Axonal changes in spinal cord injured patients distal to the site of injury

Cindy Shin-Yi Lin,1 Vaughan G. Macefield,1 Mikael Elam,3 B. Gunnar Wallin,3 Stella Engel2 and Matthew C. Kiernan1

1 Prince of Wales Medical Research Institute and Prince of Wales Clinical School, University of New South Wales, 2 Spinal Unit, Prince of Wales Hospital, Sydney, Australia and 3 Department of Clinical Neurophysiology, Sahlgren University Hospital, Gothenburg, Sweden

Correspondence to: Associate Professor Matthew C. Kiernan, Prince of Wales Medical Research Institute, Barker Street, Randwick, Sydney, NSW 2031, Australia
E-mail: m.kiernan@unsw.edu.au

It is generally assumed that the peripheral nervous system remains intact following a spinal injury. Accordingly, the electrical thresholds of motor axons in a peripheral nerve below the lesion should be similar to those in intact subjects. Yet in attempts to enter the common peroneal nerve with microelectrodes in 24 quadriplegic or paraplegic individuals it was often found that electrical stimulation over or within the nerve failed to elicit contractions in the pre-tibial flexors. To investigate whether consistent changes in axonal physiology occurred distal to the site of injury in patients with spinal cord injury (SCI), motor nerve excitability was formally tested in 15 of these patients. Threshold tracking techniques were used to measure axonal excitability parameters (stimulus–response curves, strength–duration properties, threshold electrotonus, a current–threshold relationship and the recovery cycle) of motor axons in the median and common peroneal nerves. In these patients motor axons were uniformly of high threshold and consequently, stimulus–response curves were shifted to the right. In some SCI patients, axons were completely inexcitable. Amplitudes of compound motor action potentials were reduced, consistent with axonal loss and strength–duration time constant was significantly reduced in SCI patients (SCI 0.13 ± 0.02 ms, controls 0.43 ± 0.02 ms, mean ± SE, P < 0.0001). Excitability changes were more prominent the more clinically severe the injury, with progressive deterioration over time since the original injury. While compression and traction sustained during the original injury or subsequent hospital rehabilitation may contribute in part to some of these changes, it is difficult to attribute these findings solely to such processes. Changes in axonal structure and ion channel function, but perhaps more critically decentralization and consequent inactivity, are likely to underlie the complex changes observed in axonal excitability in SCI patients.

Keywords: peripheral nerve; spinal cord injury

Abbreviations: APB = abductor pollicis brevis; CMAP = compound muscle action potential; RRP = relative refractory period; SCI = spinal cord injury; SDTC = strength–duration time constant; TA = tibialis anterior

Received January 23, 2006. Revised October 30, 2006. Accepted November 7, 2006

Introduction

Over recent years, spinal cord injury (SCI) research has tended to concentrate on enhancement of neural regeneration at the injury site, with particular emphasis on techniques that may stimulate growth, and less attention directed to nerve function distal to the level of the lesion. As a consequence there is a paucity of knowledge about the function of neuronal tissues that have been disconnected from their central control systems by trauma. Critically, peripheral nerve function has not been investigated following SCI in any systematic fashion. While the few peripheral studies undertaken have documented significant abnormalities, the basis of these findings remains unclear (Aisen et al., 1992; Blaik et al., 1989; Curt and Dietz, 1999; Kirshblum et al., 2001; Laurence et al., 1978). Inactivity of axons in paralysed limbs is likely to contribute to muscle wasting, poor wound healing and symptoms of pain. More critically in the context of future neuronal regeneration
projects, peripheral dysfunction may prevent axons from responding appropriately following regeneration of spinal pathways.

