Novel threshold tracking techniques suggest that cortical hyperexcitability is an early feature of motor neuron disease

Steve Vucic and Matthew C. Kiernan

Prince of Wales Medical Research Institute and Prince of Wales Clinical School, University of New South Wales and Institute of Neurological Sciences, Prince of Wales Hospital, Randwick, Sydney, Australia

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

The dying forward hypothesis of motor neuron disease (MND) suggests that corticomotoneurons induce excitotoxic anterior horn cell death, with involvement of the glutamatergic neurotransmitter system. The aim of the present study was to apply novel threshold tracking transcranial magnetic stimulation (TMS) techniques in conjunction with peripheral nerve excitability studies in MND patients to further investigate the dying forward hypothesis and possibly determine the site of disease onset. Studies were undertaken in 23 MND patients using a 90-mm circular coil connected to a BiStim magnetic stimulator for cortical studies and electrical stimulation for peripheral nerve excitability studies. Motor-evoked potentials and compound muscle action potentials (CMAPs) were recorded from the right abductor pollicis brevis in the same setting. Measures of cortical and peripheral nerve excitability were correlated with clinical and neurophysiological parameters of disease severity. Short-interval intracortical inhibition (SICI) was significantly reduced in MND patients compared with controls (MND group = 3.6 ± 0.8%; controls = 8.5 ± 1.0%, P < 0.001), most prominently in MND patients with limb-onset disease. Changes in intracortical inhibition were accompanied by alterations in the magnetic stimulus–response curve, cortical silent period duration and resting motor threshold, all indicative of cortical hyperexcitability. Although the reduction in SICI was more pronounced in MND patients with less severe disease, as assessed by the CMAP amplitude, it remained evident even in MND patients with advanced disease. Measures of peripheral disease burden, namely the CMAP amplitude (r = −0.6) and neurophysiological index (r = −0.6), correlated with cortical hyperexcitability changes, as did the strength-duration time constant (r = −0.6), a peripheral marker of axonal excitability. Simultaneous assessment of central and peripheral nerve excitability has established the presence of co-existent upper and lower motor neuron dysfunction, with cortical hyperexcitability an early feature in MND.

Keywords: cortical hyperexcitability; MND; threshold tracking

Introduction

Motor neuron disease (MND) is a progressive neurodegenerative disorder affecting motor neurons in the spinal cord, brainstem and motor cortex (Desai and Swash, 2002). Although the mechanisms of motor neuronal cell death in MND remain unknown, cortical hyperexcitability mediated via glutamate excitotoxicity has been proposed as a possible mechanism (Rothstein et al., 1993; Bruijn et al., 1997; Trotti et al., 1999). This hypothesis is supported...
by improvement in survival outcome of MND patients treated with riluzole, an inhibitor of glutamate release (Bensimon et al., 1994; Lacambiez et al., 1996; Miller et al., 2003; Kiernan, 2005).

While the site of disease onset in MND has not been determined, Eisen and colleagues (1992) suggested that MND was primarily a disorder of corticomotoneurons, with excitotoxic anterograde degeneration of anterior horn cells (AHC) occurring as a secondary process. This ‘dying forward’ hypothesis was based predominantly on the clinical observations that the oculomotor, abducens and Onuf’s motor nuclei, all lacking direct corticomotoneuron connections, were spared in MND. Of critical importance, pure lower motor neuron forms of MND remain rare and are usually accompanied by subtle upper motor neuron (UMN) signs. Further support for a ‘dying forward’ hypothesis has been provided by transcranial magnetic stimulation (TMS) studies, which have demonstrated increased cortical excitability early in the course of MND (Caramia et al., 1991; Eisen et al., 1993; Prout and Eisen, 1994; Mills and Nithi, 1997; Desiato et al., 2002; Mills, 2003). Another line of evidence that suggests the presence of cortical excitability in MND is provided by peristimulus time histogram studies (Kohara et al., 1996). Neuropathological studies have also provided support detailing early changes in the motor cortex, including ultrastructural changes in Betz cells and loss of specific inhibitory cortical interneurons (Leigh and Swash, 1991; Nihei et al., 1993; Eisen and Weber, 2001).

In contrast to these findings, other studies have provided evidence that would argue against a dying forward process. Specifically, neurophysiological studies have suggested that cortical hyperexcitability either increases (Zanette et al., 2002) or is unrelated to disease duration (Ziemann et al., 1997), while some neuropathological studies have reported that cortical and lower motor neuron degeneration may occur independently (Kiernan and Hudson, 1991; Pamphlett et al., 1995). In an attempt to evaluate any ‘dying forward’ process in MND, the present study has combined novel cortical and peripheral nerve excitability studies with functional assessment in MND patients.

