondary peripheral nerve dysfunction will affect muscle strength and contribute to the development of peripheral neuropathies (Burke et al. 2001; Krishnan et al. 2008) and thus leading to further loss of function and independence. Furthermore, peripheral nerve dysfunction may limit spontaneous recovery, particularly in patients with incomplete SCI (Van De Meent et al. 2010) and prevent motor axons from responding appropriately to rehabilitation and future regenerative therapies (Van De Meent et al. 2010). As such, a directed clinical investigation of potential therapies that could reverse secondary peripheral nerve dysfunction following SCI has important clinical implications. Maintenance of peripheral motor axonal function during the acute and subacute phases of SCI may lead to better functional and rehabilitation outcome later on. In an attempt to ameliorate the adverse downstream effects following SCI, the present study investigated whether altered axonal excitability could be reversed by an intensive, short-term therapeutic peripheral nerve stimulation program.
MATERIALS AND METHODS

Patient eligibility criteria

Common peroneal and median nerve excitability studies were undertaken in 22 patients with first time traumatic SCI (17 males; 5 females; age range 19-83 years; mean age 45±20.2 years), all within 6 months since injury, and the results compared to 32 healthy subjects (12 peroneal nerves and 20 median nerves). The level of functional impairment in SCI patients was categorised using the American Spinal Injury Association (ASIA) scale (Marino et al. 2003). None of the patients suffered from pressure sores, hypotension or vascular disorders at the time of experiment nor had history of peripheral neuropathy or other co-existing disease processes (such as diabetes and renal disorders) that would affect peripheral nerve function. All patients were recruited from the Spinal Medicine Department at The Prince of Wales Hospital in Randwick Sydney and gave informed consent to the experimental procedures, which were approved by the Human Research Ethics Committees of the South Eastern Sydney Local Health District (Northern Sector) and the University of New South Wales. The research procedures conformed to the Declaration of Helsinki. A subset of SCI patients were recruited to participate in a 6-week unilateral peripheral nerve stimulation program targeting the median nerve in the upper limb and/or the common peroneal nerve in the lower limb.

Axonal excitability studies

Peripheral nerve excitability was assessed using QTRAC (© Prof Hugh Bostock, Institute of Neurology, London UK) and follows a previously established protocol designed to measure a number of different excitability indices (Burke et al. 2001; Kiernan et al. 2000). Recordings were made from the median nerve (MN) and the common peroneal nerve (CPN) bilaterally before and after a 6-week unilateral peripheral nerve stimulation program (see below). For the upper limb nerve excitability studies, the MN was stimulated at the wrist and CMAPs were recorded from the APB muscle using standard surface EMG techniques, with active electrode on the motor point and the reference electrode over the inter-phalangeal joint. In the lower limb, the common peroneal nerve (CPN) was stimulated with the active electrode over the nerve around the fibular head and the reference electrode over the patella. CMAPs were recorded from the tibialis anterior (TA) muscle using surface EMG with the active electrode over the motor point and the reference electrode over the distal tibia. The current required to produce a CMAP that was of 40% of the maximal motor response (Mmax) was tracked (threshold tracking). Responses were amplified (ICP511 AC amplifier, Grass Technologies, West Warwick, USA) and electronic noise removed using Hum Bug (50/60 Hz noise eliminator, Quest, Scientific Instruments, North Vancouver, Canada). Skin temperature at the stimulation site was monitored with a thermistor thermometer (5831-A Omega Engineering, Manchester, UK) and kept above 33°C throughout the experiment. The following excitability indices were assessed and the sequence of recording followed that previously described (Kiernan et al. 2000; Krishnan et al. 2004): (i) stimulus-response relationship using a stimulus duration of 1.0 ms, with the ratio between the stimulus response curve and 4 stimulus durations (0.2, 0.4, 0.8 and 1.0 ms) being used to calculate rheobase and strength duration time constant, a measure of nodal persistent Na⁺ conductances; (ii) threshold
electrotonus using prolonged (100 ms) polarising currents, a marker of intermodal axonal membrane function; (iii) current-threshold relationship was assessed using polarizing currents of 200 ms duration, in incremental steps from +50% to 100% of threshold, providing information regarding the rectifying properties of nodal and internodal axolemma; (iv) recovery cycle was assessed using a paired-pulse paradigm, with threshold changes in response to a 1 ms (supramaximal) test stimulus as the conditioning test interval was increased from 2 to 200 ms was tracked. The latter data were then extrapolated to determine refractoriness (due to inactivation of nodal transient Na⁺ channels, and measured as the threshold change at an inter-stimulus interval (ISI) of 2.5ms), super-excitability (measured as the minimum mean threshold change of three adjacent points) and late sub-excitability (measured as the minimum mean threshold change after ISI of 10 ms). Super-excitability and late sub-excitability are dependent on juxtaparanodal fast K⁺ channels and nodal slow K⁺ channels respectively.

