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Abstract

Background: Gray matter atrophy has been implicated in the development of secondary progressive multiple sclerosis (SPMS). Cortical function may be assessed by transcranial magnetic stimulation (TMS). Determining whether cortical dysfunction was a feature of SPMS could be of pathophysiological significance.

Objectives: Consequently, novel paired-pulse threshold tracking TMS techniques were used to assess whether cortical dysfunction was a feature of SPMS.

Methods: Cortical excitability studies were undertaken in 15 SPMS, 25 relapsing–remitting MS patients (RRMS) and 66 controls.

Results: Short interval intracortical inhibition (SPMS 3.0 ± 2.1%; RRMS 12.8 ± 1.7%, $p < 0.01$; controls 10.5 ± 0.7%, $p < 0.01$) and motor evoked potential (MEP) amplitude (SPMS 11.5 ± 2.2%; RRMS 26.3 ± 3.6%, $p < 0.05$; controls 24.7 ± 1.8%, $p < 0.01$) were reduced in SPMS, while intracortical facilitation (SPMS -5.2 ± 1.9%; RRMS -2.0 ± 1.4, $p < 0.05$; controls -0.9 ± 0.7, $p < 0.01$) and resting motor threshold were increased (SPMS 67.5 ± 4.5%; RRMS 56.0 ± 1.5%, $p < 0.01$; controls 59.0 ± 1.1%, $p < 0.001$). Further, central motor conduction time was prolonged in SPMS (9.1 ± 1.2 ms, $p < 0.001$) and RRMS (7.0 ± 0.9 ms, $p < 0.05$) patients compared with controls (5.5 ± 0.2 ms). The observed changes in cortical function correlated with the Expanded Disability Status Scale.

Conclusion: Together, these findings suggest that cortical dysfunction is associated with disability in MS, and documentation of such cortical dysfunction may serve to quantify disease severity in MS.

Keywords

axonal loss, multiple sclerosis, progressive, relapsing–remitting

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Introduction

Multiple sclerosis (MS) is a chronic immune-mediated disorder of the central nervous system (CNS) and is a major cause of permanent disability in young adults.[1] While a majority of MS patients exhibit a relapsing–remitting course (RRMS), approximately 80% of patients develop progressive disease. White matter lesions, as detected by conventional magnetic resonance imaging (MRI) techniques, are an early feature of MS, but may serve as a poor predictor of disability.[2]

More recently, the development of disability in MS has been linked to atrophy involving gray matter (GM).[2-4] Specifically, pathological studies have reported that extensive and irreversible cortical damage, as indicated by focal regions of demyelination, apoptosis and activation of microglia, were early features in MS and associated with the development of physical disability.[4,5] Further, GM atrophy may develop independently of white matter lesions and evolve at faster rates.[4]

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In addition to these pathological findings, neuroradiological studies using specific MRI sequences have determined the importance of GM atrophy in the development of disability in MS.[2,3] Gray matter atrophy rates were increased threefold in patients converting from a clinically isolated syndrome (CIS) to RRMS and 14-fold in those converting to secondary progressive MS (SPMS).[3] Further, GM atrophy correlated with the Expanded Disability Status Scale (EDSS) and MS functional composite.[2] While radiological studies suggest that GM atrophy is important in the development of disability in MS, these studies have not provided information about cortical function, are not readily available in many institutions and as such may not be practical in a clinical or drug trial setting.

Cortical function in MS patients may also be assessed using non-invasive transcranial magnetic stimulation (TMS) techniques.[6] Specifically, paired-pulse TMS techniques assess the processes of intracortical inhibition and facilitation, reflecting the integrity of interneuronal circuits within the motor cortex and thereby cortical function.[7] TMS studies in MS patients to date have yielded varied results regarding cortical function. While some studies have documented cortical dysfunction in RRMS patients,[8,9] others have documented cortical dysfunction only in SPMS, thereby suggesting that cortical dysfunction was associated with development of disability.[10] Of further relevance, cortical motor reorganization along with an enhanced central motor drive, increased corticomotor excitability and increased perception of effort have also been reported in MS patients using TMS techniques.[11-13] In an attempt to clarify whether cortical dysfunction was associated with disability and progressive forms of MS, the present study applied novel threshold tracking TMS techniques combined with clinical, functional and radiological assessments in MS patients.