**Methods**

Studies were undertaken in 23 patients with clinically probable or definite MND (15 males, 8 females; age range = 44–71 years; mean age = 60.7 years) as defined by the revised El Escorial criteria (Brooks et al., 2000). All patients were referred from the multidisciplinary MND clinical service at Prince of Wales Hospital and were clinically staged using the Amyotrophic Lateral Sclerosis Functional Rating Scale—Revised (ALSFRS-R; Cedarbaum et al., 1999), the Medical Research Council (MRC) rating scale (Medical Research Council, 1976), hand function score (Triggs et al., 1999), and a UMN score (Turner et al., 2004). This UMN score comprised a sum of pathologically brisk reflexes that included assessment of biceps, supinator, triceps, finger, knee and ankle reflexes, with plantar responses, facial and jaw jerks, all bilaterally, for a maximum possible score of 16 (Turner et al., 2004).

Patients were classified according to the site of disease onset as either limb-onset or bulbar-onset. Amplitude of the compound muscle action potential (CMAP) was used as a broad marker of disease severity, using CMAP amplitude of <4 mV as a cut-off for more severe disease involvement, based on previous data obtained from control subjects (Vucic et al., 2006). Most patients (96%) were receiving riluzole treatment. All patients gave informed consent to the procedures, which were approved by the South East Sydney Area Health Service Human Research Ethics Committee.

**Cortical excitability**

Cortical excitability was assessed by applying TMS to the motor cortex by means of a 90-mm circular coil oriented to induce current flow in a posterior–anterior direction. The coil was adjusted in both antero-posterior and a medial–lateral direction until the optimal position for an evoked response was obtained from the right APB muscle. Currents were generated by two high-power magnetic stimulators that were connected via a BiStim (Magstim Co., Whitland, South West Wales, UK) such that conditioning and test stimuli could be independently set and delivered through the one coil. The circular coil was chosen over a focal (figure-of-eight) coil, as the former was easier to use with less frequent overheating of the coil itself. Studies by Abbruzzese and colleagues (1999) established no qualitative differences in the pattern of inhibition and facilitation when using either a cortical circle or a focal (figure-of-eight) coil. A previous study that incorporated threshold tracking TMS used a focal coil (Fisher et al., 2002). This study reported two phases of short-intracortical inhibition, interstimulus interval (ISI) = 1 ms and at 3 ms, identical to that reported in our own study that established normative data (Vucic et al., 2006).

**TMS threshold tracking**

In the conventional paired-pulse technique, the conditioning and test stimuli were kept at constant intensity, and changes in the motor-evoked potential (MEP) amplitude were measured. In the present study the amplitude of the MEP response was fixed and changes in the test stimulus intensity required to generate this target response, when preceded by either sub- or supra-threshold conditioning stimuli, were measured, being analogous to threshold tracking methods used in the assessment of peripheral nerve excitability as described previously (Bostock et al., 1998; Kiernan et al., 2000; Burke et al., 2001).

The threshold tracking strategy used a target response of 0.2 mV (±20%), located in the middle of the established linear relationship between the logarithm of the MEP amplitude and the stimulus intensity (Fisher et al., 2002; Vucic et al., 2006). Resting motor threshold (RMT) was defined as the stimulus intensity required to produce and maintain this target MEP response.

Initially, the stimulus–response (SR) curve for cortical stimulation was determined by increasing the intensity of the magnetic stimulus to the following levels: 60, 80, 90, 100, 110, 120, 130, 140 and 150% RMT. Three stimuli were delivered at each level of stimulus intensity. The maximum MEP amplitude (millivolts; mV) and MEP onset latency (milliseconds; ms) were recorded. Central motor conduction time (CMCT, ms) was calculated according to the F-wave method (Mills and Murray, 1986; Claus, 1990; Cros et al., 1990). Subsequently, the cortical silent period (CSP) induced by single-pulse TMS was recorded while MND patients performed a weak voluntary contraction, estimated as representing 10–30% of maximum voluntary contraction (Vucic et al., 2006).
The duration of the silent period was measured from the beginning of MEP to the return of EMG activity (Cantello et al., 1992).

Short-interval intracortical inhibition (SICI) was measured according to a previously devised protocol (Vucic et al., 2006). SICI was determined by using subthreshold conditioning stimuli (70% RMT) at increasing ISIs as follows: 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 7, 10, 15, 20 and 30 ms. Stimuli were delivered sequentially as a series of three channels: channel 1 tracked the stimulus intensity required to produce the unconditioned test response (i.e. RMT); channel 2 monitored the response to the subthreshold conditioning stimulus; and channel 3 tracked the stimulus required to produce the target MEP when conditioned by a stimulus equal in intensity to that on channel 2. Stimuli were delivered every 5–10 s (stimulus delivery was limited by the charging capability of the BiStim system) and the computer advanced to the next ISI only when tracking was stable.

SICI was measured as the increase in the test stimulus intensity required to evoke the target MEP. Inhibition was calculated off-line as follows (Fisher et al., 2002; Vucic et al., 2006):

\[
\text{Inhibition} = \frac{\text{conditioned test stimulus intensity} - \text{RMT}}{\text{RMT}} \times 100
\]

Facilitation was measured as the decrease in the conditioned test stimulus intensity required to evoke a target MEP.