**Short-term peripheral nerve stimulation therapy**

A portable neuromuscular electrical stimulation unit (NeuroTrac®) was used to deliver peripheral nerve stimulation therapy 5 times per week for 6 weeks. All therapy sessions were supervised. One limb was randomly assigned to receive peripheral nerve stimulation therapy, whilst the opposite limb acted as an internal control. Stimulus intensity was set at above motor threshold. 450 μsec pulses were delivered at 100 Hz with a 2 second on/off cycle for 30 minutes. This parameter was chosen because it produces smooth tetanic contractions of the target muscles without the need of using large electrical current (between 12-30mA). This protocol was well-tolerated by all patients and had a 100% adherence rate over the 6 week period.

During electrical stimulation therapy, the patients were encouraged to actively contract the target muscles during the “on” phase and relax completely during the “off” phase. For those who were not able to actively contract the target muscles, they were instructed to imagine moving the target muscles during the “on” phase and rest during the “off” phase. In total, each session generated approximately 450 electrically-assisted contractions. All patients continued with their usual supervised physiotherapeutic rehabilitation.

**Data analysis**

A total of 36 different excitability parameters were analysed from each nerve excitability study. Initial CPN and MN excitability data from SCI patients were compared to a group of healthy age-matched controls using t-tests for independent samples to assess for effects of SCI. Repeated measures ANOVA with post-hoc comparisons were used to assess effects of 6-week unilateral peripheral nerve stimulation program between limbs in SCI patients. All data are expressed as mean ± standard error (SE). Statistical significance was defined as P < 0.05 for all analyses.

**RESULTS**
**SCI patient characteristics**

The demographic and clinical data for the 22 SCI patients initially enrolled in the study are presented in Table 1. As expected, the majority (n=17; 77%) of the patients were males, with over half of all SCI attributed to falls (55%), followed by motor vehicle accidents (23%) and recreational or sports injuries (18%; horse riding, surfing, rugby injury, etc). All were within 6 months since injury (mean days since injury 82 ± 57 days). Thirteen patients had sustained cervical injuries (C3-C7) while eight had thoracic lesions (T1-T11); one patient had suffered a lumbar lesion at L1.

Table 1. Clinical data of all spinal cord injury patients in whom peripheral nerve excitability testing was attempted. MN: median nerve; CPN: common peroneal nerve.

<table>
<thead>
<tr>
<th>SCI patient</th>
<th>Age / Sex</th>
<th>Motor level</th>
<th>ASIA classification</th>
<th>Time from injury to test (days)</th>
<th>Nerve excitability testing</th>
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*X = unable to test due to wrist / knee flexion contractures / spasticity or fracture

Peripheral nerve excitability studies were undertaken in the median and common peroneal nerves bilaterally to elucidate the down-stream effects of SCI on axonal excitability. In some
SCI patients, the peripheral nerves were completely inexcitable (32% of CPN tested and
11.4% of MN tested respectively). Six patients had severe lower limb spasticity and were
unable to complete the full nerve excitability protocol because of positioning difficulty and
intermittent muscle spasms (see Table 1). One patient (#10) had a non-displaced spiral tibial
fracture and declined testing because of pain locally. Overall, full peroneal nerve excitability
data were successfully obtained from 10 SCI patients (18 legs) and the results were compared
to 12 healthy age-matched controls.

