Neurophysiological abnormalities in multiple sclerosis: Disease process or functional compensation?

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Abstract

Background: Intracortical inhibition is reduced in patients with multiple sclerosis. The cause and implications of this change are unknown: it may result from damage within inhibitory systems or, alternatively, may represent compensatory downregulation of cortical inhibition.

Objective: To examine the association between measures of intracortical inhibition and motor and cognitive abilities in patients with multiple sclerosis.

Methods: 36 patients with the relapsing-remitting form of multiple sclerosis were recruited from a specialty clinic and underwent evaluation of motor and cognitive ability using the Multiple Sclerosis Functional Composite scale. Cortical silent period (cSP) and short-interval intracortical inhibition (sICI) were measured in both hemispheres using transcranial magnetic stimulation. 13 healthy controls were evaluated on the same measures. We calculated correlations between functional and neurophysiological outcomes, and evaluated hemispheric asymmetries.

Results: Patients with remitting MS have significantly longer cSP durations (101.6±29.2msec) than healthy controls (82.2±22.4ms, $t(47)=-2.166$, $p=0.035$), indicating *increased* intracortical inhibition. Greater inhibition is associated with worse hand function as measured by the nine-hole peg test (dominant hand: $\rho=0.360$, $p=0.031$; non-dominant hand: $\rho=0.351$, $p=0.039$). Overall, cSP duration is comparable between cerebral hemispheres within

the patient group ($t(69)=0.633$, $p=0.529$). However, among patients with significant hemispheric asymmetry for cSP duration, the direction of this asymmetry predicts asymmetry for hand function ($\rho=0.950$, $p<0.001$).

Conclusion: These results support the hypothesis that alterations in cortical excitability in patients with MS reflect damage to inhibitory systems. In the context of earlier findings, our results also indicate that cortical excitability may change with respect to disease stage, and that the mechanisms underlying these changes may differ depending on stage. We cannot rule out the possibility that decreases in cortical excitability during the relapsing phase or in other subtypes of MS may be compensatory. Taking into account neurophysiological markers such as cSP may be useful in predicting disease severity.

Abrégé

Historique: Les mesures d'inhibition intracorticale sont réduites chez les patients atteints de sclérose en plaques. La cause et les implications de ces changements sont inconnues : cela peut résulter d'un dommage dans les systèmes d'inhibition ou, alternativement, peut représenter des régulations descendantes compensatoires de l'inhibition corticale.

Objectif: Pour examiner l'association entre les mesures de l'inhibition intracorticale et les habiletés cognitives et motrices chez les patients atteints de sclérose en plaques.

Méthodes: 36 patients ayant une forme de sclérose en plaques récurrente-rémittente ont été recrutés par une clinique spécialisée et ont subi une évaluation de leur habiletés motrices et cognitives en utilisant l'Échelle de composé fonctionnel de la sclérose en plaques. Des périodes de silence cortical (PSc) et l'inhibition intracorticale de court intervalle (IIcI) furent mesurées dans les deux hémisphères en utilisant la stimulation transcrânienne magnétique. 13 sujets normaux ont également été évalués avec les mêmes mesures. Nous avons calculé les corrélations entre les résultats fonctionnels et neurophysiologiques, et avons évalué les asymétries hémisphériques.

Résultats: Les patients avec une sclérose en plaques rémittente possèdent des PSc d'une durée significativement plus longue (101.6±29.2msec) que les sujets normaux (82.2±22.4ms, $t(47)=-2.166$, $p=0.035$), ce qui indique une

augmentation de l'inhibition intracorticale. Une inhibition plus grande est associée avec des fonctions manuelles moins bonnes telles que mesurées par le test de dextérité manuelle de neuf-trous (main dominante: ρ=0.360, p=0.031; main non-dominante : ρ=0.351, p=0.039). Généralement, la durée PSc est comparable entre les hémisphères cérébraux dans le groupe de patients (t(69)=0.633, p=0.529). Cependant, parmi les patients ayant une asymétrie hémisphérique significative pour la durée des PSc, la direction de cette asymétrie prédit une asymétrie pour la fonction manuelle (ρ=0.950, p<0.001).

Conclusion: Ces résultats supportent l'hypothèse que les changements dans l'inhibition corticale chez les patients atteints de sclérose en plaques reflètent le dommage des systèmes inhibitoires. Dans le contexte de nos découvertes précédentes, nos résultats indiquent également que l'inhibition corticale pourrait changer en ce qui concerne le stade de la maladie, et que les mécanismes reliés à ces changements pourraient différer dépendamment du stade. Nous ne pouvons omettre la possibilité que la augmentation de l'inhibition corticale durant la phase récurrente ou autre sous-forme de SP puisse être compensatoire. Il pourrait être utile de prendre en considération les marqueurs neurophysiologiques tels que la PSc afin de prédire la sévérité de la maladie.

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tolerated my bursts of intense focus and work, and furnished brilliant feedback to my occasionally less than coherent postulations.

Preface

The work presented in this thesis is part of a larger study proposed by Dr. Lisa Koski. Although Dr. Koski devised the concept for the study, I played an integral role in virtually all subsequent components, including: submitting an application for ethical approval to work with human subjects, modifying the data collection strategies and designing several tools used in data collection, performing a primary literature review focussing on cortical excitability and MS, collecting data, programming data analysis software for post-processing, performing post-processing of data and statistical analysis, and preparing this thesis in its entirety. I also proposed and designed, in collaboration with Dr. Koski, Dr. Douglas Arnold, and Dr. Sridar Narayanan, an imaging protocol that significantly extends the scope of the original study to include objective measures of disease burden. Dr. Arnold and Dr. Lesley Fellows served on my advisory committee, and made several useful suggestions pertaining to the interpretation of my results. Dr. Josephine Nalbantoglu served as my Graduate Program Mentor, and also provided useful feedback pertaining to the interpretation of my results.

Neurophysiological abnormalities in multiple sclerosis
B. Whatley

1. Introduction

Multiple sclerosis (MS) is one of the most common neurological diseases in Canada. Prevalence of MS in this country, estimated at 240/100,000, is among the highest in the world[1, 2]. A recent review of prevalence studies determined that there is a female to male ratio of approximately 2:1, although there may be considerable variation in this ratio[1].

MS is a debilitating disorder with an unclear underlying aetiology. The histopathologic hallmark of the disease is demyelination within the white matter of the central nervous system (CNS), with associated destruction of axons[3]. Pathological changes in cortical grey matter also contribute to clinical deficits[4]. Because lesions can occur anywhere within the CNS, the symptoms of MS are highly variable. Visual disturbances stemming from lesions in the anterior visual pathways are most common, and include scotomas, reduced visual acuity, and rapid afferent pupillary defects. Lesions may also result in motor and sensory disturbances, ataxias, diplopia, dysarthria, neurogenic bladder, and cognitive disturbances[5]. Fatigue is reported by 90% of patients with MS[6]. Depending on the subtype of MS, symptoms may resolve somewhat or not, and may accumulate in different domains over time. However, the disease course is often difficult to predict at the time of diagnosis, which can increase the difficulty patients have in adjusting to the disease[7].

Brain imaging and neurophysiologic techniques are shedding new light onto the processes underlying MS and its effects on the brain, and there is hope that these techniques will allow for more refined diagnosis, prognosis, and treatment options. Certainly, modern neuroimaging has already revolutionised diagnostic practices in MS, and there is promise that continuing advances may also allow us to better predict clinical impairment based on objective markers of the disease[8, 9]. However, despite numerous technical advances, correlations between conventional neuroimaging data and functional impairment are modest at best[9-11]. Findings from studies employing neuroimaging and transcranial magnetic stimulation (TMS) are beginning to generate hypotheses about the ways in which the brain *responds* to damage caused by MS, which may explain part of the discrepancy between apparent damage and disability. Increased activation of certain brain areas and network alterations may allow for maintained performance on motor and cognitive tasks despite lesion burden, implying a form of *functional compensation* [12-16]. Several studies using TMS demonstrate that alterations in cortical inhibitory systems may lead to increased cortical excitability in certain patients with MS[17-20]. Other studies show no such differences[21-23]. Whether these putative changes in excitability are entirely beneficial is far from clear: increased cortical excitability may support functional compensation, or indeed they may reflect damage to inhibitory networks – another sign of disease progression. Both models are represented in Figure 1.

The primary objective of this study is to test the hypothesis that **changes in cortical excitability represent a form of functional compensation, which allows patients with MS to maintain performance on motor and cognitive tasks despite the presence of CNS lesions.** We will measure cortical excitability using TMS, and will compare these measures to performance on motor and cognitive tasks. This study will contribute to developing notions of neuroplasticity in MS, and may lead to testing of new therapeutic options.

2. Background

§2.1 Multiple sclerosis: Brief history

Jean Martin Charcot first described three cases of *sclérose en plaques disséminée* in 1866[24]. In 1868, he presented several lectures at the Salpêtrière on this topic as part of his 'Lectures on Diseases of the Nervous System'[25]. Lecture VI contains descriptions of penetrating lesions spread throughout the cerebrum, cerebellum, pons, medulla, and spinal cord, with microscopy demonstrating a loss of 'medullary sheaths' around axons in the spinal cord. His gross anatomical depictions (Fig 2) of cerebrum are easily recognisable to neurologists today[24]. In Lecture VII, Charcot describes symptoms of disseminated sclerosis as those of an "eminently polymorphous affection" (p. 183). Principally, he draws attention to intention tremor, ocular symptoms – including diplopia, amblyopia, and nystagmus – and a particular speech disturbance whereby "the words are as if measured or scanned; there is a pause in after every syllable, and the syllables themselves are pronounced slowly" (p. 192). Later stages of the disease are marked by respiratory difficulties, as well as extremity rigidity and paresis. Lecture VIII provides an overview of the natural history of disseminated sclerosis and speculates on potential causes – mostly "appertaining to the moral order" (p. 220). Charcot concludes that the prognosis is terrible, with most known patients dying by the age of 40. However, he speculates that in the future,

physicians may take advantage of "the spontaneous tendency to remission which has been noticed in a great number of cases" (221).