**Peripheral nerve excitability**

Peripheral nerve excitability studies were performed in the same sitting according to a previously described protocol that measures multiple parameters of nerve excitability (Kiernan et al., 2000). In all studies, the median nerve was stimulated electrically at the wrist. The resultant CMAP was recorded from the abductor pollicis brevis (APB) and measured from baseline to negative peak with the threshold tracking target set to 40% of maximum CMAP.

SR curves were recorded separately for stimuli of 0.2 and 1 ms duration. From SR curves the rheobase (Bostock et al., 1998) and strength-duration time constant (\(\tau_{SD}\)) of motor axons of different thresholds were calculated using Weiss’s formula (Weiss, 1901; Mogyoros et al., 1996; Bostock et al., 1998). In addition to calculating \(\tau_{SD}\) and rheobase, the neurophysiological index (NI) was derived according to a previously reported formula (De Carvalho and Swash, 2000; De Carvalho et al., 2001):

\[
\text{NI} = \frac{\text{CMAP amplitude (mV) \times F-wave frequency}}{\text{distal motor latency (ms)}}
\]

where F-wave frequency was expressed as the number of F-responses recorded in 20 trials.

Subsequently, threshold electrotonus was measured using prolonged sub-threshold polarizing currents of 100 ms duration, set to +40% (depolarizing) and −40% (hyperpolarizing) of controlled threshold current (Bostock et al., 1998; Kiernan et al., 2000; Burke et al., 2001). Finally, the recovery of axonal membrane excitability following a supramaximal conditioning stimulus was recorded. The following parameters were measured: (i) relative refractory period (RRP, ms), defined as the first intercept at which the recovery curve crosses the x-axis; (ii) superexcitability (%), defined as the largest reduction in threshold, peaking at a conditioning–test interval of <10 ms; (iii) late subexcitability (%), defined as the largest increase in threshold following the superexcitability period after 10 ms (Kiernan et al., 2000).

Recordings of CMAP and MEP were amplified and filtered (3 Hz–3 kHz) using a GRASS ICP511 AC amplifier (Grass-Telefactor, Astro-Med Inc., West Warwick, RI, USA) and sampled at 10 kHz using a 16-bit data acquisition card (National Instruments PCI-MIO-16E-4). Data acquisition and stimulation delivery (both electrical and magnetic) were controlled by QTRACS software (©Professor Hugh Bostock, Institute of Neurology, Queen Square, London, UK).

**Statistical analysis**

Cortical excitability in MND patients was compared with control data (Vucic et al., 2006) obtained from 34 subjects (16 males; 18 females; aged 23–73 years; mean = 43.1 years) and a subgroup of 17 older age controls (8 males, 9 females; aged 42–73 years; mean age = 54.4 years). Peripheral nerve excitability parameters were compared with previously reported controls (Kiernan et al., 2000). Peripheral excitability measurements were compensated for age and temperature before statistical analysis, using the relations found in control subjects (Kiernan et al., 2000, 2001a, b). Student’s t-test was used to compare mean differences between MND patients and controls, and analysis of variance (ANOVA) for multiple comparisons. Correlations between excitability indices and clinical scales were analysed by Spearman’s rank test. A probability (\(P\)) value of <0.05 was considered statistically significant. Results are expressed as mean ± standard error of the mean.

**Results**

The clinical features and rating scores for 23 MND patients are summarized in Table 1, while conventional neurophysiological indices are detailed in Table 2. The motor cortex was inexcitable in three MND patients. These patients had protracted disease duration, and in the case of two patients high signal intensity was evident within the region of the corticospinal tract on MRI. In one patient the CMAP response was absent. As such, the TMS findings are those recorded in the remaining 19 MND patients. CMCT was prolonged in three MND patients (Patient nos 6, 9 and 11), but overall was not significantly different in MND patients as a group when compared with controls (MND = 5.6 ± 0.6 ms; controls = 5.1 ± 0.2 ms). In four MND patients the CMCT was at the lower limit of normal. Prolongation of the F-wave latency, secondary to AHC loss, may partially account for the spuriously short CMCT findings in these patients.

**Cortical excitability**

RMT, defined as the unconditioned stimulus intensity required to produce and maintain the target MEP response, was reduced in MND patients (MND = 56.5 ± 1.9%; controls = 60.7 ± 1.5%; \(P = 0.05\)). However, the RMT was only significantly reduced in a subset of MND patients with limb-onset disease (MND limb-onset = 55.2 ± 2.1%; controls = 60.7 ± 1.5%; \(P < 0.05\)). There was significant correlation between RMT and UMN score (\(r = 0.6\)), with the RMT being significantly increased in MND patients with severe UMN signs [MND group with UMN score 10–16, 62.3 ± 3.0%; MND group with UMN score ≥ 9, 54.3 ± 2.2%, \(P < 0.05\)].
To generate SR curves for TMS studies the MEP amplitude was expressed as a percentage of the CMAP amplitude recorded following electrical stimulation. The MEP : CMAP ratio was significantly increased in MND patients as a group when compared with normal controls over stimulus intensities from 110 to 150% RMT (ANOVA, \( P < 0.01 \), Fig. 1A). Subgroup analysis revealed that this increase in the MEP : CMAP ratio was only significant in MND patients with limb-onset disease (Fig. 1B). This MEP : CMAP ratio was increased in MND patients independent of CMAP amplitude, used as a marker of disease severity (Fig. 1C).