Methods

Studies were undertaken in 40 patients with MS defined according to the revised McDonald criteria.[14] In total, there were 25 RRMS (7 males, 18 females: age range 27–56 years, mean age 39 years) and 15 SPMS patients (5 males, 10 females: age range 31–68 years, mean age 48 years) recruited consecutively from the Multidisciplinary MS clinic at Westmead Hospital between January 2010 and July 2010. All patients were clinically assessed using the Expanded Disability Status Scale (EDSS).[15] The Kurtzke EDSS scale is an accepted method of quantifying disability in MS, quantifying disability in eight functional domains including pyramidal, cerebellar, brainstem, sensory, bowel and bladder, visual cerebral and 'other' (cognitive, mood, etc.) domains. The EDSS scale ranges from 0 (no disability) to 10 (death due to MS). In addition, fatigue was assessed using a Modified Fatigue Impact Scale (MFIS).[16] The MFIS is a 21-item questionnaire assessing impact of fatigue on physical, cognitive and psychosocial functioning in MS patients. Clinical disease activity was assessed by the annualized relapse rate (ARR) calculated by dividing the total number of relapses the patient has suffered by the total number of person-years at risk.[17] Informed consent for the procedures was obtained from all patients; the procedures were approved by the Human Research Ethics Committees of the South East Sydney Area Health Service and Sydney West Area Health Service.

Cortical excitability studies

Cortical excitability was assessed by applying TMS to the motor cortex by means of a 90 mm circular coil oriented such that conditioning and test stimuli could be independently set and delivered through the one coil.

The threshold tracking paired-pulse technique was used to assess cortical excitability according to a previously described protocol.[18] All responses were recorded over the APB muscle. Resting motor threshold (RMT) was defined as the stimulus intensity required to produce and maintain a target motor evoked potential (MEP) response of 0.2 mV.

Initially, the maximal MEP amplitude was recorded with the magnetic stimulus intensity set to 140% RMT. Three stimuli were delivered at this stimulus intensity and the maximum MEP amplitude (mV) and MEP onset latency (ms) were recorded. Central motor conduction time (CMCT, ms) was calculated according to the F-wave method.[19] Subsequently, the cortical silent period (CSP) induced by single-pulse TMS was recorded while patients performed a weak voluntary contraction (VC), estimated to be approximately 30% of maximal VC. The duration of the silent period was measured from the beginning of MEP to the return of EMG activity.[20]

Short interval intracortical inhibition (SICI) and intracortical facilitation (ICF) were measured according to previously devised protocols.[18] SICI was determined by using sub-threshold conditioning stimuli (70% RMT) at increasing interstimulus intervals (ISIs) as follows: 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 7 ms, while ICF was determined over the following ISIs: 10, 15, 20, 25 and 30 ms. Stimuli were delivered every 5–10 seconds (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 and ICF were calculated off-line according to a previously reported formula.[18]

In the same sitting, the median nerve was stimulated electrically at the wrist. Stimuli of 1 ms duration were delivered via 5-mm Ag-AgCl surface electrodes (ConMed, Utica, USA), with the cathode placed at the wrist crease and the anode located in the mid-forearm. The resultant compound muscle action potentials (CMAPs) were recorded from the abductor pollicis brevis (APB). Peak-to-peak amplitude and onset latency for the CMAP were determined. In addition, twenty F-wave responses were then recorded in each patient and onset latencies were determined.

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).