Today, there is still considerable debate with respect to the pathogenesis of MS. It is considered a classic autoimmune disorder, in which patients produce antibodies directed towards myelin-specific antigens, but the trigger for this attack is unknown. The resulting demyelination results in sclerotic lesions throughout the CNS. Though classically considered a white matter disease with secondary neuronal degeneration, newer neuroimaging techniques reveal extensive grey matter involvement[26-28]. This grey matter damage may even precede white matter involvement, and could be responsible for significant cognitive disability[28]. Symptoms of MS likely relate to the distribution of lesions, and include motor symptoms, visual disturbances, gait abnormalities, sexual dysfunction, difficulty with balance, and urinary incontinence among others. Motor symptoms include weakness, paralysis, spasticity, deterioration of fine motor control, and muscle wasting.

While motor and visual disturbances are the most striking features of MS, cognitive deficits, fatigue, and depression are increasingly recognised as important contributors to disability. Cognitive decline is particularly devastating and widespread. Community-based studies have estimated the prevalence of cognitive impairment to be 43%[29], while a meta-analysis of clinic-based trials reveals a prevalence of 65%[30]. While general intelligence is usually spared, processing speed, visual learning, and visual

memory are most commonly affected[31]. Cognitive impairment is progressive over time, and leads to lower health-related quality of life scores[32].

§2.2 Investigating MS pathology and neurophysiology

Neuroimaging, and in particular magnetic resonance imaging (MRI), has revolutionised the diagnosis and monitoring of MS. The development of conventional MRI techniques to evaluate white matter lesions allows for earlier assessment of patients with putative MS[8], and these techniques also allow for tracking the number, distribution, and size of lesions in longitudinal studies. However, correlations between white matter lesion measures and clinical markers are modest[33]. This apparent paradox, in addition to research revealing extensive grey matter pathology, has ultimately led to a less lesion-centric understanding of MS.

Newer imaging and image processing techniques allow for monitoring additional aspects of MS pathology, including cortical atrophy, axonal damage, and changes in functional connectivity. Changes in grey matter atrophy correlate well with clinical deterioration as measured by the MS Functional Composite (MSFC) score [34], and predict changes in cognition better than total lesion volume[35]. Magnetic resonance spectroscopy (MRS) allows for the investigation of pathology in normal-appearing white matter[36]. This latter technique provides measures that correlate well with Extended Disability Scale (EDS) scores[37], and allows detection of

pathological changes at the earliest stages of MS[36]. Finally, resting state fMRI analysis uses spontaneous fluctuations in the blood oxygen level-dependent (BOLD) signal to trace functional networks in the brain[38]. Changes in functional connectivity within several networks have been documented in patients with MS[15, 39-41], which may reflect compensation as well. All of these techniques provide a more refined analysis of the damage and reactions caused by MS than was previously available.

Another investigative modality used to measure neurophysiological changes in patients with MS is transcranial magnetic stimulation (TMS). TMS is a non-invasive technique whereby a strong, time-varying magnetic field is passed through the skull, inducing electrical stimulation of the underlying cortex according to Faraday's principle of electromagnetic induction. When TMS is applied over the primary motor cortex, it depolarises neurons in the corticospinal tract, producing descending volleys of action potentials. This results in involuntary contraction of a target muscle, which can be measured as a motor evoked potential (MEP) using electromyographic recording equipment. Simple single-pulse techniques can be used to assess MEP latency and amplitude, as well as motor threshold (MTh) – the minimum intensity of stimulation required to produce a barely detectable motor response.

Measurement of central mean conduction time (CMCT) to assess the pyramidal tracts in patients with MS was one of the earliest applications of

TMS[42]. In addition to cortical stimulation, the patient is stimulated over the C7-T1 interspace (the space between the lowest cervical vertebra and the highest thoracic vertebra, where the C8 nerve root exits the spine) to induce conduction in one of the peripheral nerves supplying the arm. Motor evoked responses (MEPs) are recorded over a target muscle in the hand, and CMCT is calculated as the difference in MEP latency between scalp and spinal stimulation. In other words, CMCT is the time required for an action potential to pass from the motor cortex to the C7-T1 level of the spine. In patients with MS, CMCT is prolonged compared with healthy controls, with an abnormality detection rate similar to that of other evoked potential techniques[42, 43]. Abnormalities in CMCT reflect central demyelination, and are helpful in identifying clinically silent lesions[42], predict disease progression in combination with other neurophysiological markers[44], and may help to monitor effects of disease modifying agents[45].

In addition to probing integrity of the corticospinal tracts, TMS can be employed to evaluate intracortical neurophysiology. Two techniques in particular, *short-interval intracortical inhibition* (sICI) and *cortical silent period* (cSP), allow for investigations of cortical excitability. sICI refers to the ratio between responses to a) conditioned stimuli, and b) unconditioned stimuli. Conditioned stimuli are involve delivering two pulses – one subthreshold 'conditioning' pulse, and one suprathreshold 'test' pulse – with interstimulus intervals of 1-6ms[46]. The conditioning pulse results in relative inhibition of the response to the test pulse, an effect likely mediated

by $GABA_A$-receptor activation[47], although presynaptic $GABA_B$ receptors may be implicated as well[48]. Greater sICI ratios therefore reflect *less* inhibition. cSP refers to the duration of suppression of EMG-activity following an MEP in a voluntarily-contracted target muscle. This effect is likely produced by a combination of spinal and cortical factors, with the first 50ms reflecting recurrent inhibition of the spinal motoneurons, and the subsequent duration of EMG suppression reflecting cortical inhibitory mechanisms[49]. $GABA_B$-receptor activation has been implicated in this measure as well[48]. Shorter cSP reflect less inhibition. Thus TMS allows evaluation of two distinct measures of intracortical inhibition.

Given the increasing appreciation for the effects of MS on grey matter, the use of TMS as an investigative tool is particularly relevant. The intracortical inhibition measures described above depend on changes within the cortex, and therefore may be sensitive to grey matter disease. Studies in patients with stroke have demonstrated that changes in sICI are significantly more likely with cortical strokes[50], and that cSP may be prolonged with subcortical, but shortened with cortical strokes[51]. The latter of these studies reports correlations between cSP duration and functional outcomes[51]. As such, TMS may provide another means of assessing the impact of grey matter disease in patients with MS, and may allow a more refined understanding of its contributions to clinical disability.

Several studies have assessed intracortical inhibition in patients with MS, with mixed results[17-23, 52]. In those studies evaluating sICI as a measure of inhibition, two found no differences between patients with MS and healthy controls [21, 23]. Liepert et al. reported that cortical inhibition as measured by sICI decreased in patients with relapsing-remitting MS (RR-MS) who report fatigue compared to patients without fatigue[17]. Caramia et al. reported that cortical inhibition is significantly decreased in patients with RR-MS who are relapsing, when measured on the affected side, compared to healthy controls[18]. Conte et al. measured sICI in both RR-MS and secondary-progressive MS (SP-MS) patients without fatigue, and determined that cortical inhibition is decreased in patients with SP-MS only[19]. None of these patients had gadolinium-enhancing lesions on MRI, nor were there any group differences in lesion load, suggesting that white matter lesions alone do not determine differences in sICI[19]. Taken together, these studies suggest that decreases in cortical inhibition, indicated by increases in the sICI ratio, may be related to disease severity. However, none of these studies specifically related changes in inhibition to functional outcomes measured across the broad range of disability recognised in MS.

Fewer studies have evaluated cSP in patients with MS. Thickbroom et al. found no difference in cSP duration between patients with MS and healthy controls[52]. However, the subtype of MS was not identified in this study, and subjects with upper-extremity symptoms were excluded. Caramia et al studied cSP in patients with RR-MS who were either in remission or

relapsing, and found that relapsing patients had significantly shorter cSPs than either patients in remission or healthy controls[18]. At the group level, there were no significant differences between hemispheres for cSP, although in patients with hemispheric asymmetries, there were monohemispheric active lesions corresponding to the side with shorter cSP. Interestingly, there were 8 subjects who were tested twice: once at the beginning of the study during a relapse, and again while in remission. In these patients, cSP changed such that it was shorter during relapse and longer in remission, comparable with the values of the larger groups in each condition. Finally, Fierro et al. conducted a double-blind trial in which patients with relapsing RR-MS were given either 1g or 2g per day of IV methylprednisolone [20]. cSP was used as one measure of response to treatment. In this study, there was a significant difference in cSP at baseline between relapsing patients and healthy controls, and lengthening of the duration of cSP correlated with clinical improvement as measured by EDSS. No correlation existed between EDSS and cSP at baseline. Although there is a paucity of information with respect to cSP in patients with MS, these studies indicate that disease severity may be related to a shortening of cSP, indicating decreased cortical inhibition. Again, however, none of these studies presents data assessing disease burden in terms of hand function or cognitive functional outcomes.

§2.3 Decreased cortical inhibition: Damage or functional compensation?

How are we to interpret findings of decreased inhibition in certain groups of patients with MS? First, there is a lack of consensus on which patients have alterations in inhibition. Liepert identified a subpopulation of relapsing-remitting patients, those with fatigue, who demonstrate reduced cortical inhibition[17], while Conte found differences only in patients with secondary-progressive MS[19]. Caramia identified reductions in cortical inhibition only in relapsing patients, and only within the affected hemisphere (although she did not generally test the unaffected hemisphere)[18]. Other studies found no effect on sICI. Findings with respect to cSP are more consistent, with decreases in cSP relating to physical deficits[18].

It is tempting to conclude based on these studies that decreases in cortical inhibition are a direct marker for disease progression, perhaps reflecting damage to inhibitory circuits. However, an alternative explanation is that decreased inhibition reflects a mechanism of functional compensation in response to increasing disease burden, whereby disinhibition supports some maintenance of function despite increasing disease severity. In the Liepert study, disability measures were similar between the patients with RR-MS +/- fatigue, but sICI was significantly less in the fatigued group[17]. The authors posit that fatigue in MS might be a reflection of disinhibition, and speculate that regulated disinhibition might compensate for premotor fatigue. If changes in sICI are consistent across cerebral hemispheres, then it may also

be reasonable to hypothesize that cortical disinhibition may also support cognitive function.