Intracortical inhibition

SICI, as reflected by an increase in the conditioned stimulus intensity required to track a constant target MEP of 0.2 mV, was significantly reduced in MND patients as a group when compared with controls \( (P < 0.001, \text{Fig. 2A}) \). The initial phase of SICI previously documented for normal controls at ISI \( \leq 1 \text{ ms} \) (Vucic et al., 2006) was completely absent in MND patients (Fig. 2A), while peak SICI occurring at ISI of 3 ms was also significantly reduced in MND patients. Subgroup analysis revealed that while SICI was reduced in MND patients with bulbar-onset disease, the biggest reduction of SICI occurred in MND patients with limb-onset disease (Fig. 2B). Furthermore, although the reduction in SICI was most prominent in MND patients with limited peripheral disease burden, as reflected by the CMAP amplitude, this reduction of SICI was also evident in patients with advanced disease (Fig. 2C). Following SICI, a period of intracortical facilitation (ICF) followed, marked by a decrease in the test stimulus intensity required to maintain the target MEP of 0.2 mV (Vucic et al., 2006). ICF was significantly increased in MND patients as a group compared with controls over a period of testing from 10 to 30 ms (Fig. 2A).

In a contracting muscle, the MEP is followed by a period of electrical silence that interferes with ongoing EMG activity, known as the CSP. In the present series, the increase in the CSP recruitment curve was non-linear in MND patients similar to the relationship established previously for control

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age (years)/sex</th>
<th>MND onset</th>
<th>Disease duration (months)</th>
<th>ALSFRS-R</th>
<th>Triggs hand score</th>
<th>MRC</th>
<th>FVC (%)</th>
<th>UMN score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>69, M</td>
<td>UL</td>
<td>6</td>
<td>45</td>
<td>1</td>
<td>4</td>
<td>52</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>64, M</td>
<td>UL</td>
<td>26</td>
<td>36</td>
<td>2</td>
<td>4</td>
<td>57</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>63, M</td>
<td>UL</td>
<td>53</td>
<td>36</td>
<td>2</td>
<td>4</td>
<td>89</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>67, F</td>
<td>UL</td>
<td>16</td>
<td>40</td>
<td>2</td>
<td>5</td>
<td>85</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>69, M</td>
<td>UL</td>
<td>17</td>
<td>40</td>
<td>2</td>
<td>5</td>
<td>67</td>
<td>7</td>
</tr>
<tr>
<td>6</td>
<td>44, F</td>
<td>UL</td>
<td>13</td>
<td>46</td>
<td>2</td>
<td>4</td>
<td>79</td>
<td>10</td>
</tr>
<tr>
<td>7</td>
<td>50, M</td>
<td>UL</td>
<td>24</td>
<td>42</td>
<td>0</td>
<td>5</td>
<td>100</td>
<td>6</td>
</tr>
<tr>
<td>8</td>
<td>71, M</td>
<td>UL</td>
<td>99</td>
<td>42</td>
<td>1</td>
<td>4</td>
<td>90</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>70, M</td>
<td>UL</td>
<td>8</td>
<td>42</td>
<td>1</td>
<td>4</td>
<td>106</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>57, M</td>
<td>UL</td>
<td>12</td>
<td>29</td>
<td>1</td>
<td>4</td>
<td>90</td>
<td>13</td>
</tr>
<tr>
<td>11</td>
<td>60, F</td>
<td>UL</td>
<td>8</td>
<td>46</td>
<td>1</td>
<td>4</td>
<td>100</td>
<td>10</td>
</tr>
<tr>
<td>12</td>
<td>53, F</td>
<td>UL</td>
<td>12</td>
<td>39</td>
<td>2</td>
<td>4</td>
<td>91</td>
<td>3</td>
</tr>
<tr>
<td>13</td>
<td>58, F</td>
<td>UL</td>
<td>10</td>
<td>39</td>
<td>2</td>
<td>4</td>
<td>86</td>
<td>10</td>
</tr>
<tr>
<td>14</td>
<td>56, M</td>
<td>UL</td>
<td>17</td>
<td>37</td>
<td>1</td>
<td>4</td>
<td>92</td>
<td>16</td>
</tr>
<tr>
<td>15</td>
<td>55, M</td>
<td>UL</td>
<td>41</td>
<td>34</td>
<td>2</td>
<td>4</td>
<td>98</td>
<td>4</td>
</tr>
<tr>
<td>16</td>
<td>60, M</td>
<td>LL</td>
<td>7</td>
<td>40</td>
<td>1</td>
<td>4</td>
<td>80</td>
<td>14</td>
</tr>
<tr>
<td>17</td>
<td>69, M</td>
<td>LL</td>
<td>29</td>
<td>36</td>
<td>0</td>
<td>4</td>
<td>89</td>
<td>0</td>
</tr>
<tr>
<td>18</td>
<td>46, M</td>
<td>LL</td>
<td>9</td>
<td>42</td>
<td>1</td>
<td>5</td>
<td>62</td>
<td>4</td>
</tr>
<tr>
<td>19</td>
<td>53, F</td>
<td>Bulbar</td>
<td>50</td>
<td>44</td>
<td>0</td>
<td>5</td>
<td>57</td>
<td>7</td>
</tr>
<tr>
<td>20</td>
<td>66, M</td>
<td>Bulbar</td>
<td>10</td>
<td>38</td>
<td>2</td>
<td>4</td>
<td>79</td>
<td>4</td>
</tr>
<tr>
<td>21</td>
<td>68, F</td>
<td>Bulbar</td>
<td>21</td>
<td>36</td>
<td>0</td>
<td>5</td>
<td>80</td>
<td>14</td>
</tr>
<tr>
<td>22</td>
<td>62, F</td>
<td>Bulbar</td>
<td>10</td>
<td>40</td>
<td>1</td>
<td>4</td>
<td>98</td>
<td>14</td>
</tr>
<tr>
<td>23</td>
<td>65, M</td>
<td>Bulbar</td>
<td>14</td>
<td>42</td>
<td>1</td>
<td>4</td>
<td>81</td>
<td>12</td>
</tr>
<tr>
<td>Mean</td>
<td>61</td>
<td>22.3</td>
<td>39.6</td>
<td>1.2</td>
<td>4.2</td>
<td>83.0</td>
<td>6.4</td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td>1.6</td>
<td>4.5</td>
<td>0.9</td>
<td>0.2</td>
<td>0.1</td>
<td>4.2</td>
<td>1.2</td>
<td></td>
</tr>
</tbody>
</table>