Conventional MRI

MR imaging was performed on a GE 1.5T Signa HDx scanner (GE Healthcare, Waukesha, WI, USA) equipped with an 8 Channel Phased array head coil. The standard MR sequence for MS included a T2 axial TR 6200 TE 124, 1.5 average, section thickness 5 mm, gap 1 mm, FOV 220 × 220, matrix 512 × 512. Axial Flair sequences were acquired with the following parameters: TR 9000, TE120, Inversion time 2200, 1.5 averages, section thickness 5 mm, gap 1 mm FOV 512 × 512, matrix 320 × 192. T1 sequences TE 14 TR 600, section thickness 5 mm, gap 1 mm, FOV 220 × 220, matrix 320 × 224. In addition, Sagittal T1, Flair and T2 Coronal images were obtained. Post gadolinium sequences were obtained in the coronal and axial planes with a standard dose of 0.1 mmol/kg (Magnevist, Berlex, NJ, USA). Images of the spine were in Sagittal T1, T2 axial T1, T2 and post gadolinium axial and Sagittal planes using a spinal coil.

All patients underwent conventional brain and spinal cord MRI imaging to assess the T2 lesion load (T2LL), T1 lesion load (T1LL) and the number of gadolinium enhanced lesions. The number of T1 and T2 lesions were manually counted to determine the lesion load according to the grading system used in MSBase, which was as follows: low, 0–2 lesions; moderate, 3–8 lesions; high ≥ 9 lesions.[21] In addition, the number of gadolinium enhanced lesions was also manually counted. Only those lesions deemed typical for MS by a neuroradiologist (LG) were included in the lesion load count numbers.

Statistical analysis

Cortical excitability in MS patients was compared with control data obtained from 66 subjects (32 males, 34 females, aged 23–73 years; mean: 44 years). While the SPMS group was older, the difference in ages was not statistically significant ($p = 0.1$), thereby indicating that the groups were age-matched. One way analysis of variance (ANOVA) was used to compare differences in TMS findings between groups. Bonferroni correction was used for post hoc analysis. Correlations between TMS findings and clinical scales were analyzed by Spearman's correlation coefficient. Multiple linear regression (stepwise method) was performed with EDSS as the dependent variable with SICI, RMT, MEP amplitude, CMCT and T2LL in the brain and spinal cord as independent variables. A p value of < 0.05 was considered statistically significant. Results are expressed as mean ± standard error of the mean.

Results

Clinical findings

The clinical features of the 40 MS patients are summarized in Table 1. Progressive MS patients were significantly older than RRMS patients (SPMS 48.6 ± 3.3 years; RRMS 39.4 ± 1.7 years, $p < 0.001$), and exhibited a longer disease duration (SPMS 9.9 ± 1.9 years; RRMS 5.8 ± 1.6 years, $p = 0.07$). In total, 68% of patients were receiving immunomodulatory therapy at the time of assessment, including glatiramer acetate (15%), interferon beta-1a (Rebif, 5%), interferon beta-1b (Betaferon, 40%), interferon beta-1a (Avonex, 7%) and alemtuzumab (20%).

Magnetic resonance imaging (MRI) findings in the brain and spinal cord

All patients underwent conventional MRI studies of the brain and spinal cord. An illustrative MRI depicting T1 and T2 lesions in one SPMS patient is included (Figure 1). All

Table 1. Clinical details for the 25 relapsing–remitting multiple sclerosis (RRMS) and 15 secondary progressive multiple sclerosis (SPMS) patients

Patients (*N*)	Age (years)	Disease duration (years)	EDSS	MFIS	ARR
RRMS (25)					
Mean	39.4	5.8	1.6	39.8	0.79
SEM	1.7	1.6	0.2	3.6	0.14
SPMS (15)					
Mean	48.6	9.9	5.9	50.8	0.8
SEM	3.3	1.9	0.3	3.3	0.3

Disease duration refers to the period from symptom onset to date of testing. The patients were clinically graded using the Expanded Disability Status Scale (EDSS), Modified Fatigue Impact Scale (MFIS) and annualized relapse rate (ARR). Results are expressed as mean ± standard error of the mean (SEM).