Motor and sensory nerve conduction studies, in combination with electromyography, have remained the method of choice for the clinicians investigating suspected peripheral nerve dysfunction. In a large study of SCI patients, Curt and Dietz (1999) found that severe axonal motor neuropathies of the tibial and peroneal nerves, indicated by loss of the compound muscle action potential (CMAP) occurred in ~70% of cases with conus or cauda equina lesions, whereas higher spinal lesions rarely resulted in loss of CMAPs. Conversely, Kirshblum and colleagues (2001) found that nerve conduction studies were abnormal in the lower limb in patients with cervical lesions, specifically that CMAPs in the tibial and peroneal nerves were often reduced and motor conduction velocities slower. Yet, while routine nerve conduction studies can document the presence of a neuropathy, they provide limited information regarding the pathophysiology. In contrast, clinical nerve excitability techniques have recently been developed to provide information about biophysical properties of the axonal membrane and the pathophysiology of disease involvement (for review, see Burke et al., 2001). Given the suggestion that function may not remain normal in peripheral axons that have become disconnected as a result of SCI, the aim of the present study was first, to apply a combination of microneurography and nerve excitability techniques to SCI patients to investigate neural function and, if there was evidence of neuropathy, to provide insight into the underlying pathophysiological mechanisms and potential contribution of these processes to symptoms of dysesthesiae, pain, fatigue and weakness in SCI patients.

**Material and methods**

Microneurography and nerve excitability studies were undertaken in 24 patients (20 males; 4 females; age range 18–55 years; mean age 30.7 years) at various stages following SCI, both subacute (<1 year) and chronic (>1 year). None of the patients had a history of peripheral nerve abnormality or co-existent disease process known to affect peripheral nerve function. Patients were recruited from the SCI unit of Prince of Wales Hospital and gave informed consent to the procedures, which were approved by the Human Research Ethics Committees of the University of New South Wales and the South Eastern Sydney Area Health Service Human Research Ethics Committee (Eastern Section). The studies were performed in accordance with the Declaration of Helsinki.

At the time of study, the majority of patients were outside a timeframe typically associated with the processes of spinal shock. All patients were clinically stable and were being managed in either a non-acute hospital care setting or had been rehabilitated to home. Patients were classified using the ASIA Impairment Scale as follows: ASIA A: complete: no motor or sensory function is preserved in the sacral segments S4–S5; ASIA B: incomplete: sensory but not motor function is preserved below the neurological level and includes the sacral segments; ASIA C: incomplete: motor function is preserved below the neurological level and more than half of key muscles below the neurological level have a muscle power MRC grade <3; ASIA D: incomplete: motor function is preserved with the muscle power MRC grade >3; ASIA E: normal.

**Microneurography**

Microneurographic studies of the common peroneal nerve were attempted in each of the SCI patients, as an initial screening process to detect altered peripheral nerve excitability. Briefly, the procedure consisted of locating the nerve at the fibular head by external stimulation (1–10 mA, 0.2–1.0 ms, 1 Hz) using constant-current cathodal pulses (Stimulus Isolator, ADInstruments, Castle Hill, Australia). In some experiments up to 32 mA was delivered from a high-current stimulus isolator (DS3, Digitimer, Welwyn Garden City, UK). In most patients, a microneurography electrode was subsequently inserted subcutaneously and electrical stimuli were delivered through the needle while searching for the nerve. These initial studies demonstrated that following SCI, the electrical thresholds for exciting motor axons within the common peroneal nerve were often elevated. In some individuals it was possible to evoke twitches in the pre-tibial flexors and to enter the nerve with a microelectrode; neurophysiological data recorded from these experiments will be reported separately. For those subjects in whom elevated electrical thresholds of the common peroneal nerve were demonstrated, detailed assessments of motor axonal physiology were then undertaken.

**Axonal excitability**

In the case of nerve excitability, studies were performed using a previously described protocol designed to measure a number of different nerve excitability parameters rapidly (Kiernan et al., 2000). In the upper limb, the median nerve was stimulated at the wrist, and CMAPs were recorded from thenar muscles using surface electrodes over abductor pollicis brevis (APB), with the active electrode at the motor point and the reference on the proximal phalanx. In the lower limb, the peroneal nerve was stimulated at the fibular neck, with the active electrode placed 1 cm distal to the fibular neck with the reference electrode over the patella, and the CMAPs were recorded from tibialis anterior (TA) using surface electrodes.

The signals were amplified and digitized by computer (486 PC) with A/D board (DT2812, Data Translation Inc., Marlboro, MA, USA), using a sampling rate of 10 kHz. Stimulus waveforms generated by the computer were converted to current with a purpose-built isolated linear bipolar constant-current source (max. output±50 mA). The stimulus currents were applied via non-polarizable Ag–AgCl electrodes (Red Dot, 3M Health Care, Borken, Germany). Stimulation and recording were controlled by QTRAC software (© Institute of Neurology, London, with multiple excitability protocol, TRONDXM).

