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**CLINICAL AND NEUROPHYSIOLOGICAL
CORRELATES OF CORTICAL EXCITABILITY
CHANGES STUDIED USING THE CORTICAL
THRESHOLD TRACKING TMS
IN HYPERKINETIC AND
HYPOKINETIC MOVEMENT DISORDERS**

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PREFACE

The studies described in this thesis were undertaken between 2010 – 2012 in the Department of Neurology, Westmead Hospital, Sydney, Australia. The works were supervised by Clinical Associate Professor Victor Fung and co-supervised by Associate Professor Steve Vucic. The submission of this thesis fulfils the requirements for the degree of Doctor of Philosophy, University of Sydney.

All the studies described were designed, performed and analysed by the author with the assistance of the supervisors. Dr. Neil Mahant and Mr. Chong Lee Lim provided the technical assistance and Ms Jane Griffith, Dr. Sharon Kemp and Dr. Zaki Aldajaani assisted with some of the data collection.

The studies were approved by the Human Research and Ethics Committee, Westmead Hospital. Informed consent was obtained from all subjects according to the Declaration of Helsinki.

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Hanna Lee has been my greatest supporter and was, is and will always be the greatest love of my life.

DEDICATION

This thesis is dedicated to my wife Hanna, my children Daniel and Rachel, and my parents Paul and Grace.

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Kim S, Ha A, Kemp S, Mahant N, Lim CL, Griffith J, Chang F, Vucic S, Fung VSC. The time course of changes in motor function with prolonged medication withdrawal – Effects of long duration response. *Movement Disorders* 2013;28 Suppl 1:242

Kim S, Vucic S, Kiernan MC, Fung VSC. Interhemispheric difference in cortical excitability in Parkinson's disease correlate with degree of asymmetry in akinesia. *Movement Disorders* 2011;26 Suppl 2:115

ABSTRACT

This thesis attempts to answer the question of “what is the significance of abnormal cortical excitability studies in movement disorders?” Remarkably similar findings have been reported in Parkinson's disease (PD) and Stiff-man syndrome (SMS), the subjects of the studies contained in this thesis, despite these conditions manifesting with widely different clinical manifestations, one with hypokinetic and the other hyperkinetic movement problems.

In SMS patients, cortical excitability studies were undertaken using the threshold tracking transcranial magnetic stimulation (TMS) along with measures of spinal excitability and anti-GAD antibodies as well as reported clinical measures in previous drug studies. Remarkably, there was significant reduction in SICI despite most of the patients still being on benzodiazepines at the time of testing with dissociation between the cortical, spinal and clinical as well as plasma levels of anti-GAD antibodies. The results suggest that spinal hyperexcitability and muscle stiffness and spasms are unlikely to be simply due to cortical disinhibition of spinal circuits.

In PD patients, cortical excitability parameters measured with threshold tracking TMS was correlated with objective measures of bradykinesia and rigidity. Relatively consistent findings were obtained with significantly reduced short intracortical inhibition (SICI) and intracortical inhibition (ICF) which appeared to be dependent on disease severity as well as plasma levodopa level. Most significant findings were found in patients who had been on levodopa carbidopa intestinal gel (LCIG), a proven therapy against motor fluctuations and dyskinesia in advanced stages of the disease. In this group of patients who had been on LCIG therapy for more than 12 months, there was lack of decrement in hand tapping speed despite marked reduction in

plasma level of levodopa which was matched by significantly reduced SICI. The results raise the possibility of restoration of the long duration effect of levodopa, perhaps related to sustained continuous dopaminergic therapy. However, these patients displayed mild-moderate dyskinesia which might have influenced the results and the alterations in SICI in this setting require further evaluation.