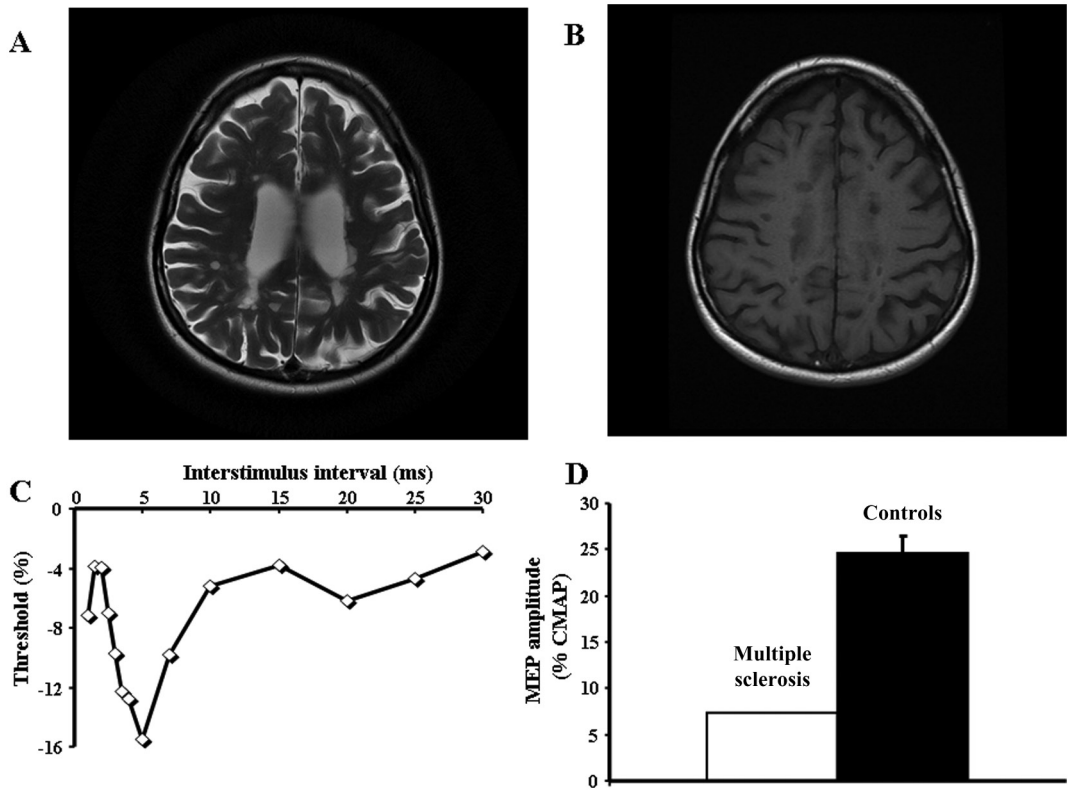

Figure 1. An illustrative case of a patient with secondary progressive multiple sclerosis from the present study with an Expanded Disability Status Scale (EDSS) score of 4. (A). Magnetic resonance imaging disclosed multiple white matter lesions on the T2 weighted sequence along with mild cortical atrophy. The extent of cortical atrophy appears to be greater than the white matter disease burden (B). In addition, multiple white matter lesions were evident on the T1-weighted sequence. (C). Short interval intracortical inhibition and (D) motor evoked potential (MEP) amplitude, expressed as a percentage of the compound muscle action potential (CMAP) response, were significantly reduced in keeping with cortical dysfunction.