Test current pulses were applied at 0.8 s intervals, combined with suprathreshold conditioning stimuli or subthreshold polarizing currents as required. The amplitude of the CMAP was measured from baseline to the initial negative peak. Skin temperature was monitored close to the stimulation site and kept >32° C for each study.

The sequence of recordings followed that previously described (Kiernan et al., 2000; Krishnan et al., 2004). Stimulus–response curves were recorded separately for test stimuli of duration 0.2 and 1 ms (Fig. 1A). The stimuli were increased in 6% steps,
with two responses averaged for each step, until three averages were considered maximal. The ratio between the 0.2 and 1 ms stimuli required to evoke the same response was used to estimate the strength–duration time constant (τSD) (Fig. 2). A target response was then set at the steepest point on the stimulus–response curve between 30 and 50% of the maximal CMAP, and the 1.0 ms test stimuli were automatically adjusted by the computer to maintain this peak compound amplitude.

To determine threshold electrotonus, prolonged subthreshold currents were used to alter the potential difference across the

Fig. 1 (A) Mean stimulus–response relationships for the median nerve recorded in the entire spinal cord injured patient group represented in Table I (open circles) and controls (filled circles) for the test stimuli of 1 ms (left of each pair) and 0.2 ms duration (right of each pair). (B) Stimulus–response data for the subgroup of SCI patients from Table I with a cervical lesion. (C) Raw data of stimulus–response recording in two representative SCI patients (patient 10 and 13 in Table I) with two stimulus widths, 0.2 and 1 ms. (D) Histograms of threshold for 50% CMAP, maximum peak response and slope of the stimulus–response curve for SCI patients (n = 12; mean ± SEM) and normal controls. SCI patient data were compared with normal controls using unpaired t-tests.
In the present protocol, test stimuli of 1-ms duration were used to track the target compound potential (40% of maximal) while threshold was changed by 100-ms polarizing currents, set to 40% (depolarizing) and −40% (hyperpolarizing) of the control threshold current (Fig. 3C). Each stimulus combination was repeated until three valid threshold estimates were recorded, as judged by the response being within 15% of the target response, or alternate responses being on either side of the target.

The current–threshold relationship (Fig. 3A) was tested at the end of 200-ms polarizing currents, which were altered in a ramp fashion from +50% (depolarizing) to −100% (hyperpolarizing) of the control threshold in 10% steps. As with the conventional threshold electrotonus protocol, stimuli with conditioning currents were alternated with test stimuli alone, and each stimulus combination was repeated until three valid threshold estimates were obtained.

The final part of the protocol recorded the recovery of excitability following a supramaximal conditioning stimulus (Fig. 3D). These changes were recorded at 18 conditioning–test intervals, decreasing from 200 to 2 ms in an approximately geometric sequence. From the recovery cycle three parameters were measured: the relative refractory period (RRP) defined as the interstimulus interval at which threshold recovered to its control value (or, in the absence of superexcitability, the first threshold minimum); superexcitability measured as the greatest percentage reduction in threshold; and late subexcitability, measured as the greatest percentage increase in threshold following the superexcitable period (Kiernan et al., 2000, 2001).

Values for each excitability parameter in the current study are expressed as mean ± SEM and comparison is made with normative data established in previous studies of 29 normal control subjects for the upper limbs (Kiernan et al., 2000, 2001) and 50 controls for the lower limbs (Krishnan et al., 2004). Data were compared using unpaired 2-tailed t-tests.

Results

The demographic and clinical data for the 24 SCI patients are presented in Table 1. Unlike able-bodied subjects, in whom electrical stimulation of the common peroneal nerve at the fibular head can evoke muscle twitches at currents usually around 2–5 mA (0.2 ms, 1 Hz), it often proved difficult to electrically stimulate the nerve in patients with SCI. It was impossible to elicit surface EMG responses or to generate overt twitches in the pre-tibial flexors in 50% of SCI patients. One paraplegic subject (Patient 8 in Table 1) who had sustained a T11 injury 5 months earlier, exhibited complete paralysis but preserved sensation in the legs and reported paraesthesiae in the cutaneous innervation territories of the common peroneal nerve at normal stimulus intensities (<5 mA). However, no EMG or twitches could be evoked in this subject, even when the stimulus duration and intensity were increased to 2 ms and 10 mA.