The strongest evidence in support of the idea of functional compensation in MS comes from imaging studies. In one study, reductions in the N-acetylaspartate:phosphocreatinine ratio (NAA/Cr), a neuroimaging indicator of axonal damage that may precede clinical signs, have been associated with increased activation of the ipsilateral sensorimotor cortex during performance of a finger-tapping task in MS patients without hand impairment[13]. The authors concluded that such increases may reflect adaptive changes in brain function that compensate for early axonal damage. Another study demonstrated that in patients with primary progressive MS, lesion burden is correlated with increased activity in non-motor regions of the brain during performance of a simple motor task[14]. Preservation of cognitive performance over time may also be supported by functional adaptations. Patients with clinically isolated syndrome who improved their performance on a test of working memory and processing speed one year after initial testing showed greater increases in prefrontal cortex activity while performing the same task, as compared with patients whose performance remained stable or declined[16]. Taken together, these studies suggest that the brain may be able to functionally compensate for damage caused by MS-type lesions, and that this compensation could allow preserved performance on cognitive and motor tasks.

No previous studies have assessed the relationship between cortical inhibition and cognitive, mobility, and dexterity functional outcomes. Evaluating this relationship is critical to understanding the implications of changes in cortical inhibition by disentangling the damage versus compensation hypotheses. The present study seeks to undertake just such an evaluation.

3. Objectives

The primary objective of the proposed study is to investigate the relationship between measures of cortical inhibition and severity of disability in a population of patients with relapsing-remitting multiple sclerosis. **If it is the case that decreases in cortical inhibition are a marker for *disease severity* only, we predict that clinical disability will be negatively correlated with cortical inhibition. If, on the other hand, decreases in cortical inhibition reflect *functional compensation*, we predict that clinical disability will be positively correlated with cortical inhibition.**

Measures of cortical inhibition are obtained using TMS, and include sICI and cSP. Disability is assessed using the Multiple Sclerosis Functional Composite (MSFC), a test that represents the breadth of disability inherent in MS[10, 53].

As secondary objectives, we will a) add to the existing literature by determining if patients with RR-MS have significant differences in cortical inhibition compared to healthy controls, b) determine whether changes in cortical inhibition are symmetric or asymmetric across cerebral hemispheres, and c) if asymmetric changes in cortical inhibition are related to asymmetric disability.

This project took place within the context of a larger study, which is also assessing a) the relationship between cortical inhibition and various measures of fatigue, and c) whether decreasing cortical excitability using repetitive TMS can lead to improvement in motor function.

4. Subjects

45 patients with multiple sclerosis were recruited from a randomly-selected sample from an MS clinic at the Montreal Neurological Hospital. Inclusion criteria included: diagnosis after 1994 (i.e. since the advent of neuroimaging criteria and disease-modifying agents). Exclusion criteria included: treatment with drugs known to affect cortical excitability (benzodiazines, gabapentin, pregabalin, neuroleptics, or any other drugs affecting GABAergic transmission), diagnosis of a health condition known to exert an effect on functioning independent of MS, and relative contraindications to TMS (personal or immediate family history of seizure disorders, pacemaker, pregnancy, metal fragments in head, prosthetic valve, pacemaker, aneurism clip, metal prosthesis)[54]. Patients in whom an MEP could not be evoked were also excluded from further participation. We did not exclude patients taking disease-modifying drugs unless they violated any of the aforementioned criteria.

14 age- and gender-matched controls were also recruited by poster.

5. Methods

Neurophysiological measures of cortical inhibition and secondary measures of cortical excitability, as well as performance-based measures of hand function, mobility and cognition were obtained in all subjects during a single session. The order of data collection did not vary between subjects, with neurophysiological assessment always preceding functional assessments. This order was implemented in order to exclude those patients in whom an MEP could not be elicited from further data collection. Table 1 displays a summary of data collected.

Each patient gave informed consent, and a local ethics committee approved our protocol.

§5.1 Clinical data

Based on a review of their clinical charts, our sample of patients was characterised by diagnosis, sex, age, height, duration of disease, most recent EDSS score, time since last relapse, and current use of medications (disease-modifying drugs, steroids).

§5.2 Functional evaluation

The MS Functional Composite (MSFC) was administered to all subjects in their preferred language of English or French and according to manualised instruction[55]. This tool better represents the spectrum of disability that may manifest in patients with MS compared to the EDSS, as it includes

measures of cognition (paced-auditory serial addition task, administered with 3-second interstimulus intervals – PASAT3), mobility (timed 25-foot walk – T25W), and hand function (9-hole peg test – 9HPT) [53].

The symbol-digit modalities test (SDMT) was also administered as a secondary test of cognitive function. Since several of our subjects had impaired hand function, this test was administered using the oral format in all but the first two subjects.

§5.3 Neurophysiological assessment with TMS

TMS measures used in this study included motor threshold (MTh), single-pulse motor-evoked potential amplitude (MEPamp), short-interval intracortical inhibition (sICI), and corticospinal silent period (cSP). These measures provide complementary information about cortical inhibition and excitability.

The primary motor cortex area representing the contralateral first dorsal interosseus (FDI) muscle was selected for targeting. When available, T1-weighted brain images of each subject were loaded into a frameless stereotactic system (Brainsight, Rogue Research, Montreal, Canada) for direct targeting of the cortical hand area. In the absence of imaging, a generic brain image was used as a basis for neuronavigation, and the coil was placed approximately over the cortical hand area, oriented tangentially to the skull, with the handle pointing back and away from the midline at approximately 45°, as per standard practice[56]. Stimulation was delivered through a

Magstim 70mm 'figure of eight' coil, connected by a Bistim module to two Magstim 200^2 stimulators (Magstim, Whitland, UK). Electromyographic (EMG) recordings were acquired via belly-tendon montages over the FDI on right and left hands, and were available for online, realtime visual inspection. Stimulating within the cortical hand area, we first determined placement and orientation of the coil corresponding to the 'hotspot' for FDI stimulation. This was defined as the stimulation site resulting in the largest peak-to-peak motor evoked potential (MEP) in the target muscle. Resting motor threshold (RMT) was then determined at the hotspot, according to standard procedures[56], and defined as the lowest intensity at which stimulation evoked a response ≥ 50μV in at least 50% of a series of 6 consecutive trials.

EMG recordings were filtered with a bandpass of 100-1000Hz and sample rate of 2000Hz for subjects MS01 – MS18, and with a bandpass of 10 – 3000Hz and sample rate of 6000Hz for subjects MS19 – MS44. The data for both hands were recorded on a personal computer for offline analysis, using a modular MATLAB time-based data analysis tool (dataWizard, version 0.7.7, A.D. Wu, UCLA). Data were stored in 250-ms samples, including a 100-ms pre-stimulus window to confirm muscle relaxation. The 150-ms post-stimulus window is longer than that normally used, but was deemed necessary to capture the entirety of responses with longer onset latencies and longer durations, such as are common in MS.

Short-interval intracortical inhibition (sICI) provides a measure of intracortical inhibition that is most likely mediated by $GABA_A$ receptors[57] (see figure 2). In the present study, we followed the paired-pulse technique outlined by Kujirai et al.[46]. A conditioning stimulus at 80% RMT was followed by a suprathreshold test pulse at 120% RMT, at various interstimulus intervals (ISIs). Previous studies indicate that there are different mechanisms underlying sICI measured with an ISI of 1ms, and sICI measured between 2-4ms[48, 57, 58]. sICI measured at an ISI of 2-4ms (sICI_2-4) likely reflects synaptic $GABA_A$ activity [59]. The mechanism behind sICI measured at an ISI of 1ms (sICI_1) is less clear. Some authors have suggested that sICI_1 reflects neural refractoriness[48], whilst others have argued that both sICI_1 and sICI_2-4 reflect GABAergic activity, but may represent the effects of different I-waves[58]. As such, we elected to collect data for sICI measured at 1, 2, & 3ms. Blocks consisted of 8 paired-pulses using each ISI as well as 8 single-pulses, delivered in a randomised order over both hemispheres, with inter-trial intervals of at least 6 seconds. The non-dominant hemisphere was always tested first, to facilitate procedural flow with other aspects of the broader study. Trials were only retained if both FDIs were completely relaxed during the pre-stimulation window, as verified immediately following each trial by visual inspection of the EMG trace. EMG traces were subsequently analysed offline. sICI was calculated as the ratio of the median peak-to-peak amplitude of the paired-pulse MEP elicited at each ISI, to the median amplitude elicited by a single pulse. As

such, a larger number for sICI reflects a *lesser* degree of inhibition. Thus, six sICI values were obtained from each subject (three per hemisphere), as well as two single-pulse amplitude values. In healthy controls, only the dominant hemisphere was tested.

Cortical silent period (cSP) provides a measure of both cortical and spinal inhibitory processes [60] (see figure 3). This measure may be mediated by $GABA_B$ receptors[48], although the specific role of these receptors is disputed[57]. Subjects were asked to contract the FDI at approximately 40% maximum contraction, as determined by a pinch gauge. When the target muscle was contracted, a single-pulse stimulus was delivered over the hotspot at 120% RMT, producing a supramaximal MEP. Ten trials were collected over each hemisphere. EMG traces were subsequently rectified and analysed offline. cSP was calculated as the time from onset of the MEP to the resumption of baseline activity, obtained from an averaged trace of the ten trials. Two cSP values were obtained from each subject (one per hemisphere). In healthy controls, only the dominant hemisphere was tested.

6. Data processing

§6.1 Post-recruitment exclusions

During or following data collection, but prior to data analysis, 9 MS and 1 HC subjects were excluded from analysis for the following reasons:

i. Given small numbers of subjects with primary-progressive or secondary-progressive MS, only those with relapsing-remitting MS (RR-MS) were included in subsequent analysis to ensure a more homogenous sample. 6 subjects were excluded for this reason.
ii. Upon chart review, one subject was found to have clinically-isolated syndrome, not RR-MS.
iii. One subject withdrew after consenting, but prior to collection of functional or neurophysiological data.
iv. One subject had excessive background electromyographic noise during neurophysiological data collection, which rendered collection of these data impossible.
v. One healthy control did not show up for the data collection session.