MS exhibited typical T2 lesions on brain MRI, while spinal cord T2 lesions were evident in 79% of patients. The majority of MS patients exhibited a high T2 lesion load, namely > 9 T2 brain lesions (74%), while 7.4% exhibited a low disease burden (0–2 T2 brain lesions) and another 7.4% exhibited a moderate T2 lesion load (3–8 T2 brain lesions). Interestingly, only 7.4% of patients exhibited a high T1 lesion load (> 9 T1 brain lesions), while 41% exhibited a low T1 lesion load (0–2 T1 brain lesions) and 30% a moderate T1 lesion load (3–8 T1 brain lesions). Gadolinium enhanced lesions were evident in 30% of MS patients (equally across RRMS and SPMS presentations). In total, 15% of patients had one gadolinium enhanced lesion, while 15% had two gadolinium enhanced lesions on brain MRI.

Conventional neurophysiological studies

Central motor conduction time was significantly prolonged in RRMS (7.0 ± 0.9 ms, $p < 0.05$) and SPMS (9.1 ± 1.2 ms, $p < 0.001$) patients when compared with controls (5.5 ± 0.2 ms). The extent of this prolongation in CMCT was greater in SPMS patients when compared with RRMS patients ($p = 0.05$). As expected, the CMAP amplitude was within normal limits in both the RRMS (9.5 ± 0.7 mV, normal > 4.3 mV) and SPMS patients (8.0 ± 0.8 mV, normal > 4.3 mV) arguing against the presence of any co-morbid peripheral nerve entrapment syndrome that could potentially influence the cortical excitability findings.

Assessment of cortical excitability

The motor cortex was inexcitable in two patients with SPMS. The resting motor threshold, defined as the unconditioned stimulus intensity required to produce and maintain the target MEP response, was significantly different across the three groups (Fisher's exact test, $[F] = 8.1$, $p < 0.01$), being increased in SPMS (67.5 ± 4.5%) when compared with RRMS (56.0 ± 1.5%, $p < 0.01$) and controls (59.0 ± 1.1%, $p < 0.001$, Figure 2A). In addition, the MEP amplitude, expressed as a percentage of the CMAP amplitude recorded following electrical stimulation, was significantly different in the three groups ($F = 4.5$, $p < 0.05$), being reduced in SPMS patients (11.5 ± 2.2%) when compared with RRMS (26.3 ± 3.6%, $p < 0.05$) and controls (24.7 ± 1.8%, $p < 0.01$, Figure 2B).

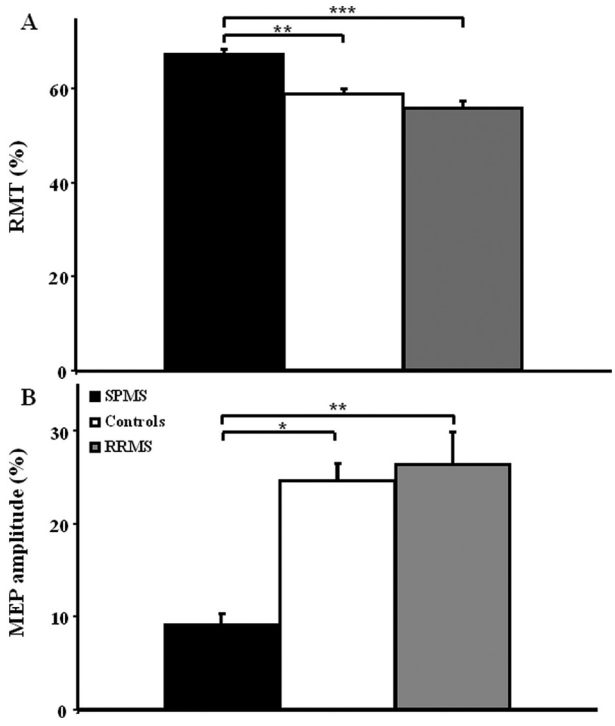

Figure 2. **(A)** Resting motor threshold (RMT) was increased in secondary progressive multiple sclerosis (SPMS) patients when compared to relapsing-remitting multiple sclerosis (RRMS) patients and controls. There was no significance difference (NS) in the RMT between RRMS and controls **(B)** The MEP amplitude was reduced in SPMS patients when compared to RRMS patients and controls. There was no significance difference in the MEP amplitude between RRMS and controls.
*$p < 0.05$ **$p < 0.01$ ***$p < 0.001$