Seven SCI patients with injury level at or below T4 demonstrated normal intrinsic hand
muscle power, hand function and median motor axonal excitability (data not shown). As such
their median nerve excitability data were not included in the final analysis. Of the remaining
15 SCI patients with injury level between C3 and T1, 4 had inexcitable median nerves (1
bilaterally and 3 unilaterally) and 2 had severe wrist and elbow flexion contracture (1
bilaterally and 1 unilaterally) which made it impossible to conduct nerve excitability study
(see Table 1). Overall, full median axonal excitability data were collected from 13 SCI
patients (22 upper limbs) and the results compared to a group of age-matched healthy
controls (N=20).

**Axonal excitability changes after SCI**

The Wilcoxin Signed Rank Test showed that there was no between-limb difference for any
peripheral nerve excitability indices in SCI patients (range for p=0.29 to 0.9), and as such, the
left and right common peroneal nerve excitability data from all SCI patients were pooled for
comparison with age-matched able-bodied controls. Compared to neurologically intact
subjects, the common peroneal motor axons in SCI patients exhibited consistently high
thresholds, with a significant right-ward shift in the stimulus-response curve, illustrated in
Fig. 1A. The mean tibialis anterior peak-to-peak CMAP amplitude was smaller in SCI
patients (4.35±1.21 mV) compared to controls (7.72±1.08 mV; p=0.024) but there was no
significant difference for the slope of the stimulus-response curves (p=0.26) between the two
groups. The strength-duration time constant (SDTC; a measure of the rate at which the
threshold current declines as the stimulus duration is increased) - which reflects the behaviour
of persistent Na⁺ conductances, was also similar between groups (spinal cord injury
0.46±0.03 ms; controls 0.44±0.02 ms; p=0.68).

The current-threshold relationship (Fig. 1B) reflects the rectifying properties of the nodal and
internodal axolemma, and the slope of the curve provides an estimate of the threshold
analogue of input conductance. A decrease in threshold is demonstrated by a right-ward shift
of the curve and an increase in threshold is indicated by a shift to the left. For SCI patients,
the current-threshold curve was shifted to the right (Fig. 1B) in the depolarising direction
(spinal cord injury 0.33±0.03; control 0.25±0.01; p=0.024). Similarly, abnormal responses
were also observed throughout threshold electrotonus, during prolonged hyperpolarising
currents at durations of 10-20 ms (p=0.002), 20-40 ms (p=0.003) and 90-100 ms (p=0.002)
and during prolonged depolarising currents at durations of 10-20 ms (p=0.001), 40-60 ms
(p=0.0003) and 90-100 ms (p=0.0016); this resulted in a “fanned in” appearance (Fig. 1C)
(Kaji 2003). The abnormalities observed in SCI patients during threshold electrotonus were
consistent with those observed during the recovery cycle (Fig. 1D). The recovery of excitability following a supramaximal conditioning stimulus was flatter in SCI patients compared to able-bodied controls, with a significant reduction in superexcitability (spinal cord injury -7.2±1.86%; controls -19.51±1.53%; p<0.0001) and late subexcitability (spinal cord injury 8.9±1.3%; controls 13±1.62%; p<0.05). Furthermore, refractoriness was increased in SCI patients (106.3±16.9%; controls 63.04±5.97% p=0.03).

Figure 1. Nerve excitability data (mean ± SE) recorded from the tibialis anterior muscle during common peroneal nerve stimulation in patients with recent traumatic spinal cord injury (○) compared with responses from healthy controls (N=12; ●). The figure shows the stimulus-response curve (for 1 ms stimulus) (A), the current-threshold relationship (B), threshold electrotonus (C) and the recovery cycle (D). SCI patient data were pooled from both lower limbs.
Compared to able-bodied controls, the median motor axons in SCI patients with neurological levels between C3 and T1 were of high threshold, evident from a shift of the stimulus-response curve to the right (Fig. 2A). The peak-to-peak CMAP amplitude for the APB muscle was similar between groups (controls 6.48±1.1 mV; spinal cord injury 5.8±1.1 mV; p=0.4) but the threshold required to produce a CMAP of 50% of maximum was increased in SCI patients (5.6±1.2 mA; controls 3.2±1.1 mA; p=0.0013). There was no significant difference in the slope of the stimulus-response curves (p=0.64) or the SDTC (p=0.4) between groups, as also observed for the common peroneal nerve. APB CMAPs in patients with SCI at or below T4 were preserved and the excitability parameters of median axons were comparable to able-bodied controls (data not shown). This is not unexpected, given that the spinal roots that contribute to the median nerve exit above T4.