The site of disease onset was classified as either upper limb (UL), lower limb (LL) or bulbar. Disease duration refers to the period from symptom onset to date of testing. The patients were clinically graded using the ALSFRS-R, with a maximum score of 48 when there is no disability. The ALSFRS-R comprises four sub-scores: bulbar (maximum score = 12), fine motor (maximum score = 8), gross motor (maximum score = 16) and respiratory (maximum score = 12). Muscle strength was clinically assessed using the Medical Research Council for the abductor pollicis brevis (APB), as this muscle was utilized for excitability testing. Forced vital capacity (FVC) was assessed in each patient. UMN involvement was graded according to the Upper motor neuron (UMN) score with a maximum score of 16 (see Material and methods).
The mean CSP duration increased from 0 to 178.8 ± 12.8 ms, as stimulus intensity increased from 60 to 150% RMT in MND patients and was significantly reduced in MND patients compared with normal controls (ANOVA, *P* < 0.0001, Fig. 3).

Cortical excitability in MND

Peripheral nerve excitability

SR curves obtained following electrical stimulation of the median nerve at the wrist were shifted to the right in MND patients for stimuli of both 0.2 and 1 ms duration relative to controls, indicating that axons in MND patients were of higher threshold. CMAP amplitude was significantly reduced in MND patients when compared with controls (MND = 4.9 ± 0.5 mV; controls = 9.8 ± 0.5 mV; *P* < 0.0005) and correlated with the ALSFRS-R fine motor sub-score (*r* = 0.7, *P* < 0.01) and MRC score (*r* = 0.7, *P* < 0.01). The NI was significantly reduced in MND patients compared with controls (MND = 0.6 ± 0.1; controls = 2.5 ± 0.2, *P* < 0.0001) and correlated with the CMAP amplitude (*r* = 0.6, *P* < 0.05). Overall, there was no difference in the CMAP amplitude between MND patients with limb-onset compared with those with bulbar-onset disease (MND limb-onset = 5.0 ± 2.7 mV; MND bulbar-onset = 4.5 ± 0.9 mV, *P* = 0.3), nor was there a significant difference in the NI between the two groups (MND limb-onset = 0.6 ± 0.2; MND bulbar-onset = 0.5 ± 0.2 mV, *P* = 0.2).

\[ \tau_{SD} \]

which reflects nodal persistent Na⁺ conductance (Bostock and Rothwell, 1997) and rheobase, defined as the threshold current for a stimulus of infinitely long duration (Bostock et al., 1998) were calculated from SR curves according to Weiss's formula (Weiss, 1901; Bostock, 1983; Mogyoros et al., 1996). As reported previously (Mogyoros et al., 1998), mean \( \tau_{SD} \) was longer in MND patients (MND group = 0.46 ± 0.03 ms; controls = 0.42 ± 0.02 ms), and rheobase was increased (MND group = 3.3 ± 1.1 mA;