Short interval intracortical inhibition has been defined as the increase in the test stimulus intensity required to track a constant target MEP of 0.2 mV, with two peaks documented, a smaller peak at ISI 1 ms and larger peak at ISI 3 ms.[18] Between-group ANOVA revealed that averaged SICI was significantly different in the three groups ($F = 7.9$, $p < 0.01$, Figure 3), being reduced in SPMS patients (averaged SICI 1–7 ms, 3.0 ± 2.1%) when compared with RRMS patients (averaged SICI 1–7 ms, 12.8 ± 1.7%, $p < 0.01$) and controls (averaged SICI 1–7 ms, 10.5 ± 0.7%, $p < 0.01$). There was no significant difference in averaged SICI between RRMS patients and controls ($p = 0.47$).

Peak SICI at ISI 1 ms was significantly reduced in SPMS patients (1.8 ± 1.6%) when compared with RRMS patients (7.4 ± 1.4%, $p < 0.01$, Fig. 4A) and controls (7.5 ± 0.8%, $p < 0.001$, Figure 4A). In addition, peak SICI at ISI 3 ms was significantly reduced in SPMS patients (6.3 ± 2.4%) when compared with RRMS patients (17.3 ± 2.0%, $p < 0.01$, Figure 4B) and controls (16.1 ± 1.2%, $p < 0.01$, Figure 3B). There was no significant difference in peak SICI between RRMS patients and controls at ISI 1ms ($p = 0.41$) and 3 ms ($p = 0.11$).

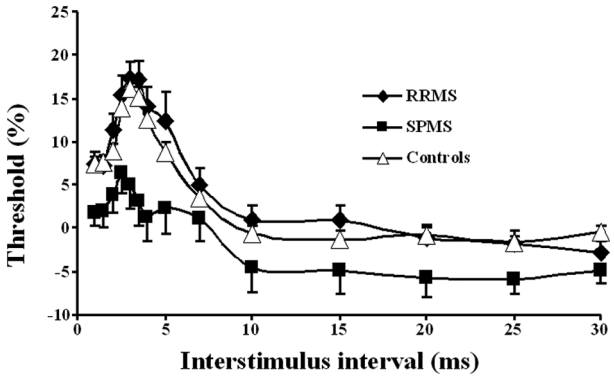

Figure 3. Short interval intracortical inhibition was reduced in secondary progressive multiple sclerosis (SPMS) patients when compared with relapsing–remitting multiple sclerosis (RRMS) patients and controls.

Intracortical facilitation (ICF) develops at ISIs of 10–30 ms and is reflected by a decrease in the test stimulus intensity required to maintain the target MEP response.[18] Between-group ANOVA revealed a significant difference of ICF in the three groups ($F = 3.8$, $p < 0.05$), with ICF being significantly increased in SPMS patients (-5.2 ± 1.9%) when compared with RRMS patients (-2.0 ± 1.4, $p < 0.05$) and controls (-0.9 ± 0.7, $p < 0.01$, Figure 3).

Cortical silent period

The cortical silent period is defined as interruption of voluntary electromyography activity following an MEP response. In the present series, the CSP duration, with stimulus intensity set to 140% RMT, was reduced in SPMS patients (180.5 ± 17.1 ms) when compared with RRMS patients (202.8 ± 9.3 ms) and controls (208.2 ± 3.1 ms), although this reduction was not significant.