In contrast to the motor axons studied in the common peroneal nerve, the nerve current-threshold relationship and the threshold electrotonus for motor axons in the median nerve were relatively unaffected following SCI (Fig 2B and 2C; range for p=0.07 to 0.89).

However, as for the common peroneal motor axons, the recovery cycle of median nerve motor axons was flatter in SCI patients, with a significant reduction in both superexcitability (spinal cord injury -17.14±2.3%; controls -23.51±1.42%; p=0.036; Fig 2D) and subexcitability (spinal cord injury 12.17±1.77%; controls 16.5±0.9%; p=0.034; Fig.2D).

Taken together, the results from the present study demonstrate that in patients with recent SCI, axonal excitability changes were generally more abnormal in the lower limbs (common peroneal nerve) than in the upper limbs (median nerve) and suggests that the adverse downstream effects of SCI may be distance or length dependent.
Effects of 6-week unilateral peripheral nerve stimulation therapy on axonal excitability

Ten patients with recent SCI (mean days since injury 65±55 days) participated in the 6-week unilateral peripheral nerve stimulation program targeting the common peroneal nerve (CPN). One leg was randomly assigned to receive CPN stimulation therapy, whilst the opposite leg acted as an internal control, except in two patients in whom electrical stimulation therapy was applied to the CPN that remained excitable (patient #21 and #22). Nerve excitability data pre and post 6-weeks unilateral CPN stimulation were compared between limbs and between the
two time points to assess the effects of short-term electrical stimulation therapy. Repeated measures ANOVA with post hoc analyses showed no significant difference in any excitability parameters between limbs in SCI patients at baseline (range for \( p=0.29 \) to 0.9). However, six weeks of unilateral CPN stimulation ameliorated a number of abnormal excitability parameters identified previously following SCI, including the increased superexcitability of the recovery cycle (pre -8.9±2.6%; post -16.85±2.9%; \( p=0.04 \); Fig. 3D) and threshold electrotonus during both prolonged hyperpolarising and depolarising currents (\( p<0.007 \)), resulting in a more “fanned out” appearance (Fig. 3C) towards the normal limits. Furthermore, the current-threshold curve was shifted to the left after 6-weeks CPN stimulation, in the hyperpolarising direction, a trend towards normality (Fig. 3B). In addition, mean peak CMAP amplitude for the tibialis anterior was maintained over the 6-week period (pre 4.15±1.7 mV; post 4.1±1.2 mV; \( p=0.92 \)).

Figure 3. Common peroneal nerve excitability data (mean ± SE) recorded from the tibialis anterior muscle in patients with spinal cord injury prior to (○) and after 6-weeks of unilateral common peroneal nerve stimulation
therapy (●). Data from age-matched healthy controls (N=12) is shown in solid black line with SE indicated by
dotted lines. The figure shows changes in stimulus-response curve (for 1 ms stimulus) (A), the current-threshold
relationship (B), threshold electrotonus (C) and the recovery cycle (D). 6-weeks of common peroneal nerve
stimulation therapy ameliorated abnormalities in current-threshold relationship (B), the threshold electrotonus
(C) and the recovery cycle (D) toward the normal range.

In contrast, there were no significant changes in any CPN excitability parameters recorded
from the opposite, non-stimulated (control) leg over the 6-week period (Fig. 4). The averaged
peak CMAP of the non-stimulated TA muscle was smaller at 6 weeks (baseline; 6.1±1.2 mV;
at 6 weeks 4.05±1.3 mV), however this decrement in amplitude was not statistically
significant (p=0.25).
Figure 4. Common peroneal nerve excitability data (mean ± SE) recorded from the non-stimulated (control) tibialis anterior muscle in SCI patients at baseline (○) and after 6-weeks of contralateral common peroneal nerve stimulation (●). The figure shows the stimulus-response curve (for 1 ms stimulus) (A), the current-threshold relationship (B), threshold electrotonus (C) and the recovery cycle (D). There were no significant changes in any excitability parameters over the 6-week period.