Table of Contents

CHAPTER 1	TRANSCRANIAL MAGNETIC STIMULATION AND PARKINSON'S	
	DISEASE: INSIGHTS INTO PATHOPHYSIOLOGY	1
1.1	INTRODUCTION	2
1.2	SINGLE-PULSE TMS AND INSIGHTS INTO MOTOR CORTEX PHYSIOLOGY	4
1.2.1	<i>Motor threshold</i>	4
1.2.2	<i>Magnetic Evoked Potential</i>	6
1.2.3	<i>Cortical silent period (CSP)</i>	7
1.3	PAIRED PULSE TMS TECHNIQUES	8
1.4	CONCEPTS OF PD PATHOPHYSIOLOGY	14
1.5	CONCLUSIONS	16
CHAPTER 2	DYSFUNCTION OF CORTICAL AND SPINAL INHIBITORY	
	CIRCUITS UNDERLIE DEVELOPMENT OF STIFF-MAN SYNDROME	18
2.1	INTRODUCTION	21
2.2	METHODS	22
2.2.1	<i>Clinical disease severity</i>	23
2.2.2	<i>Cortical excitability</i>	23
2.2.3	<i>Spinal excitability</i>	25
2.2.4	<i>Peripheral studies</i>	25
2.2.5	<i>Anti-GAD antibody titre</i>	26
2.2.6	<i>Statistics</i>	26
2.3	RESULTS	26
2.3.1	<i>Clinical disease severity</i>	26
2.3.2	<i>Spinal excitability</i>	27
2.3.3	<i>Cortical excitability</i>	28
2.3.4	<i>Peripheral studies</i>	29
2.3.5	<i>Relationship between clinical, immunological, and neurophysiological findings</i>	29
2.4	DISCUSSION	31
2.4.1	<i>Pathophysiology of cortical dysfunction in SMS</i>	32
2.4.2	<i>Relationship between cortical dysfunction and ant-GAD antibodies</i>	33
2.4.3	<i>What underlies the generation of symptoms in SMS?</i>	33
2.4.4	<i>Limitations of the present study</i>	34
2.5	CONCLUSIONS	35
CHAPTER 3	HAND DOMINANCE INFLUENCES SIDE-TO-SIDE DIFFERENCES	
	IN TAPPING SPEEDS BUT NOT SICI IN NORMAL SUBJECTS	36
3.1	INTRODUCTION	39
3.2	METHODS	41
3.2.1	<i>Subjects</i>	41
3.2.2	<i>Handedness</i>	41
3.2.3	<i>Objective measures</i>	41
3.2.4	<i>STATISTICS</i>	43
3.3	RESULTS	43
3.3.1	<i>Cortical Excitability</i>	44
3.3.2	<i>Objective hand function</i>	46
3.4	DISCUSSION	47

CHAPTER 4 INTERHEMISPHERIC DIFFERENCES IN CORTICAL	
EXCITABILITY IN PARKINSON’S DISEASE	49
4.1 INTRODUCTION	52
4.2 METHODS	54
4.2.1 <i>Subjects</i>	54
4.2.2 <i>Clinical staging of PD</i>	56
4.2.3 <i>Experimental set up</i>	56
4.2.4 <i>STATISTICAL ANALYSIS</i>	59
4.3 RESULTS	60
4.3.1 <i>Clinical measures of disease of severity (modified MDS-UPDRS)</i>	60
4.3.2 <i>Objective bradykinesia - hand tapping speed</i>	60
4.3.3 <i>Objective rigidity</i>	61
4.3.4 <i>Cortical excitability measurements</i>	62
4.3.5 <i>Relationship between clinical measures of disease severity and cortical excitability in PD subjects</i>	65
4.4 DISCUSSION	66
4.4.1 <i>Limitations of this study</i>	71
4.5 CONCLUSION	72
CHAPTER 5 EFFECTS OF LEVODOPA ON CORTICAL EXCITABILITY IN	
PARKINSON’S DISEASE	73
5.1 INTRODUCTION	75
5.2 METHODS	77
5.2.1 <i>Experiment 1 – Short duration response to levodopa</i>	77
5.2.2 <i>Experiment 2 – Long duration response to levodopa</i>	82
5.3 STATISTICAL ANALYSIS	83
5.4 RESULTS	83
5.4.1 <i>Experiment 1</i>	83
5.4.2 <i>Experiment 2</i>	94
5.5 DISCUSSION	100
5.5.1 <i>Experiment 1 - SDR</i>	100
5.5.2 <i>Experiment 2 - LDR</i>	102
5.5.3 <i>Limitations</i>	103
5.6 CONCLUSION	104
CHAPTER 6 CORTICAL EXCITABILITY IN PATIENTS WITH PARKINSON’S	
DISEASE ON LEVODOPA CARBIDOPA INTESTINAL GEL THERAPY	105
6.1 INTRODUCTION	107
6.2 HYPOTHESES	110
6.3 METHODS	110
6.3.1 <i>Subjects</i>	110
6.3.2 <i>Experimental procedure</i>	112
6.3.3 <i>Clinical assessment of disease severity</i>	113
6.3.4 <i>Cortical excitability</i>	114
6.3.5 <i>Hand tapping speed</i>	115
6.3.6 <i>Plasma levodopa concentration</i>	115
6.3.7 <i>Data analysis</i>	116
6.4 STATISTICAL ANALYSIS	116
6.5 RESULTS	117
6.5.1 <i>Clinical measures of disease of severity (modified MDS-UPDRS)</i>	117