---

### Table 2: Summary of clinical neurophysiological indices

<table>
<thead>
<tr>
<th>Patients</th>
<th>CMAP amplitude (mV)</th>
<th>NI</th>
<th>RMT (MSO%)</th>
<th>MEP amplitude (mV)</th>
<th>CMCT (ms)</th>
<th>MEP : CMAP ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.6</td>
<td>0.6</td>
<td>46</td>
<td>3.2</td>
<td>4.4</td>
<td>0.6</td>
</tr>
<tr>
<td>2</td>
<td>6.5</td>
<td>1.3</td>
<td>57</td>
<td>2.9</td>
<td>7.0</td>
<td>0.5</td>
</tr>
<tr>
<td>3</td>
<td>7.0</td>
<td>1.2</td>
<td>45</td>
<td>4.6</td>
<td>4.9</td>
<td>0.7</td>
</tr>
<tr>
<td>4</td>
<td>3.5</td>
<td>0.5</td>
<td>53</td>
<td>2.1</td>
<td>6.3</td>
<td>0.6</td>
</tr>
<tr>
<td>5</td>
<td>9.4</td>
<td>2.0</td>
<td>48</td>
<td>5.8</td>
<td>6.1</td>
<td>0.6</td>
</tr>
<tr>
<td>6</td>
<td>6.8</td>
<td>0.8</td>
<td>57</td>
<td>1.0</td>
<td>7.6</td>
<td>0.2</td>
</tr>
<tr>
<td>7</td>
<td>6.9</td>
<td>0.2</td>
<td>54</td>
<td>4.5</td>
<td>6.0</td>
<td>0.7</td>
</tr>
<tr>
<td>8</td>
<td>2.2</td>
<td>0.1</td>
<td>52</td>
<td>0.6</td>
<td>5.2</td>
<td>0.3</td>
</tr>
<tr>
<td>9</td>
<td>3.1</td>
<td>0.1</td>
<td>64</td>
<td>0.7</td>
<td>14.7</td>
<td>0.2</td>
</tr>
<tr>
<td>10</td>
<td>1.7</td>
<td>0.2</td>
<td>74</td>
<td>0.4</td>
<td>5.9</td>
<td>0.2</td>
</tr>
<tr>
<td>11</td>
<td>9.8</td>
<td>1.7</td>
<td>57</td>
<td>2.8</td>
<td>7.5</td>
<td>0.9</td>
</tr>
<tr>
<td>12</td>
<td>2.9</td>
<td>0.5</td>
<td>43</td>
<td>1.4</td>
<td>4.0</td>
<td>0.5</td>
</tr>
<tr>
<td>13</td>
<td>3.3</td>
<td>0.5</td>
<td>IE</td>
<td>0</td>
<td>NR</td>
<td>0</td>
</tr>
<tr>
<td>14</td>
<td>0</td>
<td>0</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>15</td>
<td>4.2</td>
<td>0.2</td>
<td>IE</td>
<td>0</td>
<td>NR</td>
<td>0</td>
</tr>
<tr>
<td>16</td>
<td>5.4</td>
<td>0.6</td>
<td>IE</td>
<td>0</td>
<td>NR</td>
<td>0</td>
</tr>
<tr>
<td>17</td>
<td>4.0</td>
<td>0.2</td>
<td>55</td>
<td>1.8</td>
<td>3.0</td>
<td>0.5</td>
</tr>
<tr>
<td>18</td>
<td>7.8</td>
<td>0.3</td>
<td>79</td>
<td>2.1</td>
<td>3.1</td>
<td>0.3</td>
</tr>
<tr>
<td>19</td>
<td>7.5</td>
<td>0.8</td>
<td>71</td>
<td>1.6</td>
<td>4.3</td>
<td>0.2</td>
</tr>
<tr>
<td>20</td>
<td>3.6</td>
<td>0</td>
<td>57</td>
<td>0.8</td>
<td>NR</td>
<td>0</td>
</tr>
<tr>
<td>21</td>
<td>4.9</td>
<td>0.9</td>
<td>63</td>
<td>0.7</td>
<td>4.1</td>
<td>0.2</td>
</tr>
<tr>
<td>22</td>
<td>4.6</td>
<td>0.6</td>
<td>55</td>
<td>1.2</td>
<td>3.1</td>
<td>0.3</td>
</tr>
<tr>
<td>23</td>
<td>1.9</td>
<td>0.1</td>
<td>67</td>
<td>1.0</td>
<td>3.5</td>
<td>0.2</td>
</tr>
</tbody>
</table>

**Mean ± SEM**

<table>
<thead>
<tr>
<th>CMAP amplitude (mV)</th>
<th>NI</th>
<th>RMT (MSO%)</th>
<th>MEP amplitude (mV)</th>
<th>CMCT (ms)</th>
<th>MEP : CMAP ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.6 ± 0.5</td>
<td>0.5 ± 0.1</td>
<td>56.5 ± 1.9</td>
<td>1.8 ± 0.3</td>
<td>5.6 ± 0.6</td>
<td>0.4 ± 0.04</td>
</tr>
<tr>
<td>Control mean ± SEM</td>
<td>9.8 ± 0.5</td>
<td>2.5 ± 0.1</td>
<td>60.7 ± 1.5</td>
<td>2.3 ± 0.2</td>
<td>5.1 ± 0.2</td>
</tr>
</tbody>
</table>