A subset of 11 SCI patients with injury levels between C3 and T1 (mean days since injury 55±54 days) participated in the 6-week median nerve stimulation program (three of whom also took part in the unilateral peroneal nerve stimulation program concurrently). One arm was randomly assigned to receive median nerve stimulation, whilst the opposite arm acted as an internal control. Three out of 11 patients were subsequently excluded from the study because of unforeseen medical complications (unrelated to the electrical stimulation therapy) that are known to affect peripheral nerve function. One patient developed acute inflammatory demyelinating polyneuropathy (AIDP), one reported increase hand numbness and was diagnosed with carpal tunnel syndrome and another developed brachial plexus palsy. Figure 5 and 6 show the mean median nerve excitability data (± SE) pre and post 6-week unilateral median nerve stimulation from the remaining eight SCI patients. Similar to the nerve excitability recordings obtained from the lower limb, short-term median nerve stimulation increased superexcitability of the recovery cycle (pre -17.14±2.3 %; post -23.16±2.4 %; p=0.005; Fig. 5D) and threshold electrotonus during both prolonged hyperpolarising currents at durations of 10-20 ms (p=0.03), 20-40 ms (p=0.009) and 90-100 ms (p=0.004) and during depolarising currents at durations of 10-20 ms (p=0.006), and thus produced a “fanned out” appearance (Fig. 5C). There were no significant differences in the stimulus-response curve and current-threshold relationship (Fig 5A and 5 B respectively). In contrast to the stimulated limb, there were no significant differences in any of the excitability parameters recorded from the opposite, non-stimulated median nerve (Fig. 6).
Figure 5. Median nerve excitability data (mean ± SE) recorded from the APB muscle in SCI patients prior to (○) and after 6-weeks of unilateral median nerve stimulation therapy (●). The figure shows changes in stimulus-response curve (for 1 ms stimulus) (A), the current-threshold relationship (B), threshold electrotonus (C) and the recovery cycle (D). 6-weeks of median nerve stimulation therapy produced a “fanned out” appearance in threshold electrotonus (C) and increased superexcitability of the recovery cycle (D).
Figure 6. Median nerve excitability data (mean ± SE) recorded from the non-stimulated (control) APB muscle in SCI patients at baseline (○) and at the 6th week (●). There were no significant changes the stimulus-response curve (for 1 ms stimulus) (A), the current-threshold relationship (B), threshold electrotonus (C) or the recovery of excitability following a supramaximal stimulus (D) over the 6 week period.

Effects of long-term unilateral peripheral nerve stimulation therapy on axonal excitability

One patient with an incomplete cervical spinal cord injury (patient #7; C4 ASIA D) who commenced the 6-week unilateral CPN stimulation program 75 days post injury voluntarily continued with the same stimulation regimen (30 minute per day, 4-5 times per week) for another 24 months after completing the initial 6-week stimulation program. Follow-up common peroneal nerve excitability studies were conducted bilaterally at 6, 12 and 24 months, and CMAP of tibialis anterior recorded. The results are presented in Figures 7 and 8. Long-term CPN stimulation reinforced the effects produced by the 6-week stimulation
therapy, that is, a left-ward shift of the current-threshold curve in the hyperpolarising
direction (Fig. 7A), increased superexcitability of the recovery cycle (Fig. 7C) and
normalisation of threshold electrotonus during both prolonged hyperpolarising and
depolarising currents towards normal limits (Fig. 7B). There was some spontaneous
improvement in threshold electrotonus and the recovery cycle in the opposite non-stimulated
leg at 6 months follow-up but there was no further improvement thereafter (Fig. 7 E & F).
Long-term CPN stimulation improved TA CMAP amplitude after 6 months and maintained it
over the next 24 months (Fig. 8). In contrast, TA CMAP of the non-stimulated leg
deteriorated gradually over time (by approximately 1/3).