As such, data were analysed for 36 subjects with RR-MS, and 13 healthy controls.

§6.2 Neurophysiological data processing and post-processing exclusions

What follows is a synopsis of data processing decisions and techniques, where those differ from standard practice. By virtue of the fact that we were working with subjects who had neuropathology known to affect cortical excitability, EMG traces resulting from targeted stimulation were not always amenable to standard processing. The following decisions were made in an effort to include as much data as possible, while impartially dealing with anomalies.

When evaluating MEP amplitude, the automated data processing relies on being able to identify key values: MEP max, and MEP min. However, in many trials across several subjects, there were no responses. Counting these trials as 'zero' amplitude would likely underestimate the true value, whilst excluding these trials would certainly overestimate the value. As such, we determined time markers for these critical events from an averaged trace for each condition within each subject, and took the amplitude values at those time points on traces where no distinct response could be observed.

One expects that stimulating at 120% RMT will normally produce an MEP with an amplitude of between 0.5 and 1.0 mV in the FDI. However, in our sample, we obtained MEP amplitudes that were considerably smaller from several subjects. Because these single-pulse MEP amplitudes form the denominator of the ratio that represents sICI, a sufficiently small single-pulse response will necessarily lead to inaccurate sICI measurements, skewed

towards over-estimation. As such, we decided to exclude single-pulse MEP amplitude and sICI data when single-pulse amplitude for a given hemisphere was less than 0.1mV. This decision resulted in the exclusion of single pulse MEP amplitude and sICI data for one hemisphere in six RR-MS subjects (three in non-dominant hemisphere, three in dominant hemisphere) and one healthy control (dominant hemisphere).

Additional post-processing exclusions or imputations are as follows:

i. Non-dominant hemisphere data for single-pulse MEP amplitude and sICI excluded in one subject due to excessive unilateral background EMG.
ii. Non-dominant hemisphere data for single-pulse MEP amplitude and sICI excluded in two subjects due to immeasurable motor thresholds, where no MEPs were generated using stimulation at 100% MSO.
iii. Non-dominant hemisphere sICI measured with an ISI of 2ms in one subject was imputed by averaging the sICI calculated for ISI of 1ms and 3ms, because there were no responses in any of the eight trials at an ISI of 2ms.

§6.3 Statistical analysis

Given significant deviation of both neurophysiological and functional measures from normal distributions, all analyses were conducted using non-parametric tests. The exception to this practice was comparison of cSP

durations between patients and HCs, as both were normally distributed. Mann-Whitney tests were used to compare measures of cortical excitability between patients and healthy controls. These comparisons were conducted for ICI measured at 1, 2, and 3ms, cSP, single-pulse MEP amplitude, and motor threshold.

Kruskal-Wallis one-way analysis of variance testing was used to assess the effect of ISI on sICI in both subjects with RR-MS and healthy controls. Significant effects were further characterised using Mann-Whitney tests. Ultimately, sICI values for each subject with RR-MS were averaged across hemispheres, and across ISI = 2 and 3, to create the variables ICI_1 and ICI_23. Averaging across these ISIs is consistent with their likely sharing an underlying mechanism, as described above.

Results for subjects with RR-MS for all components of the MSFC were transformed into standardised scores using data from the National MS Society database, according to manualised directions[55]. Component scores were then combined to create a composite score. These transformations were performed to confirm that our population of subjects with RR-MS is comparable on these tests to the reference population. Raw scores on all components of the MSFC as well as the SDMT were compared across RR-MS and healthy control groups. Again, Mann-Whitney tests were used to assess for significant differences.

To evaluate the functional compensation hypothesis, we calculated correlations between functional scores and neurophysiological scores. Spearman's rank correlation coefficients were calculated between neurophysiological measures (sICI_23, sICI_1, cSP, single-pulse MEP amplitude, motor threshold) and functional scores (MSFC composite score, 9-NPT for dominant and non-dominant hand, T25W, PASAT3, SDMT).

To further explore the associations between intracortical inhibition and functional impairment, we created asymmetry scores for 9HPT, sICI_1, sICI_23, and cSP. For 9HPT, Z-scores were calculated based on an age- and dominance-matched reference population [61]. Using this transformation, higher Z-scores would correspond to longer 9HPT times, or worse performance. For consistency with MSFC scores, z-scores were therefore multiplied by -1, such that higher z-scores reflect better performance. The asymmetry score was then calculated as 'Dom_z-score – NDom_z-score', such that a higher asymmetry score reflects better performance in the dominant hand. Asymmetry scores for sICI_1 and sICI_23 were calculated as 'Dom_sICI – NDom_sICI', such that higher asymmetry scores indicate a higher sICI ratio – and therefore less inhibition – within the dominant hemisphere. Asymmetry scores for cSP were calculated as -('Dom_cSP – NDom_cSP'), such that higher asymmetry scores reflect shorter cSP – and thus less inhibition – within the dominant hemisphere. To assess whether asymmetric cortical inhibition predicts asymmetric hand function, outlying asymmetry scores were first identified as those greater than one standard deviation from the

mean. Spearman's rank correlation coefficients were then calculated to determine whether outlying asymmetries in cortical inhibition are related to asymmetries in functional impairment.

7. Results

§7.1 Subject characteristics

Patient and healthy control characteristics are summarised in Table 2. Data were analysed from 36 patients with RR-MS diagnosed after 1994, and 13 healthy controls. These groups did not significantly differ in age [$t(47) = -0.44$, $p = 0.664$], or sex, [$X^2(1) = 0.271$, $p = 0.602$]. Within the RR-MS group, EDSS scores ranged from 0 (no disability) to 6.5 (requiring nearly constant bilateral support for mobilisation of 20 meters) ($M = 2.35$, $SD = 2.0$).

§7.2 Functional measures

Functional outcomes are reported in Table 3. Standardised *z*-scores demonstrate that our sample of patients with RR-MS is consistent with the reference population of patients for the composite MSFC score (M=0.06, SD=0.64), 9-HPT (M=0.23, SD=0.88), T25W (M=0.01, SD=2.4), and PASAT3 (M=-0.41, SD=1.0). Compared with healthy controls, patients with RR-MS required significantly longer to complete the 9-HPT with the dominant hand (U=91.5, n_1=13, n_2=36, $p = 0.001$), 9-HPT with the non-dominant hand (U=66.5, n_1=12, n_2=36, $p = 0.000$), and T25W (U=94.0, n_1=13, n_2=36, $p = 0.002$). Patients with MS scored fewer answers in the allotted time on the SDMT (U=108.0, n_1=13, n_2=34, $p = 0.007$), but did not differ significantly in performance on the PASAT3 (U=160.5, n_1=13, n_2=36, $p = 0.096$).

§7.3 Neurophysiological measures

sICI: Between subjects analysis

Neurophysiological outcomes are reported in Table 4. sICI data were not normally distributed for either patients or healthy controls. Within the dominant hemisphere, Mann-Whitney testing revealed no significant differences between patients and healthy controls for sICI_1 [U=159.0, n_1=12, n_2=34, p = 0.260], sICI_2 [U=199.0, n_1=12, n_2=34, p = 0.900], or sICI_3 [U=168.0, n_1=12, n_2=34, p = 0.368].

Effect of hemisphere and ISI on sICI

The effect of hemisphere could be assessed only in the patient group, as only dominant-hemisphere data were collected in the healthy control group. Due to the non-normal distribution of data for these measures, and the lack of an appropriate non-parametric substitute for the two-way ANOVA, we could not assess the possibility of a hemisphere x ISI interaction. However, visual inspection of Figure 4 reveals that a significant interaction is unlikely. Within the patient group, Kruskal-Wallis one-way analysis of variance reveals no effect of ISI within the non-dominant [X^2=1.937, df=2, p=0.380] or dominant [X^2=4.359, df = 2, p=0.113] hemisphere (see Figure 4). There is no effect of dominant versus non-dominant hemisphere at any ISI (1ms: [U=401.0, n_1=33, n_2=31, p = 0.138]; 2ms: [U=465.0, n_1=33, n_2=31, p = 0.532]; 3ms: [U=379.0, n_1=33, n_2=31, p = 0.075]).

Within the healthy controls (dominant hemisphere only), Kruskal-Wallis one-way analysis of variance reveals a trend towards an effect of ISI on sICI [X^2=4.950, df=2, p=0.084], which appears to be driven by the difference between ISI=1ms and ISI=2ms (see Figure 5).

Based on these findings, which support previous reports regarding different mechanisms behind sICI_1 and sICI 2-4[48, 58], sICI_2 and sICI_3 were averaged to create the variable sICI_23. For subsequent correlations with non-lateralised functional measures (i.e. MSFC, PASAT3, T25W, and SDMT), sICI_1 and sICI_23 were each averaged across hemispheres.

Neurophysiological measures: cSP

Data for cSP were normally distributed in both patient and healthy control groups. Within the dominant hemisphere, cSP was significantly longer in the patient group (M=101.5 ± 29.2ms) compared to the healthy controls (M=82.2 ± 22.4ms) [t(47)=-2.166, p=0.035]. Within the patient group, there was no effect of hemisphere on cSP duration [t(69)=0.633, p=0.529]. For subsequent correlations with non-lateralised functional measures (MSFC, T25W, PASAT3, and SDMT), cSP was therefore averaged across hemispheres.

Neurophysiological measures: Secondary outcomes

Secondary outcomes are reported in Table 5. For single pulse MEP amplitude (MEPamp) and motor threshold (MTh) outcome measures, only data from patients recruited after correcting the amplifier settings were analysed

(MS18-MS44), as it is felt that the differences in band-pass filtering almost certainly altered these measures in a non-systematic manner. MEPamp and MTh were not normally distributed. Within the dominant hemisphere, motor threshold did not differ significantly between patients (M=48.3 ± 11.3%MSO) and healthy controls (M=42.8 ± 7.0%MSO) [U=90.5, n_1=13, n_2=21, p=0.102]. Healthy controls (M=1.27 ± 1.0mV) had significantly higher MEPamp than patients (M=0.42 ± 0.33mV) [U=42.0, n_1=12, n_2=19, p=0.004].