Correlations between clinical assessment, cortical function and MRI

Combining measures of cortical function, clinical assessment, and disease severity, it became evident that disability, as measured by EDSS, correlated with SICI (Rho = -0.72, $p < 0.001$), MEP amplitude (Rho = -0.42, $p < 0.05$), central motor conduction time (Rho = 0.6, $p < 0.001$) and resting motor threshold (Rho = 0.50, $p < 0.01$). In addition, EDSS correlated with fatigue as measured by the modified fatigue inventory (Rho = 0.55, $p < 0.05$). Although the CMCT correlated with the T2 brain lesion load (Rho = 0.54, $p < 0.05$), there was no correlation between other TMS parameters, such as SICI, RMT and MEP amplitude, and the T2 or T1 lesion load in the brain, and T2 lesion load in the spinal cord. There was a weak correlation between the EDSS and the T2LL (Rho = 0.37, $p < 0.05$) and T1LL (Rho = 0.37, $p < 0.05$), but no correlation of EDSS with the

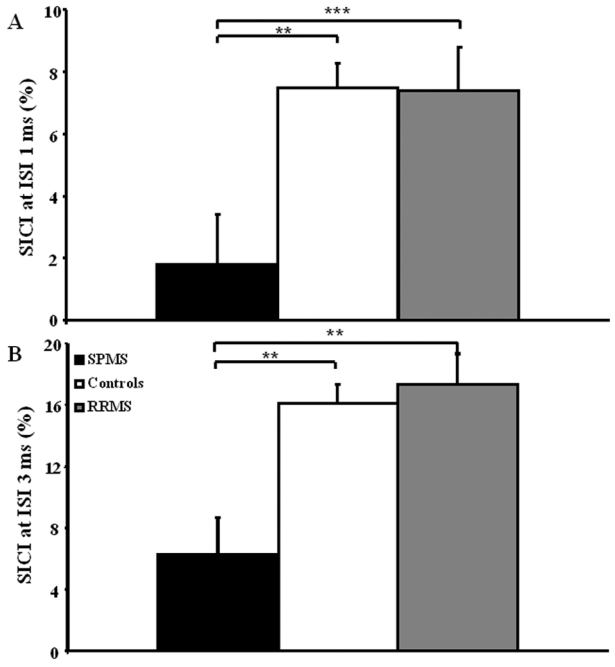

Figure 4. Short interval intracortical inhibition (SICI) peaks at an interstimulus interval (ISI) of 1 and 3 ms. There was a significant reduction of SICI at ISI 1 ms (A) and ISI 3 ms (B) in secondary progressive multiple sclerosis (SPMS) patients when compared with relapsing–remitting multiple sclerosis (RRMS) patients and controls.
$^{**}p < 0.01$ $^{***}p < 0.001$.

number of gadolinium enhanced lesions (Rho = 0.1, p = 0.25) and ARR (Rho = -0.1, p = 0.23). Multiple linear regression analysis (step wise) revealed that a model incorporating SICI and CMCT predicted the EDSS (Beta = -0.672, $p < 0.01$, adjusted $R^2 = 0.631$).

Discussion

Using novel threshold tracking TMS techniques combined with clinical and radiological assessment, the present study has established that abnormalities of cortical function were more prominent in SPMS and were linked to disability. These abnormalities of cortical function were evident by a significant reduction in short-interval intracortical inhibition and MEP amplitudes, and increased resting motor threshold, intracortical facilitation and prolonged central motor conduction time. Further, cortical dysfunction correlated with the EDSS, a conventional measure of disability in MS, thereby suggesting a link between cortical dysfunction and disability in MS. In contrast, cortical function appeared preserved in relapsing–remitting forms of MS, although the central motor conduction time was prolonged. White matter disease burden, as measured by T2LL, also correlated with disability, although the degree of correlation was not as strong as that for cortical dysfunction. Taken together, these findings suggest that cortical dysfunction may be a better predictor of development of disability in MS than simple assessment of white matter disease burden by radiological means.