6.5.2	<i>Modified AIMS</i>	119
6.5.3	<i>Objective bradykinesia - hand tapping speed</i>	119
6.5.4	<i>Plasma Levodopa Concentration</i>	119
6.5.5	<i>Cortical excitability measures</i>	121
6.5.6	<i>Relationship between Cortical excitability and hand tapping speed and plasma levodopa concentration</i>	126
6.6	DISCUSSION	127
6.6.1	<i>Limitations of the study</i>	131
6.7	CONCLUSION	131
6.7.1	<i>Clinical implications</i>	131

**Chapter 1 Transcranial magnetic stimulation and
Parkinson's disease: Insights into pathophysiology**

1.1 INTRODUCTION

Parkinson's disease (PD) is a prototypical basal ganglia disorder with cardinal motor features of tremor, bradykinesia and rigidity [1]. There is mounting evidence that altered oscillatory activity in the basal ganglia-thalamo-cortical loop is central to the pathophysiology, which has been shown to correlate with rigidity and bradykinesia [2, 3]. It is recognised that bradykinesia is not primarily the result of deficit in the motor cortex given that the movements elicited by direct stimulation of the motor cortex are the same whether the stimulus is given when patients are in the "off" state or "on" with dyskinesia [4]. However, the motor cortex is the final output pathway of the basal ganglia motor circuitry and thus understanding its contribution to the pathophysiology of Parkinson's disease could provide further insights into the motor manifestations of the disease and carry potential therapeutic implications.

Transcranial magnetic stimulation (TMS) is a robust method of interrogating the functions of the motor cortex and its circuitry and it has been extensively applied in PD, with reports of abnormalities in various parameters of motor cortical excitability, thereby suggesting involvement of the motor cortex in the disease pathophysiology [5-7].

Significantly, based on the understanding that altered motor cortical excitability is a feature of PD and that plastic changes in excitability can be induced in the motor cortex with repetitive transcranial magnetic stimulation (rTMS) [8-11], a growing number of studies has explored the effect of applying rTMS as a therapeutic measure although to date only transient improvements in motor function have been reported [12, 13].

TMS studies in PD have produced mixed results and their significance remains uncertain, particularly in regards to the relationship between the excitability changes and clinical manifestations of PD. The difficulty in unravelling the role of cortical excitability changes in PD pathophysiology arises from several factors related to the disease and technical factors. With respect to disease related factors, the fact that PD is associated with impairments in a number of motor domains, including muscle tone and movement scaling, i.e. rigidity and bradykinesia, which are further complicated by the development of dyskinesia and motor fluctuations related to dopaminergic therapy, create a significant challenge in dissecting out the relationship between cortical excitability changes and impairments in individual motor domains. A major limitation of cortical excitability studies reported thus far is that they do not allow definite conclusions to be drawn regarding whether the reported abnormalities are primarily due to the underlying disease or secondary to compensatory changes in the brain in an attempt to optimise motor performance or even still medications.

The present review will firstly focus on the mechanism of generation of TMS parameters and critically analyse the reported changes in PD. It will then consider the way in which these have contributed to the current understanding of PD pathophysiology and highlight areas of interest for further research.

1.2 SINGLE-PULSE TMS AND INSIGHTS INTO MOTOR CORTEX

PHYSIOLOGY

A standard single-pulse TMS involves exciting a network of motor cortical neurons and recording over the contralateral hand muscle, usually first dorsal interosseous (FDI) or abductor pollicis brevis (APB) muscle. Single-pulse TMS provides basic information about the functions of the corticomotoneuronal system, with the main parameters being motor threshold (MT), motor evoked potential (MEP), and cortical silent period (CSP).