When intraneural stimulation was successful, apparently normal afferent activity could be recorded from mechanoreceptors in muscle or skin, though it did appear that ‘active’ sites within the nerve were rather sparse. In some subjects (e.g., Patient 9 with a T8+L1–L2 lesion), it was possible to electrically stimulate and enter the right common peroneal nerve but impossible to stimulate the left side at intensities of 50 mA (1 ms). This was confirmed on four occasions and presumably reflected the complex nature of this particular traumatic lesion, which may have involved the spinal roots. In other subjects twitches could be evoked by intraneural stimulation at intensities <20 μA, currents that represent in normal subjects the levels typical of a location of the microelectrode tip within a motor fascicle, yet no neural activity from muscle afferents could...
be recorded. In subjects in whom it was not possible to stimulate motor axons within the nerve, blind searching within the nerve failed to uncover any sites of spontaneous or mechanically evoked afferent activity.

In 15 of the total of 24 SCI patients in whom the above microneurographic studies were undertaken, the electrical excitability of the median and common peroneal nerve was examined formally, using the threshold-tracking paradigm. The full sequence of excitability measurements described in Material and methods was attempted in each patient, 60% of whom had clinical features of spasticity (Table 2). In regard to those patients that did not have features of spasticity, Patients 7, 9, 11 and 14 from Table 2 had incomplete thoracic lesions (mostly Asia C and D); while Patients 1 and 11 were studied at 1 month and were also incomplete. In 30.8% of the group of patients from Table 2, lower limb nerves were inexcitable compared to only 7.7% of upper limb nerves.

In the upper limb studies, stimulus–response curves for the median nerve established that axons were of high threshold in the combined SCI patient group, as indicated by a shift to the right of the stimulus–response curve (Fig. 1A and B). In SCI patients, as a consequence of the high electrical threshold, the peak CMAPs could not be

![Fig. 3](Image)

**Fig. 3** Changes in excitability of motor axons from median nerve studies in SCI patients ($n=12$, mean ± SE) compared to 95% confidence limits for normal controls Kiernan et al., 2000. **(A)** Current–threshold relationships. **(B)** Strength–duration time constants. **(C)** Threshold electrotonus for 100 ms polarizing currents, ±40% of the resting threshold, i.e. the changes in threshold during and after subthreshold depolarizing and hyperpolarizing currents (±40% of threshold) lasting 100 ms. **(D)** Recovery of axonal excitability following a single supramaximal conditioning stimulus.
reach the short stimulus duration (0.2 ms) up to the maximal stimulator output (50 mA), but maximal CMAPs could be generated with the longer stimulus duration (1 ms) as shown in Fig. 1C. The threshold for a CMAP 50% of maximum using 1-ms stimuli was significantly higher for SCI patients than for control subjects (SCI: 9.9 ± 1.1 mA; control: 4.5 ± 1.1 mA, \( P<0.001 \)). Moreover, the slope of the stimulus–response curve was steeper in SCI (SCI: 8.5 ± 1.2; control: 4.9 ± 1.0, \( P<0.001 \); Fig. 1C). Each of these changes were more prominent in the subgroup of SCI patients from Table 1 with a cervical lesion (Fig. 1B), with further reduction in their CMAP amplitude (SCI: 2.5 ± 1.5 mV).

The strength–duration time constant (SDTC) is a measure of the rate at which the threshold current for a target potential declines as stimulus duration is increased (Mogyoros et al., 1996; Fig. 2A). The voltage dependence of the strength–duration properties is determined by persistent \( \text{Na}^+ \) currents that are active near threshold (Bostock and Rothwell, 1997). SDTCs were calculated for different fractions of the compound potential (Fig. 3B). SDTC (for a CMAP 40% of maximal) was significantly less for SCI patients when compared to controls (SCI: 0.13 ± 0.02 ms, control: 0.43 ± 0.02 ms; \( P<0.001 \), Fig. 2B) possibly indicating reduction in persistent \( \text{Na}^+ \) currents (Bostock et al., 1998; Burke et al., 2001). Again, there were more prominent changes in the subgroup of SCI patients from Table 1 with a cervical lesion (SDTC: 0.11 ± 0.02 ms).