Figure 7. Common peroneal nerve excitability data from a patient with incomplete cervical SCI (C4 ASIA D) on
day 75 post injury (●), and after 6 months (○), 12 months (∇) and 24 months (Δ) of unilateral common peroneal
nerve stimulation. The figure shows the current-threshold relationship (A and D); threshold electrotonus (B and
E) and the recovery cycle (C and F), with 95% confidence intervals obtained from healthy controls (n=12)
indicated by dotted lines. The top panel (A-C) depicts data from the leg that received CPN stimulation therapy
and the bottom panel (D-F) shows data from the opposite, non-stimulated (control) leg.
367 Figure 8. Tibialis anterior (TA) CMAP (mV) recorded from a patient with incomplete cervical spinal cord injury 368 who continued with unilateral common peroneal nerve stimulation for a further 24 months after completing the 369 6-week stimulation program. Data from the stimulated leg is depicted by empty bars and data from the non- 370 stimulated (control) leg is represented by grey-filled bars. Mean TA CMAP (± SE) from 12 healthy controls is 371 shown in black for comparison. TA CMAP amplitude is maintained in the stimulated leg, and in contrast, 372 CMAP of the non-stimulated TA deteriorated over time.

373 DISCUSSION

374 There is accumulating evidence that peripheral nerve function is compromised following 375 spinal cord injury (Boland et al. 2009; Boland et al. 2011; Lin et al. 2007; Nogajski et al. 376 2006; Van De Meent et al. 2010), and the results from the present study are in full agreement 377 with this view. Using novel threshold tracking nerve excitability techniques, we have 378 demonstrated that peripheral motor axons in both the upper and lower limbs underwent 379 significant functional modifications within a few months of SCI. The distal axons were more 380 severely affected, accompanied by significant reduction in CMAP amplitude. The magnitude 381 of CMAP loss (~44 %) was significant enough to affect muscle strength and function (Van 382 De Meent et al. 2010). Since motor axonal dysfunction is apparent in the early phases of SCI, 383 the aim of the present study was to investigate whether the dysfunction could be reversed by 384 a targeted peripheral nerve stimulation program. We have demonstrated that the addition of
an intensive, 6-week peripheral nerve stimulation program in the early phase of SCI ameliorated abnormalities in motor axonal excitability. Furthermore, peripheral nerve function could be maintained with long-term stimulation. It is important to note at this juncture that the abnormalities in axonal excitability did not improve with “standard” rehabilitation, but only with the addition of peripheral nerve stimulation. It is presumptuous to assume that peripheral nerve stimulation alone ameliorated axonal dysfunction. A more likely scenario is that peripheral nerve stimulation improved the biophysical properties of axonal membrane by normalising various energy-dependent processes and this in turn enhanced the responsiveness of motor axons to other rehabilitation therapies.

The results from our study suggest that peripheral nerve stimulation provides an opportunity to prevent chronic changes in axonal and muscular function following SCI. Maintenance of peripheral nerve function may help to reduce the risk of developing secondary peripheral neuropathy and peripheral nerve diseases which are extremely prevalent amongst SCI patients, especially within the first 12 months of injury (Nogajski et al. 2006). Maintenance of peripheral nerve function in the early phases of SCI may improve long-term rehabilitation outcomes and the responsiveness of motor axons to future regenerative therapies. A longitudinal investigation with a larger cohort will be necessary to test this hypothesis.

Mechanisms underlying excitability abnormalities following SCI

A number of mechanisms are likely to contribute to the complex downstream excitability changes after SCI, these included compression and ischemia related to the primary mechanical injury as well as delayed secondary processes such as cord inflammation, disuse atrophy and increased sedentariness of motor neurones that have been disconnected from the central nervous system (Balentine 1978; Boland et al. 2011; Dietz 2010; Noble and Wrathall 1989). The “fanning-in” responses during threshold electrotonus, and a reduction in superexcitability during the recovery cycle, as well as shifting of the current-threshold curve to the right and increased refractoriness, all suggest some degree of axonal depolarisation in SCI nerves. However, no change in the SDTC was observed and a decrease in late subexcitability would argue against pure membrane depolarisation as the primary pathogenic process. Ischemia is known to cause axonal depolarisation through its inhibitory actions on the Na⁺/K⁺ pump (Kiernan and Bostock 2000) and is likely to contribute in part to the “depolarisation-like” changes observed in SCI nerves. This may be compounded by delayed secondary metabolic processes such as upregulation of proinflammatory cytokines following SCI (Hayes et al. 2002), which is known to affect Na⁺ and K⁺ conductances similar to that observed in various channelopathies (Waxman 1998), which further disrupt the homoestasis of energy-dependent processes and together with decentralisation contributed to the abnormalities in axonal excitability (Boland et al. 2011; Lin et al. 2007).