The indices are corresponding for the 23 MND patients detailed in Table 1. CMAP amplitude was recorded from abductor pollicis brevis (APB). The CMAP response was absent in one patient and this precluded further studies (NR = non-recordable). The neurophysiological index (NI) was calculated according to an established formula (see Material and methods). RMT was defined as the unconditioned stimulus intensity, expressed as percentage of maximum stimulator output (MSO%), required to maintain a target of 0.2 mV (see Material and methods). In three MND patients the cortex was inexcitable (IE). The motor evoked potential (MEP) amplitude was also recorded from the APB. Central motor conduction time (CMCT) was calculated according to an established method (see Material and methods). As the F-wave responses were absent in three patients, the CMCT could not be calculated in these patients. Furthermore, the cortex was inexcitable in three patients, and as such calculation of CMCT was not possible. In two patients with an inexcitable cortex, the F-wave responses were absent.

---

subjects (Vucic et al., 2006). The mean CSP duration increased from 0 to 178.8 ± 12.8 ms, as stimulus intensity increased from 60 to 150% RMT in MND patients and was significantly reduced in MND patients compared with normal controls (ANOVA, *P* < 0.0001, Fig. 3).
controls = 3.1 ± 1.1 mA) although these differences were not significant.

The type I abnormality of threshold electrotonus, in which there is a greater change in response to a sub-threshold depolarizing pulse (Bostock et al., 1995), is depicted for an illustrative MND patient (Fig. 4A), and was evident in 20% of MND patients from the present series. The type II abnormality, in which there is a sudden decrease in membrane excitability marked by an abrupt increase in threshold (Bostock et al., 1995), was not evident in any of the MND patients studied. Overall, threshold electrotonus established greater changes in threshold to depolarizing and hyperpolarizing sub-threshold conditioning pulses in MND patients (Fig. 4B–D) similar to the ‘fanned out’ response that occurs with membrane hyperpolarization. These findings in threshold electrotonus were accompanied by a significant increase in superexcitability as recorded during the recovery cycle of excitability (Fig. 5A and B). There was no significant change in other parameters of the recovery cycle including the relative refractory period (MND = 3.1 ± 1.0 ms; controls = 3.2 ± 1.0 ms; P = 0.6) and subexcitability (MND = 15.2 ± 0.9%; controls = 14.9 ± 0.7%; P = 0.8).

In addition, there was no significant difference in the hyperpolarizing current/voltage slope between MND patients and controls (MND = 0.38 ± 0.04; controls = 0.37 ± 0.01).

Combining measures of cortical and peripheral excitability, clinical assessment and disease severity, it was evident that SICI correlated with measures of peripheral disease burden, particularly CMAP amplitude (Fig. 6A) and the NI (Fig. 6B). Furthermore, the MEP:CMAP ratio correlated with a peripheral marker of axonal excitability, namely τSD, which increases with peripheral nerve degeneration (Fig. 6C). Together, these correlations suggest that cortical
hyperexcitability is most evident in early MND, when peripheral disease burden is small, and that cortical hyperexcitability may be an underlying mechanism of neurodegeneration, supporting a dying forward process.

Discussion

The present study, using a combination of novel threshold tracking techniques to explore central and peripheral neuronal excitability in MND patients, has established widespread abnormalities throughout the neural axis. Cortical hyperexcitability was evident in MND patients with reductions in the RMT, SICI and CSP duration and increases in the maximum MEP:CMAP ratio and in the cortical SR curve gradient. The increase in cortical excitability correlated with traditional measures of peripheral nerve function such as the CMAP amplitude and NI, and with axonal excitability parameters, including $\tau_{SD}$. Together, these findings confirm co-existent dysfunction in upper and lower motor neuron systems in MND patients with evidence of cortical hyperexcitability as an early feature in MND when peripheral disease burden is small.

Mechanisms mediating abnormalities of intracortical inhibition and facilitation

SICI was first reported by Kujirai and colleagues (1993), who demonstrated that a subthreshold conditioning stimulus could suppress the response to a later suprathreshold test stimulus when the ISI was <5 ms. Recently, two physiologically distinct phases of SICI have been reported, with the
first phase occurring at an ISI of <1 ms and the second phase at ISI of 2.5–3 ms (Fisher et al., 2002; Roshan et al., 2003; Vucic et al., 2006). Although there is debate regarding the first phase of SICI, with some arguing that it reflects local excitability properties of the cortical axon (Fisher et al., 2002) and others arguing that it reflects activation of inhibitory circuits different from that which mediates inhibition at ISI of 2.5–3 ms (Roshan et al., 2003), the mechanism of the second phase of SICI (ISI 2.5–3 ms) is believed to be cortical in origin and mediated by GABA-secreting inhibitory cortical interneurons via GABAA receptors (Ziemann et al., 1996; Ziemann, 2004a, b).