1.2.1 Motor threshold

Motor threshold reflects of how readily a group of corticomotoneurons can be excited. It is a measure of the excitability of individual neurons and their local density [14-16] and similarly, the density of corticomotoneuronal projections onto the spinal motor neuron [17-19]. In addition, MT may also be a biomarker of cortical neuronal membrane excitability [20-22], whereby pharmacological blockade of voltage-gated sodium channels increases MT [23], while glutamate excitotoxicity reduces MT [24]. MT may be defined as the minimum stimulation intensity required to generate an MEP response of 50 μ V in the target muscle in 50% of the trials, using the constant stimulus technique [15, 25]. The more recently described threshold tracking technique on the other hand defines MT as the stimulus intensity required to elicit and maintain a target MEP response of 0.2 mV [26]. Of significance, MT correlates strongly with ability to perform independent (fractionated) finger movements as tested with finger tapping and pegboard dexterity, suggesting that MT is a clinically relevant measure [27].

In PD, mixed but generally consistent findings have been reported. Several studies have reported reduced MT, both at rest (resting motor threshold, RMT) and during a weak voluntary muscle contraction (activated motor threshold, AMT) [28, 29] while others have reported normal thresholds [5, 7, 30-33]. This discrepancy likely arises from several factors, most significant of which is the fact that until recently there has been a lack of consensus in defining the parameters of TMS. In particular, MT has been variably defined, such that in some studies the target MT amplitudes differed by more than 100% [5, 7, 32, 34, 35]. Secondly, despite PD being a heterogeneous disorder, some studies failed to differentiate PD patients into subgroups based on disease severity and predominant motor manifestations, reporting averaged results from a small number of patients with widely different disease duration, e.g. disease duration ranging from 4 months to 20 years in one study [34, 36].

Notably, several studies have reported normal RMT but increased AMT, consistent with the notion of relative failure of voluntary facilitation in PD [34], which has been postulated to be a possible cortical mechanism underlying the development of bradykinesia, i.e. *volitional activation failure* [37]. However, the relationship between stimulus intensity and response amplitude, i.e. Stimulation Response (SR) curve, has been reported to be steeper than normal when tested *at rest* in PD [34, 38]. The steeper SR curve implies that the distribution of cortical excitability is skewed towards higher values than normal, possibly as a compensatory mechanism that attempts to overcome slow recruitment of commands to move by making it easier to recruit activity from a resting state. Of relevance, TMS studies in PD patients who have had deep brain stimulation (DBS) surgery have shown that RMT in OFF-DBS condition is not significantly altered by switching to ON-DBS state or administration

of dopamine agonist, apomorphine, despite clear improvements in motor function [39], suggesting that RMT is not related to motor performance in PD unlike in normal controls where MT correlates with fine finger movements, and hence that clinical manifestations of PD are not primarily the result of any deficit in the excitability of individual motor neurons and their local density.

1.2.2 Magnetic Evoked Potential

Magnetic Evoked Potential (MEP) amplitude reflects a summation of complex corticospinal volleys consisting of D (direct) and I (indirect)-waves onto the motor neurons [20, 40]. At threshold, TMS elicits I1 waves, with the frequency and amplitude of I waves increasing with higher stimulus intensities [40]. The MEP amplitude increases with higher stimulus intensities, thereby enabling the generation of a stimulus-response (SR) curve that follows a sigmoid function [41]. The slope of the SR curve reflects the motor cortex excitability [41]. As with motor thresholds, the MEP amplitude reflects the density of corticomotoneuronal projections onto motor neurons [42], although the neuronal population mediating MEP responses appears to be different to those mediating MTs.

The MEP amplitude should be expressed as a percentage of the supramaximal compound muscle action potential (CMAP) response [25], thereby accounting for any lower motor neuron contribution and providing an insight into the percentage of the motor neurone pool activated in the MEP. Normative values for the MEP/CMAP ratio demonstrate a large inter-subject variability thereby limiting the value of this measure for detecting abnormalities of the corticomotoneurons [15, 43].