Our results argue against a generalised polyneuropathy as the cause of peripheral nerve dysfunction as excitability of motor axons above the lesion was completely normal. Moreover, the cause of peripheral nerve dysfunction could not be attributed to direct trauma
to the peripheral nerves because the excitability changes were observed in both upper and lower limb nerves and not just confined to one single nerve. This is further supported by a previous study which showed different patterns of excitability changes in patients with focal common peroneal nerve palsy (Boland et al. 2011).

In accordance with previous studies, we also confirmed that the abnormalities in motor axonal excitability were more prominent in the lower limbs than in the upper limbs (Boland et al. 2011; Lin et al. 2007). The paradox that a SCI has a more detrimental effect on excitability properties of distal peripheral axons is perplexing and has been ascribed to trans-synaptic degeneration after SCI (Boland et al. 2011; Van De Meent et al. 2010). In our SCI cohort (all within 6 months of injury), there was already evidence of significant atrophy and axonal loss in the tibialis anterior (reduced TA CMAP by approx. 44% and increased electrical threshold) whilst CMAP of APB was only mildly affected. A reduction in CMAP amplitudes after SCI has been reported previously (Boland et al. 2011; Dietz 2010; Kirshblum et al. 2001; Lin et al. 2007; Van De Meent et al. 2010). A recent, multi-centre study with a large sample size (345 SCI patients) has also reported greater CMAP reduction in the lower limb (abductor hallucis; 36-57%) than the upper limb (abductor digiti minimi; 15-24%) in the first year after SCI, with the lowest CMAP amplitudes found between 5 and 9 months post injury (Van De Meent et al. 2010). The results of our study are in line with these previous studies.

Clinical implications

The data from the current study support the view that a SCI has a profound downstream effect on the peripheral nervous system below the level of injury. As such, rehabilitation efforts must take this into account. Irrespective of the precise pathophysiological mechanisms responsible for the abnormalities in axonal excitability that developed following SCI, the present study has clearly demonstrated that an intensive 6-week peripheral nerve stimulation program was beneficial in improving nerve excitability parameters toward the normal range. Importantly, the improvement could be maintained with long-term stimulation. The results of our study have several significant clinical implications for the management and rehabilitation of patients with SCI, particularly in the acute phase as well as in the context of future neuro-regenerative projects. Firstly, assessment of peripheral nerve function must commence in the acute phase of SCI and needs to be investigated routinely - especially in patients with complaints of new motor weakness, sensory loss or pain. Secondly, therapies that help to maintain peripheral nerve function (such as the peripheral nerve stimulation paradigm used in the current study) need to be incorporated into the mainstream neurorehabilitation program in the early phases of SCI. Lastly, peripheral nerve stimulation maybe used as a preventative strategy to maintain neural function in peripheral nerves that are more prone to chronic compression, such as the median and ulnar nerves at the wrist and elbows, as well as the common peroneal nerve near the fibula head and the posterior tibial nerve in the popliteal fossa. In summary, maintenance of peripheral nerve function in the early phases of SCI may improve long-term rehabilitation outcomes.
ACKNOWLEDGEMENTS

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DISCLOSURES

The authors declared no conflict of interest.

AUTHOR CONTRIBUTIONS

Author contributions: ML, MCK, VGM and CSYL - Conception and design of research; ML - performed experiments; ML and BBL – recruitment of SCI patients; ML – data analyses; ML and VGM - interpretation of results; ML - preparation of figures and table; ML - drafted manuscript.

REFERENCES


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*X = unable to test due to wrist / knee flexion contractures / spasticity or fracture*
Table 1. Clinical data of all spinal cord injury patients in whom peripheral nerve excitability testing was attempted. MN: median nerve; CPN: common peroneal nerve.