The current–threshold relationship (Fig. 3A) reflects the rectifying properties of the axon (both nodal and internodal axolemma), and the slope of the curve can be used to provide an estimate of the threshold analogue of input conductance. The plots are orientated such that decreases in threshold occur to the right and increases in threshold to the left. The steepening of the curve towards the top right with increasingly strong depolarizing currents results from outward rectification, due to activation of fast and slow \( \text{K}^- \) channels, while the less prominent steepening towards the bottom left with increasingly strong hyperpolarizing currents indicates inward rectification, due to activation of the hyperpolarization-activated conductance \( (I_{\text{HI}}) \). The current–threshold relationships for SCI patients were within 95% confidence limits established for control subjects in the depolarizing direction, as illustrated in Fig. 3A for SCI patients. There was a trend for a smaller change in threshold for the same currents during hyperpolarization, consistent with the changes in threshold electrotonus (see above and Fig. 3C). The mean changes in excitability occurring during and after subthreshold depolarizing and hyperpolarizing currents lasting 100 ms are plotted for SCI patients in Fig. 3C. More abnormal changes in threshold occurred with hyperpolarizing currents, with a smaller increase for SCI patients than controls \( [\text{TEh} (90–100 \text{ ms}): \text{SCI}, 73.2 ± 4.0\%; \text{control}, 120.8 ± 2.8\%; \text{P}<0.0001] \).

The recovery of excitability following a supramaximal conditioning stimulus was flatter in SCI patients: late subexcitability was significantly reduced (Fig. 3D) in SCI patients (7.7 ± 1.3% for SCI patients and 14.6 ± 0.8% for controls, \( P<0.0001 \)), and there was a trend for reduction in RRP, refractoriness and superexcitability, although these changes were not statistically significant.

Inexcitability became more evident in lower limb studies (Table 2). As with upper limb studies, there was a trend for

### Table 1

<table>
<thead>
<tr>
<th>SCI patient</th>
<th>Age/sex</th>
<th>Level of injury</th>
<th>ASIA</th>
<th>Microneuronal could be activated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>28 M</td>
<td>C6/C7</td>
<td>B</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>24 M</td>
<td>C5/C6</td>
<td>C</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>26 M</td>
<td>C5</td>
<td>C</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>33 M</td>
<td>T10</td>
<td>A</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>34 F</td>
<td>C5</td>
<td>C</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>45 M</td>
<td>C3/C4</td>
<td>A</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>55 M</td>
<td>T8/T9</td>
<td>D</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>21 M</td>
<td>T11</td>
<td>C</td>
<td>–</td>
</tr>
<tr>
<td>9</td>
<td>44 M</td>
<td>T8, L1–L2</td>
<td>C</td>
<td>–</td>
</tr>
<tr>
<td>10</td>
<td>28 M</td>
<td>C6</td>
<td>A</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>47 M</td>
<td>T1</td>
<td>C</td>
<td>–</td>
</tr>
<tr>
<td>12</td>
<td>51 M</td>
<td>C4</td>
<td>D</td>
<td>–</td>
</tr>
<tr>
<td>13</td>
<td>37 M</td>
<td>C4/C5</td>
<td>C</td>
<td>–</td>
</tr>
<tr>
<td>14</td>
<td>42 F</td>
<td>T12</td>
<td>B</td>
<td>–</td>
</tr>
<tr>
<td>15</td>
<td>43 M</td>
<td>T3</td>
<td>A</td>
<td>–</td>
</tr>
<tr>
<td>16</td>
<td>36 M</td>
<td>T6</td>
<td>A</td>
<td>–</td>
</tr>
<tr>
<td>17</td>
<td>34 M</td>
<td>C4/C5</td>
<td>A</td>
<td>–</td>
</tr>
<tr>
<td>18</td>
<td>18 M</td>
<td>C6/C7</td>
<td>A</td>
<td>–</td>
</tr>
<tr>
<td>19</td>
<td>50 M</td>
<td>T10</td>
<td>C</td>
<td>–</td>
</tr>
<tr>
<td>20</td>
<td>48 M</td>
<td>C6</td>
<td>C</td>
<td>–</td>
</tr>
<tr>
<td>21</td>
<td>36 M</td>
<td>C5</td>
<td>A</td>
<td>+</td>
</tr>
<tr>
<td>22</td>
<td>18 F</td>
<td>C4</td>
<td>A</td>
<td>+</td>
</tr>
<tr>
<td>23</td>
<td>32 F</td>
<td>L1</td>
<td>B</td>
<td>+</td>
</tr>
<tr>
<td>24</td>
<td>36 M</td>
<td>T5</td>
<td>A</td>
<td>+</td>
</tr>
</tbody>
</table>