Within the patient group, there was no effect of hemisphere on MTh [U=208.0, n_1=21, n_2=21, p=0.753] or MEPamp [U=179.0, n_1=19, n_2=19, p=0.965]. For subsequent correlations with non-lateralised functional measures, MTh and MEPamp were therefore averaged across hemispheres.

§7.4 Functional x neurophysiological correlations

Input variables for correlations included:

- Primary neurophysiological outcomes: sICI_1, sICI_23, cSP
- Secondary neurophysiological outcomes: MEPamp, MTh
- Primary Functional outcomes: 9HPT, T25W, PASAT3, MSFC
- Secondary Functional outcomes: SDMT

For 9HPT, which is lateralised by hand, correlations were calculated between hand and contralateral hemisphere. For all non-lateralised functional outcomes, correlations were calculated with neurophysiological outcomes averaged across hemispheres. Spearman's rank correlations for the patient

group are displayed in Table 6. There were no significant correlations within the healthy control group. Within the patient group, there were significant correlations between dominant hand 9HPT performance and contralateral cSP (ρ=0.360, p=0.031) and between non-dominant hand 9HPT and contralateral cSP (ρ=0.351, p=0.039). Significant correlations are depicted in Figure 8.

§7.5 Asymmetry analysis

The 95% confidence intervals of asymmetry scores for sICI_1 (M=-0.28 ± 0.71), sICI_23 (M=-0.18 ± 0.62), and cSP (-1.89 ± 25.3ms) all included zero. There were three outlying asymmetry scores for sICI_1, two outlying asymmetry scores for sICI_23, and nine outlying asymmetry scores for cSP. There was a significant correlation between 9HPT asymmetry scores and outlying cSP asymmetry scores (ρ=0.950, p<0.001), depicted in Figure 7. Even when the apparent outlier at the cSP asymmetry of ∼-90 is removed, the correlation remains significant (ρ=0.929, p<0.001). The correlation between 9HPT asymmetry scores and outlying sICI_1 asymmetry scores was not significant (ρ=0.5, p=0.667), and the correlation with outlying sICI_23 scores could not be calculated. Furthermore, there were no outlying 9HPT asymmetry scores that were not associated with an outlying cSP score.

8. Discussion

§8.1 Functional compensation or damage?

This is the first study to evaluate correlations between neurophysiological measures of cortical inhibition and performance on the MSFC in patients with MS. The primary objective of this study was to assess two hypotheses concerning abnormalities in intracortical inhibition among people with this disease. We determined that there is a significant association between decreases in one measure of cortical excitability and maintained hand function. Specifically, shorter cSPs, measured over the primary motor cortex, are associated with better contralateral hand function. The relationship held true for both the dominant and non-dominant hand. This relationship appears consistent with what we had proposed as the 'functional compensation' hypothesis, which stated that decreased cortical inhibition in patients with MS represents a form of compensation that may protect against functional decline.

However, we did not find decreased intracortical inhibition in our patient group, as measured by either cSP or sICI. Rather, we found that our patient group had significantly longer cSPs than healthy controls, indicating *increased* intracortical inhibition. Taken together, the direction of the functional x neurophysiological association and the difference between patients and healthy controls for cSP cannot provide support the functional compensation hypothesis. The more prudent interpretation is that patients

with increased intracortical inhibition as measured by cSP have greater upper extremity motor impairment. This result is more in keeping with the damage hypothesis. In order to understand this finding, we must consider the impact of disease subtype and activity on intracortical inhibition.

We exclusively studied patients with the relapsing-remitting subtype of MS, and within that subtype only those in the remitting phase. Our findings indicate that cSP is significantly longer among these patients, compared to healthy controls. This finding is consistent with that of Caramia et al., who found that cSP is significantly shorter in relapsing patients, and longer in remitting patients, compared with healthy controls[18].

Interestingly, Fierro et al. also found that cSP is shortened in patients with relapsing disease. However, these authors report that lengthening of the cSP with treatment corresponds with clinical improvement as measured by the EDSS[20]. This suggests that shorter cSP is associated with worse performance, which is contrary to our present findings. However, if we pull together the admittedly sparse data on cSP, disease phase, and functional impairment, we might conclude that there is an ideal mid-range, at which cSP is at an intermediate level, and functional status is optimised. During the relapsing phase, cSP is shorter, and the more it deviates from baseline, the worse the functional impairment becomes. During the remitting phase, cSP returns to and may even exceed baseline, and our findings demonstrate that

the extent to which cSP lengthens is related to the degree of functional impairment. In both cases, cSP functions as a marker of disease severity.

While Fierro et al. found a relationship between EDSS score and cSP duration[20], we did not find any correlation between cSP and T25W. This is somewhat surprising, given that both are primarily measures of mobility. This raises the possibility that shortened cSP during relapse and lengthened cSP during remission reflect different underlying processes. Shortened cSP may reflect a global shift in inhibition throughout the cerebral hemispheres, which may be secondary to the accompanying inflammatory state. During remission, however, increased cSP may be secondary to residual damage and therefore specific to the site of TMS stimulation. In the latter case, then, we would expect cSP duration to reflect damage only within the area being stimulated, which in our study was the hand area. If changes in cSP duration depend on lesion locations during the remitting phase of RR-MS, this may explain the lack of correlations detected between cSP and cognitive or lower extremity measures in our study.

§8.2 Cortical silent period

To better understand the implications of altered cSP in patients with MS, it may be useful to consider the neurophysiological underpinnings of this measure. The duration of cSP depends on several factors: spinal mechanisms, including recurrent inhibition and after-hyperpolarisation, are implicated in the first 50ms of cSP, whilst the later segments depend on cortical

inhibition[49]. The cortical component of cSP may reflect the interruption of voluntary motor drive[62], but the neurochemical basis for this interruption is unclear. Tigabine, a presynaptic GABA-reuptake inhibitor, causes an increase in cSP duration[63-65], implicating a role for GABA-transmission. One case study, involving a single patient with dystonia, found that cSP duration was significantly increased following intrathecal administration of baclofen, a $GABA_B$ agonist[66]. Subsequent studies, however, found no effect of intravenous or oral baclofen administration[57, 67, 68]. Diazepam, a $GABA_A$ agonist, causes a shortening of cSP duration[67], which reduces the likelihood of a direct role for $GABA_A$ transmission causing inhibition as measured by this technique. $GABA_B$ transmission is more likely involved, given the relatively long duration of inhibition involved in cSP[58]. Administration of tigabine results in an interesting dissociation in measures of intracortical inhibition, insofar as it causes increased cSP, but decreased sICI[65]. All of these findings may be best explained by viewing cSP as the manifestation of spinal mechanisms plus modulation of pre-synaptic $GABA_B$ receptors.

Given the somewhat ambiguous neurochemistry behind cSP, it is difficult to understand what might be occurring on a molecular level to explain the results of our study. However, changes in cSP have been observed in relation to other neuropathology, particularly stroke[69-71]. In the affected hemisphere, cSP duration decreases between the sub-acute and chronic phases following stroke[71]. The decrease in cSP between these phases

predicts improvement in hand function[69]. Our data indicate a similar trend, with longer cSPs associated with worse hand function. However, taking into account the findings of Caramia et al., there are clear differences in the temporal shifts in cSP among patients with MS. Indeed, cSP is at its shortest during the relapsing phase, and longest during the remitting phase[18]. It has been postulated that decreases in intracortical inhibition may support reorganisation and use-dependent plasticity within the motor cortex[50, 72], and we have not ruled out the possibiltity that such compensatory changes are operating during the relapsing phase of MS. However, this theory cannot account for why cSP should be lengthened during the remitting phase.

Another possibility is that changes in cSP may relate to the location of MS lesions. Again, there is evidence from stroke research to support this possibility. Liepert et al. determined that in a group of patients with acute strokes and similar clinical disability, the lesion location determines a specific pattern of changes in cortical excitability[51]. Cortical lesions of M1 result in shorter cSP durations, as well as disinhibition as measured by sICI[51]. Subcortical internal capsule or paramedian pontine lesions result in prolongation of the cSP, but no changes in sICI[51]. Interestingly, these authors also reported a significant correlation between increasing cSP duration and worsening dexterity, but only in the subgroup with internal capsule lesions[51]. The pattern of changes in cortical excitability and relationship to hand function is entirely consistent with our data in patients

with remitting MS. It is possible that our data are driven by a subgroup of patients with MS plaques within the internal capsule, but unlikely. Caramia et al. documented a dynamic change in cSP duration within the same group of patients, depending on disease phase, which argues against the idea of lesion location determining patterns of cortical inhibition in these patients.

More recently, Honaga et al. reported that sICI is increased in the unaffected hemisphere of some patients with chronic stroke, but that this change is significantly more likely when the location of the stroke is cortical rather than subcortical[50]. cSP was not evaluated in that study, but it nonetheless adds credence to the possibility that grey- vs white- matter lesions in MS may also result in different effects on measures of intracortical inhibition. Combining future TMS studies with an imaging protocol may prove useful in further investigating this hypothesis.

§8.3 Short-interval intracortical inhibition

We did not find any difference in sICI between patients and healthy controls. However, among patients with RR-MS in the remitting phase, only those with fatigue have previously been found to have differences in sICI[17]. It is possible that if we were to take fatigue into account, we may identify a subgroup within our sample displaying these differences. Indeed, the present study is part of a larger study that also seeks to further evaluate the relationship between fatigue and cortical inhibition, and results of that sub-

study are forthcoming. Otherwise, our results with respect to sICI in this patient population are compatible with previous findings [19, 21, 23, 73].