Figure 1. Nerve excitability data (mean ± SE) recorded from the tibialis anterior muscle during common peroneal nerve stimulation in patients with recent traumatic spinal cord injury (○) compared with responses from healthy controls (N=12; ●). The figure shows the stimulus-response curve (for 1 ms stimulus) (A), the current-threshold relationship (B), threshold electrotonus (C) and the recovery cycle (D). SCI patient data were pooled from both lower limbs.

Figure 2. Nerve excitability data (mean ± SE) recorded from the abductor pollicis brevis (APB) muscle during median nerve stimulation in spinal cord injury patients with neurological level between C3 and T1 (○) compared with responses from normal controls (N=20; ●). The figure shows the stimulus-response curve (for 1 ms stimulus) (A), the current-threshold relationship (B), threshold electrotonus (C) and the recovery cycle (D). SCI patient data were pooled from both upper limbs.

Figure 3. Common peroneal nerve excitability data (mean ± SE) recorded from the tibialis anterior muscle in patients with spinal cord injury prior to (○) and after 6-weeks of unilateral common peroneal nerve stimulation therapy (●). Data from age-matched healthy controls (N=12) is shown in solid black line with SE indicated by dotted lines. The figure shows changes in stimulus-response curve (for 1 ms stimulus) (A), the current-threshold relationship (B), threshold electrotonus (C) and the recovery cycle (D). 6-weeks of common peroneal nerve stimulation therapy ameliorated abnormalities in current-threshold relationship (B), the threshold electrotonus (C) and the recovery cycle (D) toward the normal range.

Figure 4. Common peroneal nerve excitability data (mean ± SE) recorded from the non-stimulated (control) tibialis anterior muscle in SCI patients at baseline (○) and after 6-weeks of contralateral common peroneal nerve stimulation (●). The figure shows the stimulus-response curve (for 1 ms stimulus) (A), the current-threshold relationship (B), threshold electrotonus (C) and the recovery cycle (D). There were no significant changes in any excitability parameters over the 6-week period.

Figure 5. Median nerve excitability data (mean ± SE) recorded from the APB muscle in SCI patients prior to (○) and after 6-weeks of unilateral median nerve stimulation therapy (●). The figure shows changes in stimulus-response curve (for 1 ms stimulus) (A), the current-threshold relationship (B), threshold electrotonus (C) and the recovery cycle (D). 6-weeks of median nerve stimulation therapy produced a “fanned out” appearance in threshold electrotonus (C) and increased superexcitability of the recovery cycle (D).

Figure 6. Median nerve excitability data (mean ± SE) recorded from the non-stimulated (control) APB muscle in SCI patients at baseline (○) and at the 6th week (●). There were no significant changes the stimulus-response curve (for 1 ms stimulus) (A), the current-threshold relationship (B), threshold electrotonus (C) or the recovery of excitability following a supramaximal stimulus (D) over the 6 week period.

Figure 7. Common peroneal nerve excitability data from a patient with incomplete cervical SCI (C4 ASIA D) on day 75 post injury (●), and after 6 months (○), 12 months (●) and 24 months (○) of unilateral common peroneal nerve stimulation. The figure shows the current-threshold relationship (A and D); threshold electrotonus (B and E) and the recovery cycle (C and F), with 95% confidence intervals obtained from healthy controls (n=12) indicated by dotted lines. The top panel (A-C) depicts data from the leg that received CPN stimulation therapy and the bottom panel (D-F) shows data from the opposite, non-stimulated (control) leg.

Figure 8. Tibialis anterior (TA) CMAP (mV) recorded from a patient with incomplete cervical spinal cord injury who continued with unilateral common peroneal nerve stimulation for a further 24 months after completing the 6-week stimulation program. Data from the stimulated leg is depicted by empty bars and data from the non-stimulated (control) leg is represented by grey-filled bars. Mean TA CMAP (± SE) from 12 healthy controls is shown in black for comparison. TA CMAP amplitude is maintained in the stimulated leg, and in contrast, CMAP of the non-stimulated TA deteriorated over time.