Abnormalities of cortical function in MS

Short interval intracortical inhibition is mediated by GABA-secreting cortical inhibitory interneurons acting via $GABA_A$ receptors.[7,22] In the present study, the finding of marked SICI reduction in SPMS patients and the strong correlation of SICI reduction with EDSS suggest that cortical neuronal degeneration or dysfunction contributes to the development of neurological disability in MS. These findings are in keeping with one previous TMS study[10] and are supported by pathological studies documenting irreversible cortical neuronal damage in the form of neuronal apoptosis and atrophy of cortical gray matter structures in MS.[4,5,23,24] Of further support are findings that gray matter atrophy, as documented by specialized MRI techniques, is strongly correlated with development of disability and secondary progressive forms of MS.[2,3,25]

In addition to reduced SICI, further evidence of cortical dysfunction in SPMS was heralded by marked reduction in MEP amplitude and an increase in the resting motor thresholds. The MEP amplitude reflects the density of corticomotoneuronal projections, while the RMT reflects the excitability of cortico-cortical and thalamo-cortical axons which are located in the deep layers of the motor cortex.[26] Degeneration or dysfunction of these cortical neurons may account for the observed findings in the present cohort of MS patients, and is further in keeping with previous pathological and radiological studies reporting an association between cortical dysfunction and disability in MS.[2-5,24]

Of further relevance, central motor conduction time has also been regarded as a marker of cortical function reflecting the time to activation of cortical pyramidal cells and conduction down the corticospinal tracts.[27] Although CMCT was prolonged in both RRMS and SPMS, the extent of CMCT prolongation was greater in SPMS patients and correlated with the EDSS. While demyelination of corticospinal axons may in part contribute to the observed findings, the greater degree of CMCT prolongation in SPMS patients together with reduced MEP amplitude and a higher resting motor threshold suggests that degeneration of corticospinal axons accounts for this greater prolongation of CMCT. These findings are in accordance with previous studies[6,10,28,29] and further support the notion that cortical dysfunction is associated with disability in MS.[6,28,30,31]

A potential limitation of the study was that groups were not matched for gender. Specifically, the male:female ratio was 1:2.6 for RRMS patients, 1:2 for SPMS patients and 1:1.1 for controls. Given that there were no significant differences in cortical function between RRMS and controls, despite a greater gender difference, it seems unlikely that

gender differences accounted for the findings in the present study.

Neurodegeneration and the development of disability in MS

While the mechanisms underlying the development of disability in MS remain to be fully elucidated, degeneration or dysfunction of cortical neurons, as revealed by TMS studies in the present cohort of MS patients, may be an important mechanism. Glutamate excitotoxicity may in part underlie the development of this cortical neuronal dysfunction. Specifically, reduced expression of glutamate transporters located on astrocytes, which are crucial in maintaining low extracellular glutamate concentrations, have been reported in demyelinating cortical lesions of MS patients in post-mortem studies.[32] The finding in the present cohort of SPMS patients of increased intracortical facilitation, mediated in part by cortical glutamate activity[6,22] suggests that glutamate excitotoxicity may contribute to development of cortical dysfunction in SPMS.

Glutamate excitotoxicity may induce cortical neuronal degeneration by excessively stimulating the ionotropic N-methyl-D-aspartate (NMDA) receptors.[33] Specifically, glutamate excitotoxicity leads to an influx of intracellular Na^+ and Ca^2 ions, ultimately resulting in neurodegeneration via activation of Ca^{2+}-dependent mechanisms.[34] The importance of NMDA receptors, in particular the newly recognized NR3 subunits, in mediating neuronal injury in MS has been recently reported in MS.[35,36] From a therapeutic perspective, the use of adjuvant glutamate receptor antagonists in conjunction with established neuromodulatory agents may provide an additional therapeutic benefit in MS. As such, further clinical trials utilizing adjuvant neuroprotective agents may be warranted in MS patients.

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Conflict of interest statement

SV serves on the scientific advisory board for Novartis, Merck Serono Australia and Bayer Schering Australia and serves as a medical consultant for Merck Serono Australia.

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