### Table 2

<table>
<thead>
<tr>
<th>SCI patient</th>
<th>Level of injury</th>
<th>Time from injury to test (months)</th>
<th>Excitability testing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Upper limbs</td>
</tr>
<tr>
<td>1</td>
<td>C6/C7</td>
<td>1</td>
<td>Y</td>
</tr>
<tr>
<td>2</td>
<td>C5/C6</td>
<td>5</td>
<td>Y</td>
</tr>
<tr>
<td>3</td>
<td>C5</td>
<td>10</td>
<td>Y</td>
</tr>
<tr>
<td>4</td>
<td>T10</td>
<td>2</td>
<td>Y</td>
</tr>
<tr>
<td>5</td>
<td>C5</td>
<td>1165</td>
<td>Y</td>
</tr>
<tr>
<td>6</td>
<td>C3/C4</td>
<td>64</td>
<td>Y</td>
</tr>
<tr>
<td>7</td>
<td>T8/T9</td>
<td>3</td>
<td>Y</td>
</tr>
<tr>
<td>8</td>
<td>T11</td>
<td>9</td>
<td>Y</td>
</tr>
<tr>
<td>9</td>
<td>T8, L1–L2</td>
<td>34</td>
<td>Y</td>
</tr>
<tr>
<td>10</td>
<td>C6</td>
<td>4</td>
<td>Inexcitable</td>
</tr>
<tr>
<td>11</td>
<td>T1</td>
<td>1</td>
<td>Y</td>
</tr>
<tr>
<td>12</td>
<td>C4</td>
<td>2</td>
<td>Y</td>
</tr>
<tr>
<td>13</td>
<td>C4/C5</td>
<td>5</td>
<td>Y</td>
</tr>
<tr>
<td>14</td>
<td>T12</td>
<td>1179</td>
<td>Y</td>
</tr>
<tr>
<td>15</td>
<td>T3</td>
<td>18</td>
<td>Y</td>
</tr>
</tbody>
</table>
a ‘fanned-in’ appearance during threshold electrotonus (Fig. 4), with significant decreases in both populations of axons studied (e.g. TEd (10–20 ms): SCI 54.7 ± 1.5, control 69.3 ± 0.9, \( P < 0.0001 \)). In the hyperpolarizing direction, the threshold change was also significantly smaller for SCI patients [TEd (10–20 ms): SCI –57.1 ± 1.8, control –63.8 ± 1.2, \( P = 0.0011 \), and TEh (90–100 ms): SCI –75.8 ± 7.1, control –108 ± 4.2, \( P = 0.0004 \)].

Given the heterogeneous nature of the SCI group studied, patients were divided according to their level of injury to further dissect the basis for the changes in axonal excitability. In particular, thoracic SCI patients became...
a specific focus, given that upper limb values may be expected to be relatively unaffected. In these Patients (4, 7, 8, 9, 11, 14, 15), median nerve CMAP remained preserved (5 ± 1.5 mV), and there were no significant abnormalities in median nerve excitability parameters. In contrast, lower limb studies in this subgroup confirmed prominent abnormalities, with a significant increase in threshold (16.7 ± 1.1; P < 0.0001), reduction in CMAP amplitude (0.8 ± 2 mV; P < 0.001), associated with a flattening of their recovery cycles and significant reduction in SDTC (0.14 ± 0.01; P < 0.0001).