Despite consistency with previous studies, our sICI findings require qualification. sICI data from six patients and one healthy control were excluded because the MEP amplitude in response to single-pulse stimulation was below 100μV. In healthy controls, stimulating at 120% RMT is sufficient to produce an MEP of 0.5 – 1 mV [46]. However, MEP morphology is known to be different in patients with MS[22], perhaps because the temporal dispersion of descending corticospinal volleys results in prolongation of the compound action potential. We documented a significant difference in MEPamp between patients and healthy controls, with patients having smaller values. sICI represents a ratio of paired-pulse MEPamp to single-pulse. As such, when single-pulse MEP amplitude is very low, a floor effect may result in determinations of sICI, whereby the paired-pulse MEPamp cannot be smaller than the single-pulse MEPamp without becoming indistinguishable from background EMG activity. We cannot rule out the possibility that excluding these data introduces a bias in our findings. In order to address this problem, future studies should ensure that single-pulse stimuli are calibrated to elicit MEPs of a fixed amplitude for each subject.

§8.4 Asymmetry of neurophysiological findings

Few studies have specifically compared measures of intracortical inhibition in both hemispheres in patients with MS. Consistent with previous findings

[18], we did not find any group level inter-hemispheric differences for any of our measures of intracortical inhibition within our patient group (see Figure 4). Having created asymmetry scores for these measures, we defined subjects with significant inter-hemispheric asymmetry as those with scores greater than one standard deviation from the mean. Within the nine subjects displaying significant asymmetry for cSP duration, there was a significant correlation with 9HPT performance asymmetry. The direction of the correlation indicates that where hemispheric asymmetries exist in cSP duration, the hemisphere with shorter duration corresponds to the hand with better 9HPT performance. This finding may lend support to the idea that longer cSPs during the remitting phase of RR-MS are specific to the location of pathology incurred during the remitting phase. The absence of outlying asymmetry scores for 9HPT performance without an accompanying asymmetry in cSP duration further suggests that the latter measure is a sufficient predictor of the former.

§8.5 Limitations

Evaluating the objectives of this project was hampered by a lack of objective measures of disease burden. With respect to the first objective of determining whether changes in cortical inhibition relate to damage or functional compensation, we might imagine two hypothetical patients with the following characteritics: Patient A has good hand function, and high low cortical inhibition, in keeping with the functional compensation hypothesis. Patient B also performs well on the 9HPT, but exhibits no change in cortical inhibition. Does the latter patient have a milder disease, or is she maintaining performance by some other mechanism? Without quantifying the burden of disease in these participants, it is difficult to disambiguate the role of cortical inhibition in these two cases. Similarly, while we have determined that asymmetries in cSP predict asymmetries in hand function, we can only speculate on underlying mechanisms. To this end, we have developed an imaging protocol in collaboration with Dr. Doug Arnold's group. While data are accruing, the inclusion of these data here is beyond the scope of this project.

As outlined in the methods section, we changed band-pass settings part way through data collection, after having realised that the original settings were not ideal. While this change did not have any effect on MEP latency, cSP duration, or sICI, it very likely influenced MTh and MEP amplitude findings in a non-systematic way. As such, we only analysed these latter outcomes for

subjects assessed after the amplifier settings were changed. This necessarily led to a loss of power in looking at effects involving motor threshold or MEP amplitude.

§8.6 Implications

We have demonstrated the utility of a combined approach using TMS and a multi-dimensional functional assessment, the MSFC, to better understand the clinical changes associated with MS. The finding that hand function may be impaired in patients with longer cSPs, implies that changes in cortical inhibition reflect damage caused by MS pathology. Where cSP is significantly longer in one hemisphere, function in the corresponding hand is relatively more impaired, lending further support to the damage hypothesis. However, we cannot comment on the relationship between changes in cortical inhibition and function where those changes are towards disinhibition, as is reported in other subtypes of MS. Further study, with inclusion of an imaging component, will provide more clarity to the interaction between disease burden, neurophysiological changes, and functional status, and may contribute to better understanding poor correlations between imaging findings and functional impairment.

Figures and Tables

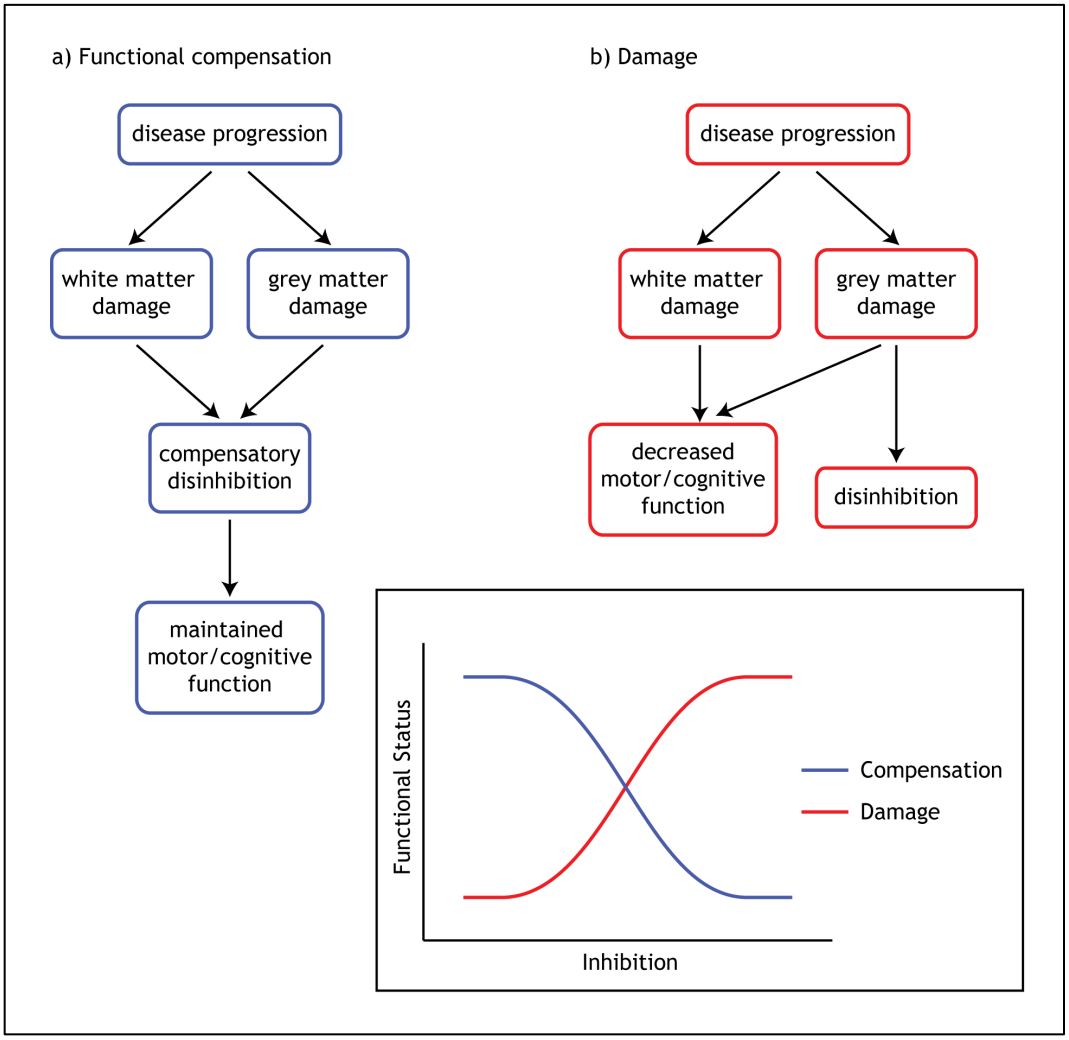

Figure 1: Schematic representation of a) the functional compensation hypothesis, and b) the damage hypothesis. If decreased cortical inhibition reflects functional compensation, the functional compensation hypothesis predicts an inverse association between inhibition and cognitive and/or motor function. If decreased cortical inhibition reflects damage caused by MS pathology, the damage hypothesis predicts a direct association between inhibition and cognitive and/or motor function.

Table 1: Summary of data to be collected.

Variable	Construct	Method	Scale
Predictor	ICI	Paired-pulse, sICI	MEP amplitude (% of single-pulse MEP amplitude)
	ICI	Silent period	Duration of silent period (msec)
Primary Outcome	Cognitive function	Performance-based	PASAT# number correct
	Motor function-arm	Performance-based	Nine-hole peg test# time (sec)
Secondary Outcome	Cognitive function	Performance-based	SDMT score
	Motor function-leg	Performance-based	25-foot Walk Test# time (sec)
Covariate	Clinical variables	Chart review	Age, sex, disease course, last relapse, EDSS score, medical therapies

Component measures of the Multiple Sclerosis Functional Composite.
ICI: intracortical inhibition. sICI: short-interval intracortical inhibition. TMS: transcranial magnetic stimulation. MEP: motor-evoked potential. PASAT: paced auditory serial addition test. SDMT: symbol-digit modalities test. EDSS: expanded disability status scale.

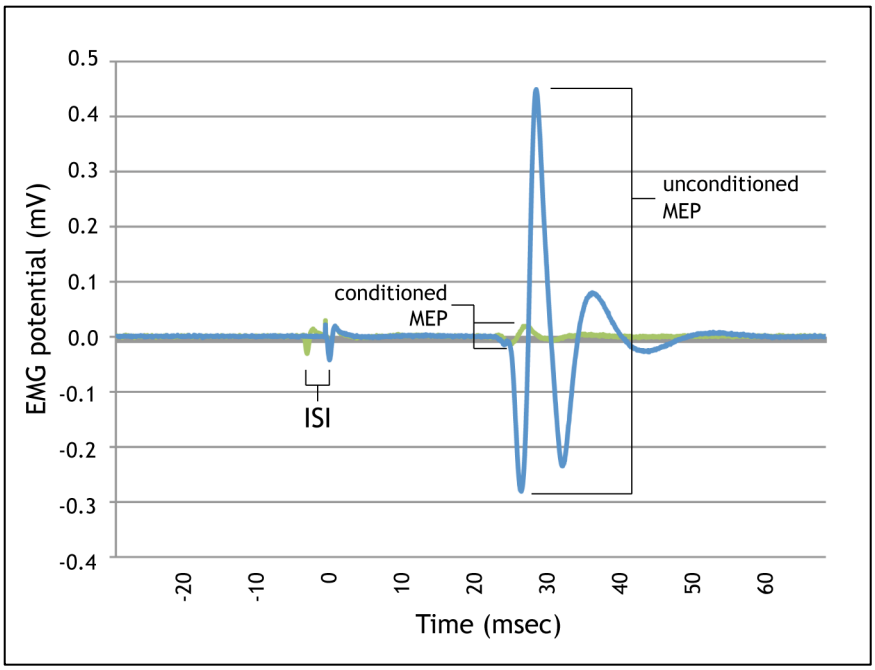

Figure 2: Short-interval intracortical inhibition (sICI). Paired-pulse stimulation: green; single-pulse stimulation: blue. When a sub-threshold conditioning pulse is delivered 1-4ms before a suprathreshold test pulse, there is a resultant suppression of the motor evoked potential. sICI is expressed as the ratio between the conditioned MEP amplitude and the unconditioned MEP amplitude.