Previous TMS studies have reported mixed findings in MEP amplitudes in PD patients. A number of studies have reported significantly higher MEP amplitudes at rest [28, 29, 44], consistent with the notion of increased membrane excitability of cortical neurons in PD, while others have reported normal MEP amplitudes [5, 7, 34, 45].

One plausible explanation for reduced MT and increased MEPs, is that the studies were contaminated by incomplete relaxation or tremor which can exert facilitatory effects on cortical neurons and spinal cord [46]. This problem can be overcome by undertaking the studies during a uniform weak voluntary muscle contraction, as this controls for unwanted muscle activation contaminating the results. Indeed, a recent study in early drug naïve PD patients showed that average AMT was not different from that of normal controls although there was increased variability, with greater oscillations around an average value [47]. Further evidence comes from a study, which found no differences in MTs and MEPs between PD patients and normal controls when studies were performed while subjects were generating a 20% voluntary contraction [48].

1.2.3 Cortical silent period (CSP)

CSP is defined as interruption of voluntary electromyographic (EMG) activity in a target muscle following TMS of the contralateral motor cortex [49]. The CSP duration is measured from the onset of the MEP response to resumption of voluntary EMG activity [23, 49] and increases with stimulus intensity [49-51].

The initial phase of the cortical silent period is mediated by inhibition of the spinal alpha motor neurons, while the contributing mechanism of the late part of CSP is not exactly known but GABA_B receptor mediated cortical inhibition most likely plays an important role, as pharmacological activation of GABA_B receptors leads to significant CSP lengthening [49, 50, 52-55]. Of further relevance, the CSP duration appears to be influenced by the degree of corticomotoneuronal projections onto motor neurons, being longest in intrinsic hand muscles [15].

Abnormalities of the CSP are well established in PD, with TMS studies documenting a reduction in CSP duration, a finding which is most prominent with TMS intensity set to near-maximal [6, 7, 31, 48]. The CSP duration is significantly shorter on the more affected side [6, 47], and correlates with more severe clinical dysfunction as measured by the Unified Parkinson's Disease Rating Scale (UPDRS) [6]. Of further relevance, the CSP duration is shorter when PD patients are in the OFF-medication state, whereas the CSP duration lengthens in the ON-medication state when the motor function, as measured by finger tapping speed, has improved [56]. Taken together, TMS studies suggest that dysfunction of the GABAergic neurotransmitter system, acting via GABA_B receptors, may contribute to the generation of motor impairment in PD.

1.3 PAIRED PULSE TMS TECHNIQUES

Motor cortex excitability can also be assessed by utilising paired-pulse TMS techniques in which a conditioning stimulus modulates the effect of a second test

stimulus [16]. Several different paired-pulse TMS techniques have been developed to date [15, 23, 26, 57], but all techniques utilise a subthreshold conditioning stimulus, which modulates the effects of a second test stimulus. In PD research, short interval intracortical inhibition (SICI) and intracortical facilitation (ICF) have been most frequently utilised to assess the extent of cortical dysfunction.

Short interval intracortical inhibition and ICF are best assessed by delivering a subthreshold conditioning stimulus at pre-determined time intervals prior to a suprathreshold test stimulus [26, 58-60]. In the early TMS paradigms [58, 60, 61], the conditioning and test stimuli were kept constant, and changes in the test MEP amplitude were evaluated, such that when the interstimulus interval (ISI) was set between 1-5 ms, the test response was inhibited (*SICI*), and increasing the interstimulus interval to between 7 and 30 ms resulted in facilitation of the test response amplitude (*ICF*) [15]. A potential limitation of this “constant stimulus” technique related to marked variability in MEP amplitudes with consecutive stimuli that may be up to 200%, making it difficult to discern whether changes in the MEP produced are secondary to a true change in cortical excitability or, alternatively, represent fluctuations related to the delivery of the test stimuli [59, 62]. In an attempt to overcome this limitation, a *threshold tracking technique* was developed, whereby a constant target MEP response (0.2 mV) was tracked by a test stimulus, such that SICI was heralded by higher and ICF by lower conditioning-test stimulus intensities [26, 57]. By setting a tracking target of 0.2 mV, located in a steep portion of the nonlinear SR curve [41], marked variability in MEP amplitudes translate to smaller changes in stimulus intensity, potentially overcoming this limitation and provides the added advantage of speeding up the recording process thereby allowing multiple

recordings to be made in quick succession. Two physiologically distinct phases of SICI, a smaller phase at $ISI \leq 1$ ms and a larger phase at $ISI \geq 3$ ms, were identified by the novel technique [26, 57, 63, 64].