The relative flattening of recovery cycle in SCI patients had a similar timecourse and pattern to recovery cycles of normal controls, suggesting that the cause did not lie with any single parameter that determines the separate periods of the cycle. Rather, these variations may represent a difference in the amplitude of the initial disturbance to the resting equilibrium state (Krishnan and Kiernan, 2005). It is possible that this difference could result from a reduced nodal driving current, with smaller action potentials in decentralized axons of SCI patients, compared to controls (Kiernan et al., 1996; Vogel and Schwarz, 1995).

In support of such a hypothesis, the RRP duration correlated with the severity of SCI as graded using the ASIA classification (Fig. 5). In other words, the more clinically severe the injury, the greater the decrease in RRP duration, possibly reflecting the overall reduction in activity and thereby neural transmission that inevitably occurs in decentralized axons of SCI patients. Similarly, the ASIA level demonstrated significant correlation with the extent of refractoriness at 2 ms (r = 0.8; P < 0.05) and the CMAP amplitude (r = 0.5; P < 0.05).

Of further relevance, previous studies established that the adaptation that occurs in neuronal circuitry below the level of the lesion progressively evolves with time (Hiersenzenz et al., 2000). When the excitability findings from the present series were compared with the time that had elapsed since SCI, progressively severe abnormalities became evident in SDTC, the recovery cycle of excitability and threshold electrotonus, with the strongest negative correlation evident during refractoriness (r = 0.9).

**Discussion**

The aim of the present study was to investigate whether changes after a central motor lesion were reflected in peripheral nerve excitability. Microneurographic and nerve excitability studies undertaken in motor axons of 24 SCI patients at various stages following injury, both subacute and chronic, were markedly abnormal below but not above lesion. Clinically, peripheral muscle wasting was evident in some patients far remote from their site of injury. As a group, compound motor action potentials were small in amplitude, and nerves below lesion were uniformly of high threshold and difficult to activate electrically in SCI patients.

Before interpreting the complex axonal excitability findings in SCI patients, an important consideration remains that changes in peripheral axons may reflect the transneuronal axonal fallout of motor neurons and their axons. As such, the reduction in CMAP amplitude and muscle wasting may suggest a dying back process. Of relevance, transsynaptic degeneration of neuronal systems has been previously demonstrated in animal
models (Ginsberg and Martin, 2002) and has recently been implicated as a mechanism for the exhaustion of activity that has been observed in SCI patients undergoing locomotor training (Dietz and Muller, 2004). Consequently, the abnormalities detected in the present series may well reflect similar processes, with less severe changes present in the axons that have been studied than would be evident in those axons that have already died, and that therefore could not have contributed to any observed change.

Excitability abnormalities in SCI

Some of the excitability changes obtained from SCI patients may suggest that a degree of axonal depolarization was present in their peripheral nerves. A number of mechanisms are involved in the injury and secondary processes which may have contributed to such changes, including ischaemia (acute and chronic) and abnormal ionic shifts across cell membranes (for review see Tator and Fehlings, 1991). Given that compression and ischaemia (anoxia) occur in traumatic SCI, could the excitability changes be ischaemic in origin? Ischaemia is known to paralyse energy-dependent processes including the axonal membrane Na\(^+\)/K\(^+\) electrogenic pump, with resultant axonal depolarization. Previous excitability studies in control subjects have established that ischaemia produces an increase in the slope of the current–threshold relationship, a ‘fanning-in’ of responses during threshold electrotonus and a decrease in superexcitability (Kiernan and Bostock, 2000), changes observed in SCI patients.

Other nerve excitability parameters obtained from the present study would argue against membrane depolarization as the sole underlying cause for the excitability abnormalities. Specifically, \(\tau_{SD}\) was significantly reduced in SCI patients suggesting that there may be other mechanisms interfering with the function of axonal ion channels, particularly persistent Na\(^+\) channels (Bostock and Rothwell, 1997). In addition, SCI patients demonstrated changes in the recovery cycle of excitability following a single impulse. The reduction in refractoriness and the duration of the RRP, due to the inactivation of nodal transient voltage-gated Na\(^+\) channels, would be unexpected with membrane depolarization (Kiernan and Bostock, 2000).