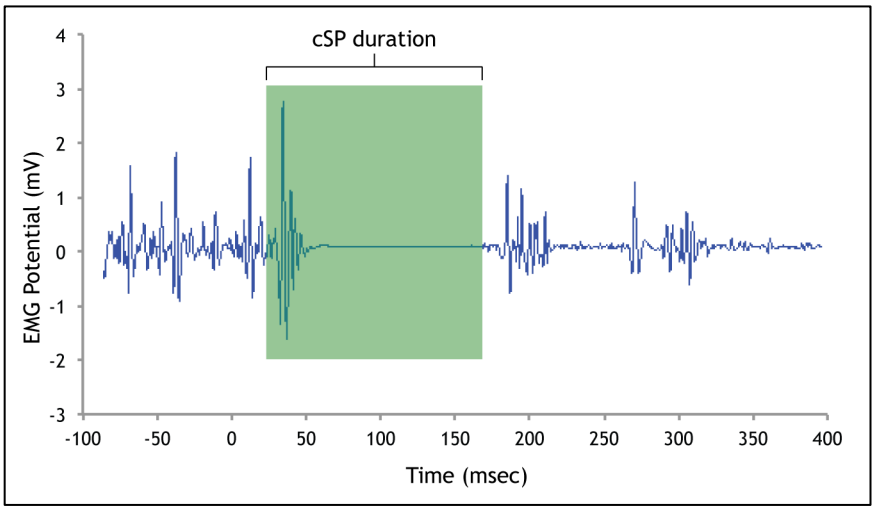

Figure 3: Cortical silent period (cSP). When a suprathreshold pulse is delivered during tonic contraction of the target muscle, a supramaximal motor-evoked potential (MEP) results. Following this MEP, there is a period of electrical silence in the EMG. The duration of this silence, measured from the beginning of the MEP to resumption of tonic contraction, is the 'cortical silent period'.

Table 2: Subject characteristics

		MS	HC
N		36	13
Age (years)	Mean (SD)	45.7 (11.0)	44.2 (10.1)
	Range	23.2 – 68.6	30.6 – 59.2
Gender	Female	25 (69%)	8 (62%)
	Male	11 (31%)	5 (38%)
Handedness	Right	31 (86%)	13
	Left	5 (14%)	0
EDSS	Mean (SD)	2.35 (2.0)	
	Range	0 – 6.5	

Table 3: Functional outcomes

	MSFC score (SD)*	Mean raw score (SD)		Significance (*p*)**
	MS	MS	HC	
MSFC	0.06 (0.64)			
9-hole peg test	0.23 (0.88)			
Dominant hand (s)		23.0 (9.2)	18.2 (1.9)	0.001*
Non-dominant (s)		22.6 (5.2)	17.9 (1.6)	0.000*
Timed 25' walk (s)	0.01 (2.4)	9.3 (26.7)	3.6 (0.62)	0.002*
PASAT-3" (# correct)	-0.41 (1.0)	40.1 (12.4)	46.9 (8.9)	0.096
SDMT (# complete)		47.9 (12.5)	58.0 (7.2)	0.007*

* MSFC z-scores calculated for patients, standardised using data from the National MS Society database[55]. Positive scores indicate better performance than the reference group, whilst negative scores indicate poorer performance.

** Raw score differences between patients and healthy controls assessed using Mann-Whitney *U*.

Table 4: Primary neurophysiological outcomes (mean ± SD)

	MS (NDom)	MS (Dom)	HC (Dom)	Significance (*p*)*
N++	31/31/31/35	33/33/33/36	12/12/12/13	
SICI_1	0.52±0.78	0.27±0.21	0.27±0.37	0.259
SICI_2	0.49±0.34	0.49±0.41	0.40±0.27	0.918
SICI_3	0.75±1.15	0.36±0.30	0.29±0.33	0.305
cSP (ms)	96.8±34.1	101.6±29.2	82.2±22.4	0.035*

SICI: short-interval intracortical inhibition, tested at 1, 2, and 3ms. cSP: cortical silent period. MS: patients with RR-MS. HC: healthy controls. Dom: dominant hemisphere by handedness. NDom: non-dominant hemisphere by handedness.

* Raw score differences between patients and healthy controls, within the dominant hemisphere, assessed using Mann-Whitney *U* (sICI) or Student t-test (cSP).

++ N: number of subjects for SICI_1/SICI_2/SICI_3/cSP.

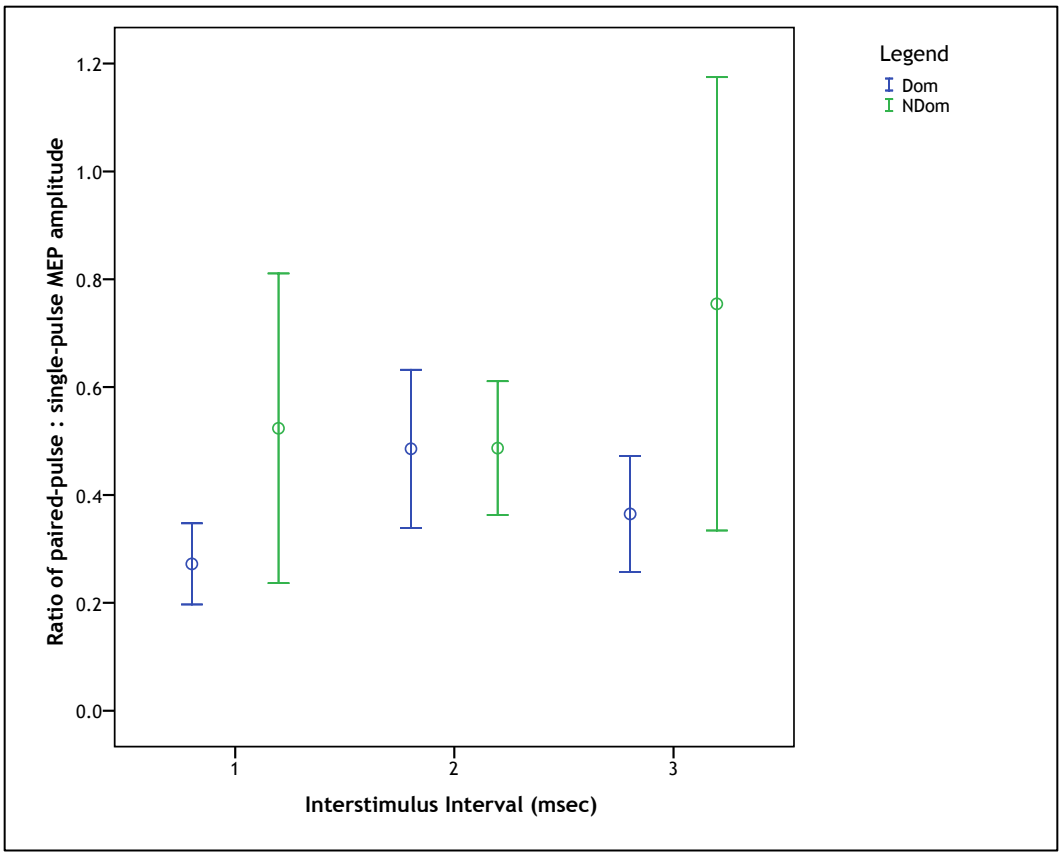

Figure 4: Short-interval intracortical inhibition (sICI) in patients with RR-MS in remission. Depicted as mean sICI at each ISI, clusters represent dominant and non-dominant hemispheres. Error bars represent 95% CI. There is no significant effect of ISI in either dominant [X^2=4.359, df = 2, p=0.113] or non-dominant [X^2=1.937, df=2, p=0.380] hemisphere. There is no significant effect of hemisphere at any ICI (1ms: [U=401.0, n_1=33, n_2=31, p = 0.138]; 2ms: [U=465.0, n_1=33, n_2=31, p = 0.532]; 3ms: [U=379.0, n_1=33, n_2=31, p = 0.075]). We cannot evaluate for ISI x hemisphere interaction, but visual inspection does not suggest a significant interaction.