Short interval intracortical inhibition and ICF originate at the level of the motor cortex, mediated by synaptic processes, a notion supported by studies assessing the effects of paired-pulse TMS on descending corticospinal volleys [60, 65]. Specifically, SICI is associated with a reduction in the number and amplitude of later I-waves, namely I₂ and I₃, with I-wave suppression remaining up to an ISI of 20 ms, the typical duration of the inhibitory postsynaptic potential mediated through GABA_A receptors [59, 66]. In addition, GABAergic agonists acting via GABA_A receptors appear to enhance I wave amplitudes and SICI [66-69]. Importantly, SICI and ICF appear to be physiologically distinct as indicated by a lower threshold for activation of SICI, and that SICI remains independent of the direction of current flow in the motor cortex, at least in healthy subjects, while ICF appears to be preferentially generated by current flowing in a posterior-anterior direction [61].

Long interval intracortical inhibition (LICI) is elicited by delivering a suprathreshold conditioning stimulus 50-200ms prior to a suprathreshold test stimulus [70]. Although both SICI and LICI produce MEP inhibition by reducing the late indirect (I) waves, there is evidence that SICI and LICI are mediated by different population of neuronal cells given that markedly different pattern of response is seen when test stimulus intensity is varied [71]. This is further supported by the fact that there is lack

of correlation between the extent of SICI and LICI in that instead of potentiating the SICI, LICI reduces or even reverses SICI, which has been postulated to be due to direct inhibition of the circuits mediating SICI as well as the output neurons [71]. Lastly, SICI and LICI are mediated by different receptor subtypes, with LICI being mediated by GABA_B receptors and SICI by GABA_A receptors [54].

As with other TMS parameters, the changes in SICI have been varied and inconsistent in PD. Specifically, while some studies have failed to document any significant changes [31, 72, 73], others have documented a significant reduction in SICI at rest, with at least partial normalisation with dopaminergic therapy and “ON-DBS” condition, matched by improvement in clinical motor scores as measured by the UPDRS [7, 11, 30, 32, 33, 74], suggesting that abnormalities in thalamo-cortical motor pathways may in part underlie the reduced SICI and thereby development of motor symptoms in PD. However, there are also studies that showed significantly reduced SICI despite the fact that patients were on medication [32, 75] and others which showed no changes in SICI [5].

It has been argued that the discordant SICI findings in PD patients may relate to methodological issues. Specifically, MacKinnon and colleagues [5] reported that SICI was reduced only when the CSI was set to 90% or 100% of the RMT while stimulation with CSIs 40-80% RMT were normal. Given that facilitatory circuits, i.e. short interval intracortical facilitation (SICF) may be activated at these higher conditioning stimulus intensities [63], it was postulated that abnormalities in SICI in PD were due to activation of the facilitatory pathways on the basis of reduced

threshold rather than abnormalities in the inhibitory pathways. However, the possibility that the reduced SICI resulted from the summation of descending volleys from both the conditioning and test stimuli cannot be excluded because CSIs above AMT were used [5]. This was underscored by the finding that dopaminergic medications failed to significantly influence SICI (MacKinnon et al 2005). Of further relevance, others have failed to establish a relationship between changes in SICI and clinical measures of motor impairment in PD [7, 47]. Recently, Ni et al. [76] provided further evidence that the reduced SICI may be due to enhanced facilitation rather than reduced inhibition. They examined the time course of SICI and SICF, and demonstrated that SICF was enhanced in the medication “off” state and that this normalised with dopaminergic medications, supporting the hypothesis that reduced SICI in the OFF state may be due to increased SICF. Of interest, they showed that there was a small reduction in SICI in the absence of significant SICF, which only partially normalised with dopaminergic medication. Together, there appears to be both dopamine responsive facilitatory networks and non-dopaminergic GABA_A pathways contributing to motor cortical dysfunction in PD [76, 77].