An alternative explanation would be that axons below lesion level in SCI patients have an overall reduction in the concentration of nodal Na\(^+\) channels. Such a reduction in Na\(^+\) conductances may also underlie the reduction in SDTC in SCI patients. Reductions in SDTC and parameters of the recovery cycle have recently been established to occur with tetrodotoxin, a potent inhibitor of Na\(^+\) channel function (Isbister et al., 2002). Reductions in refractoriness, superexcitability and late subexcitability, as occurred in patients following ingestion of tetrodotoxin, were successfully reproduced using a mathematical model of the human axon in which Na\(^+\) conductances were reduced by 50% (Kiernan et al., 2005).

While as a combined group, the level of the injury did not appear to correlate with the changes in axonal excitability, subgroup analysis with a focus on thoracic SCI confirmed marked changes in lower limb studies, with preservation of upper limb compound potentials and excitability indices, much as may be expected. In the subgroup of patients with high (cervical) SCI from the present series in whom both median and peroneal nerve excitability could be recorded, the striking finding was that there was no significant difference between the upper limb and lower limb excitability recordings, suggesting that similar processes may be active in these SCI patients at both levels, underlying such changes. High cervical injuries with consequent contusions will involve tract fibres but also inevitably the motor neurons and roots over at least two to four segments that innervate arm muscles (Curt and Dietz, 1996; for review, see Dietz and Curt, 2006).

The pathophysiology of traumatic SCI relates to a primary mechanical injury to the cord tissue and delayed secondary injury processes (Balentine, 1978a, b; Bresnahan, 1978; Noble and Wrathall, 1989). A cascade of secondary tissue damage may induce further loss of tissue and function (Dusart and Schwab, 1994; Taupin et al., 1993; Yang et al., 2005). Hayes and colleagues (2002) reported an upregulation in proinflammatory cytokines in the sera of infection-free, chronic (>12 months), traumatically injured SCI patients. Of relevance, similar cytokines were previously implicated in the pathogenesis of disrupted ion channel (Na\(^+\) and K\(^+\)) conductances resulting in ‘channelopathy’ (Waxman, 1995, 1998), and demyelination (‘myelinopathy’) in chronic inflammatory disease (Kuwabara et al., 1999).

Clinical implications

Complex changes in axonal function evolve in SCI patients. Of relevance to such changes and their consequent effects on muscle tone and function, diaphragmatic pacing by phrenic nerve stimulation in high cervical lesion patients may fail with time due to hardware failure, or alternatively because of excitability changes (Lieberman et al., 1980; Tibbals, 1991). When interpreting the findings from subacute and chronic SCI patients in the present study, an important consideration remains that axonal dysfunction may reflect a multitude of factors. In the process of sustaining the original trauma, a second peripheral lesion may also have occurred below the level of the SCI. In the acute phase there tends to be a clinical focus on the critical SCI, and consequently a peripheral lesion may not be detected. Furthermore, spinal contusions will inevitably affect motor neurons and roots over at least two to four segments below the level of the lesion, and myelomalacia may develop distal to the injury, secondary to the effects of ischaemia. Compression and traction of peripheral nerves may also be sustained during the course of subsequent
inpatient acute treatment and rehabilitation processes and may thereby contribute in part to some of the changes observed in the present study. In addition, it is possible that distinct patterns of change develop for different peripheral nerves after SCI (Hansen et al., 2005), and that medications may alter excitability, although in the case of peripheral excitability at least, this would seem unlikely (Maddison et al., 1999).

In conclusion, ischaemia-induced depolarization could not explain the present findings in entirety. The fact that similar excitability changes were observed in median and peroneal nerves following cervical SCI, would suggest that a primary peripheral lesion is unlikely. Similarly, an inflammatory mechanism may not be expected to leave nerves above a lesion relatively unaffected, as occurred in thoracic SCI patients. Consequently the present data would suggest that decentralization and consequent inactivity underlie the complex changes in axonal excitability. Finally, irrespective of the specific mechanism, the high frequency of impaired peripheral neural function observed in SCI patients from the present series needs to be considered in future treatments aiming at bridging the spinal lesion.

References