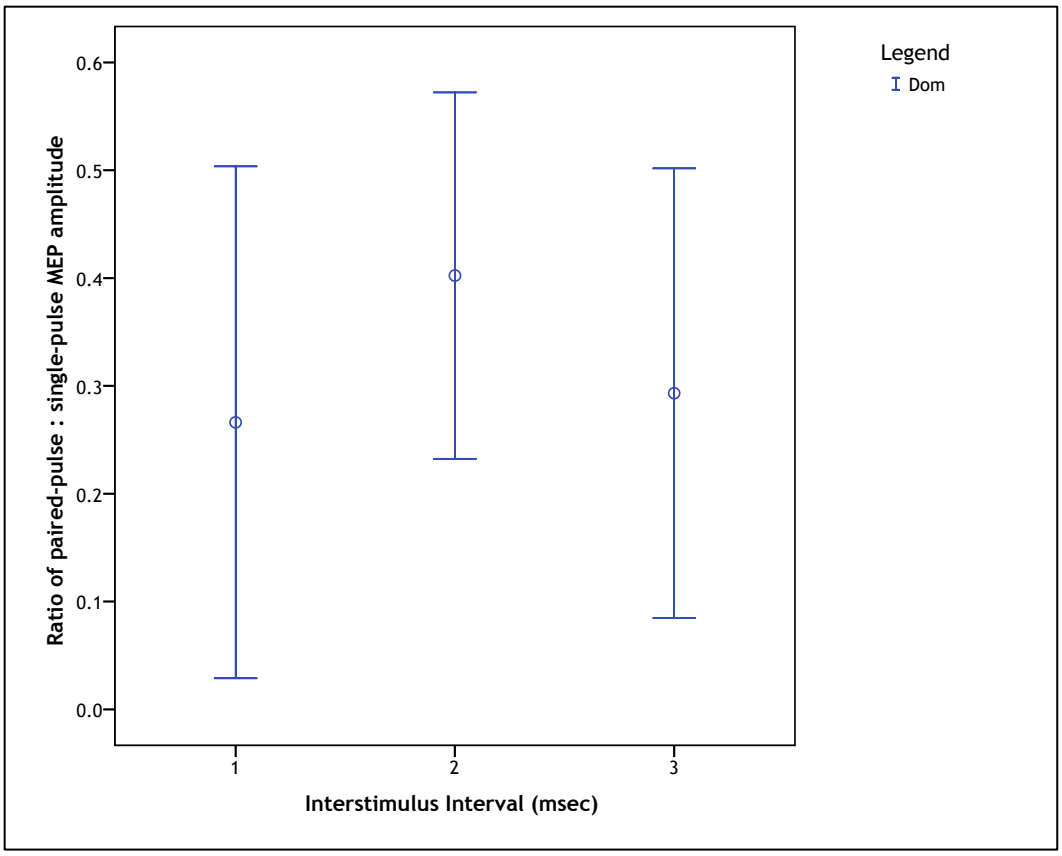

Figure 5: Short-interval intracortical inhibition (sICI) in healthy controls. Depicted as mean sICI at each ISI. Error bars represent 95% CI. There is no significant effect of ISI in the dominant hemisphere [X^2=4.950, df=2, p=0.084]. Only the dominant hemisphere was tested in healthy controls.

Table 5: Secondary neurophysiological outcomes

	MS (NDom)	MS (Dom)	HC (Dom)	Significance (*p*)*
N++	21/19	21/19	13/12	
MTh (%MSO)	49.7±12.8	48.3±11.3	42.8±7.0	0.102
MEPamp (mV)	0.39±0.33	0.42±0.33	1.27±1.0	0.004**

MTh: motor threshold, expressed as % of maximum stimulator output. MEPamp: amplitude of the motor-evoked potential resulting from a single pulse at 120% resting motor threshold. Dom: dominant hemisphere by handedness. NDom: non-dominant hemisphere by handedness.

* Raw score differences between patients and healthy controls, within the dominant hemisphere, assessed using Mann-Whitney *U*.

++ number of subjects for MTh/MEPamp.

Table 6: Neurophysiological x functional correlations, patients with RR-MS. For 9HPT, correlations were calculated between performance in each hand and neurophysiological outcome in the corresponding hemisphere. For all other functional outcomes, correlations were calculated with neurophysiological outcomes averaged across hemispheres. Values displayed are Spearman's ρ, with *p*-values given for significant correlations only. N = number of ranked pairs. There were no significant correlations within the healthy control group.

	ICI_1	ICI_23	cSP	MEPamp	MTh
Dom_9HPT N	0.29 33	-0.31 33	0.36* (*p*=0.031) 36	-0.43 19	0.14 21
NDom_9HPT N	0.025 31	-0.04 31	0.35* (*p*=0.039) 35	-0.21 19	-0.22 21
PASAT3 N	-0.027 36	0.23 36	-0.060 36	0.026 21	0.33 21
T25W N	0.057 36	-0.049 36	0.15 36	-0.40 21	-0.02 21
MSFC N	-0.15 36	0.22 36	-0.21 36	0.35 21	0.23 21
SDMT N	-0.005 34	0.13 34	-0.16 34	0.008 20	0.35 20

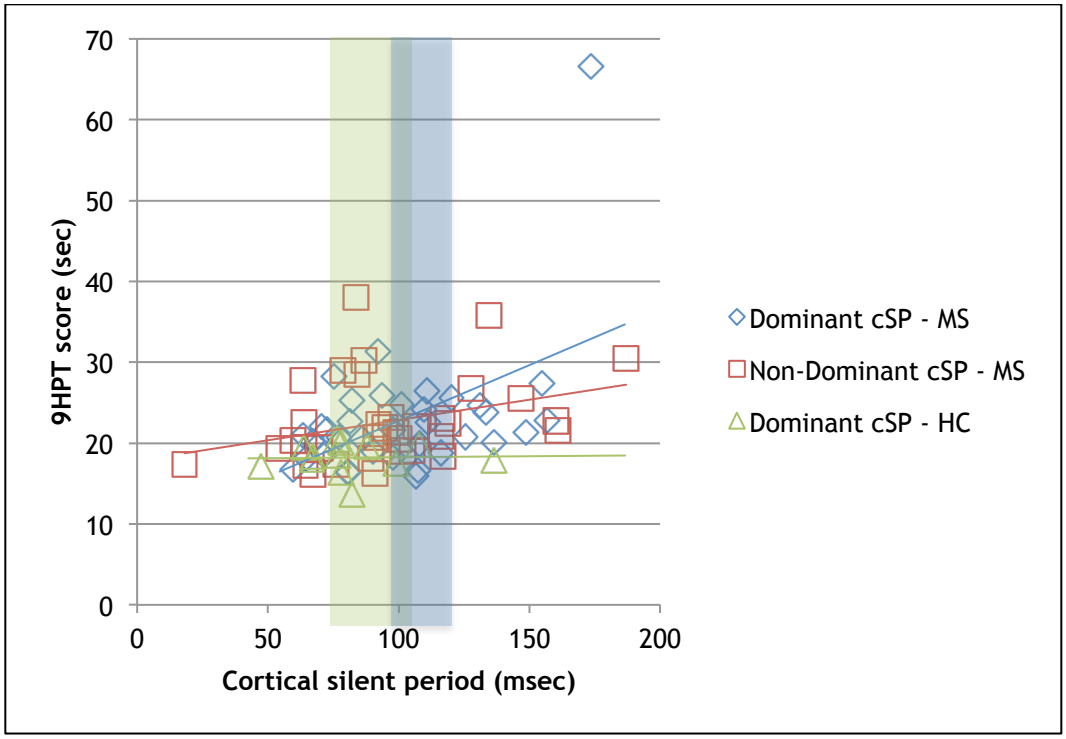

Figure 6: Correlations between 9-hole peg test (9HPT) performance and cortical silent period (cSP). Within the patient group, there were significant correlations between hand performance and cSP on both the dominant side [ρ=0.360, p=0.031] and non-dominant side [ρ=0.351, p=0.039]. This indicates that 9HPT performance is faster with shorter cSP, or decreased cortical inhibition. There is no significant correlation in the healthy control group. However, cSP duration on average is longer in the patient group than in the healthy control group [t(47)=-2.166, p=0.035]. Shaded areas represent 95% CI for cSP duration in patients (blue) and healthy controls, dominant hemisphere only.

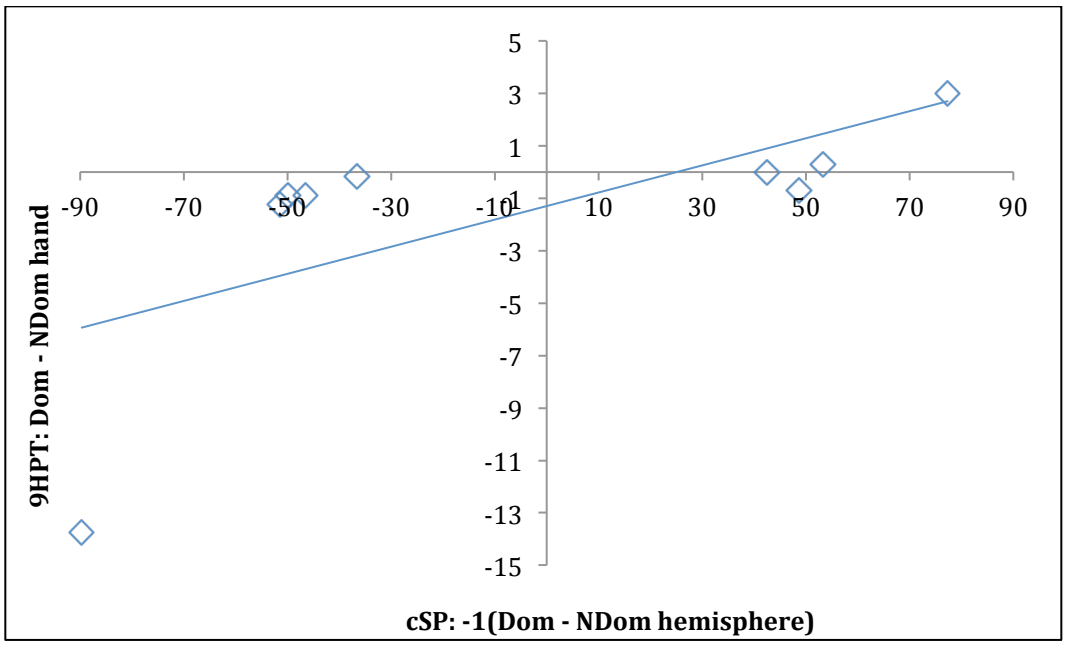

Figure 7: Asymmetry plot for 9-hole peg test (9HPT) vs cortical silent period (cSP) scores. Only outlying asymmetry scores for cSP are used to predict 9HPT asymmetry scores. Positive y-axis values reflect better 9HPT performance with the dominant hand. Positive x-axis values reflect shorter cSP, or lesser cortical inhibition, in the dominant hemisphere. There is a significant correlation between 9HPT asymmetry scores and outlying cSP asymmetry scores (ρ=0.950, p<0.001).

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