In the present series, SICI was significantly reduced in MND patients in keeping with previous studies (Hanajima et al., 1996; Yokota et al., 1996; Ziemann et al., 1997; Sommer et al., 1999; Stefan et al., 2001; Zanette et al., 2002). One possible mechanism for reduction of SICI is loss of inhibitory cortical interneurons. Evidence for this is provided by neuropathological studies that have revealed a loss of parvalbumin-positive inhibitory cortical interneurons in MND patients (Nihei et al., 1993).

Loss of inhibitory cortical interneurons is not the only mechanism mediating SICI reduction, as SICI may be rapidly restored in MND patients treated with pharmacological agents such as riluzole (Stefan et al., 2001; Ziemann, 2004a). This would suggest that glutamate-mediated down-regulation of SICI may be another mechanism mediating the reduction of SICI in MND. In addition to the effects on SICI, riluzole also decreases ICF and inhibits persistent Na+ currents (Liepert et al., 1997; Kuo et al., 2005). As such, the neurophysiological findings may have been even more severely affected in patients from the present study had they not been treated with riluzole.

Another possible explanation for reduction in SICI and the concomitant increase in MEP:CMAP ratio may be related to reduced phase cancellation of corticomotoneuron discharges in MND. However, peristimulus time histogram studies have established that corticomotoneuron volleys to the AHC are desynchronized to a greater extent in MND compared with normal controls (Kohara et al., 1996; Weber and Eisen, 2000; Weber et al., 2000). Furthermore, methods such as the triple stimulation technique, which resynchronize the MEP (Magistris et al., 1998), would not be as sensitive at detecting UMN dysfunction in MND compared with
conventional TMS techniques were the corticomotoneuron volley to be less desynchronized in MND (Rosler et al., 2000; Komissarow et al., 2004).

The reduction in SICI, along with other TMS changes suggestive of cortical hyperexcitability, although uniform across all clinical MND phenotypes, were most evident in MND patients with limb-onset disease. While it remains possible that cortical hyperexcitability is less prominent in MND patients with bulbar-onset disease, it is more likely that the difference lies in the choice of target muscles. That is, all studies from MND patients in the present series were undertaken using a peripheral target muscle. Desiato and colleagues (2002) have reported on the presence of cortical hyperexcitability in MND patients with bulbar-onset disease, with reduction of CSP duration when recordings were undertaken directly from affected bulbar muscles.

In addition to reduction of SICI, the CSP duration was uniformly reduced in all clinical MND phenotypes. Given that the CSP duration is mediated by inhibition of AHC in the early phase (Cantello et al., 1992; Inghilleri et al., 1993; Chen et al., 1999; Ziemann, 2004b) and cortical processes, through GABA\(_B\) receptors, in later segments (Connors et al., 1988; Inghilleri et al., 1993; Avoli et al., 1997; Werhahn et al., 1999; Ziemann, 2004b), this finding is in keeping with previous studies in MND patients documenting both disinhibition of AHC (Raynor and Shefner, 1994; Drory et al., 2001) and dysfunction of cortical inhibitory interneurons acting via GABA\(_B\) receptors (Zanette et al., 2002).

**Is there a dying forward process in MND?**

In the present study, coincidental dysfunction of upper and lower motor neuron systems was evident in MND patients. While the study design does not lend itself to absolutely determining the site of disease onset, the fact that cortical hyperexcitability inversely correlated with measures of peripheral disease burden, particularly CMAP amplitude and NI, would suggest that cortical hyperexcitability is an early feature of MND, thereby providing indirect support for a dying forward process. Furthermore, the fact that cortical hyperexcitability was evident in MND patients with advanced disease (CMAP < 4 mV, Figs 2C and 3C) suggests that cortical hyperexcitability is a persistent and ongoing process.

Further indirect support for a ‘dying forward’ process, which proposes that corticomotoneurons drive AHC loss (Eisen et al., 2002), may be derived by the inverse correlation between the \(\tau_{SD}\) and MEP : CMAP ratio. Given that the prolongation of \(\tau_{SD}\) has been previously linked to the processes of axonal regeneration and sprouting (Kanai et al., 2003), this correlation may suggest that cortical hyperexcitability is responsible for motor neuron loss in MND, although compensatory upregulation of the UMN system attempting to overcome lower motor neuron dysfunction cannot be excluded.

In conclusion, the present study has established the presence of cortical hyperexcitability in all MND phenotypes, being most prominent in patients with limb-onset disease. Furthermore, correlation studies suggest that cortical hyperexcitability is an early feature of MND that persists throughout the disease process, possibly serving as a mechanism driving neuronal dysfunction in MND.

**Acknowledgements**

S.V. was awarded the Clinical Fellowship of Motor Neuron Disease Research Institute of Australia (MNDRIA), with funding provided by the Motor Neuron Disease Association of NSW. Grant support was also received from the NSW Ministry for Science and Medical Research Spinal Cord Injury and Related Neurological Conditions Research Grant Program. Input from Professor Hugh Bostock, particularly with threshold tracking software, is again gratefully acknowledged. These studies were presented in part at the 18th World Congress of Neurology, Australia, and the 16th International Symposium on ALS/MND, Republic of Ireland.

**References**