It is noteworthy that studies that have reported normal SICI have used higher test stimulation intensities above 120% RMT [11, 31]. Disease severity – Several studies that have reported significant reduction in SICI were in patients with relatively advanced disease [7, 74]. Studies that have examined de novo PD patients or those with mild to moderate disease reported equivocal changes in SICI, with some reporting normal [5, 11] while others reduced SICI [75, 78].

Mixed findings have been reported in ICF. Several groups have reported normal ICF in both ON and OFF-medication states, consistent with the notion that decreased SICI in PD is not mediated by changes in ICF [7, 30, 76], but others have reported significantly reduced ICF, consistent with abnormalities in facilitatory motor inputs and cortical connections in PD [78-80]. Reduced ICF has been demonstrated in both early and advanced PD patients [31, 78] (although it should be noted that these studies have been associated with variable changes in SICI). Reduced ICF could be due to reduction of facilitatory inputs involved in ICF and could underlie the impaired muscle activation [81] as well as movement scaling deficit [82] that underlies bradykinesia in PD.

LICI has been reported to be abnormal in PD, but there are mixed reports with increased as well as decreased intracortical inhibition at long ISIs (100-250ms) [45]. The fact that there is reasonably good evidence for decreased cortical inhibition, with SICI being reduced and CSP shortened, makes determining the changes in LICI an important question in terms of understanding the cortical mechanisms in PD pathophysiology. Reduced LICI would support the hypothesis that loss of dopaminergic transmission in PD patients is associated with impaired motor cortical inhibitory mechanisms [74], while increased LICI would support the hypothesis that there is differential modification of the motor cortex in PD [47]. Pierantozzi et al. [74] reported significantly reduced LICI, which was maximal at 80-100 ms and reversed with Apomorphine, but on the other hand, Beradelli et al. [48] reported increased LICI in the setting of shortened CSP [48], which has been reproduced by other groups, including one that used a similar protocol used by Beradelli et al. [47, 83]. In addition, this would be consistent with the finding that SICI is reduced when

tested in the presence of LICI [71], suggesting that LICI can inhibit SICI through activation of presynaptic GABA_B receptors.

In summary, results from TMS studies in PD have been somewhat inconsistent, most likely owing to the methodological issues within and between studies. Studies have employed varying stimulation intensities for conditioning and test stimuli and often study subjects were not carefully selected out according to disease severity, medication states and presence or absence of treatment related complications such as motor fluctuations and dyskinesia.

Notwithstanding this, there is reasonably good evidence that SICI is reduced and CSP is shortened in PD and that these improve to an extent with dopaminergic therapy. These findings need to be confirmed in carefully selected patient groups and further research into the relationship between the changes in cortical excitability and clinical as well as objective measures of disease status will help to clarify the significance of cortical excitability changes in PD pathophysiology and whether modern TMS techniques might be of use in drug trials as an objective biomarker of disease severity.

1.4 CONCEPTS OF PD PATHOPHYSIOLOGY

Marsden proposed that the role of the basal ganglia in motor control is to facilitate desired movements and to inhibit unwanted movements [84, 85]. Likewise, the surround inhibition motor control model, proposes that basal ganglia is essential in inhibiting competing circuits so that the motor cortex can execute the optimal motor program [86]. This notion is supported by the TMS finding that in normal subjects, SICI is reduced in the agonist muscle prior to voluntary movement but not in the antagonist muscle, thereby focussing the excitatory drive to produce the intended

movement [87, 88]. Although it is uncertain whether the TMS changes in PD are primary or secondary or even effects of dopaminergic therapy, one could speculate that the finding of reduced SICI in PD would be consistent with the idea that the reduced intracortical inhibition in PD allows the “unhelpful” competing motor circuits to be active during an intended movement, interrupting the focus of neuronal activity onto the appropriate pathways thus leading to reduced movement speed and amplitude [56]. However, the recent evidence that reduced SICI arises from enhancement in the SICF subcomponent of SICI would be consistent with the notion that there is increased excitability of corticospinal neurons in PD patients [76, 89]. This could be a compensatory strategy against decreased excitatory input from the motor thalamus to the primary motor cortex arising from reduced excitatory activities in the direct pathway and increased activities in the indirect pathway, resulting from loss of dopaminergic neurons in the substantia nigra [90, 91].

In addition to the abnormalities in the inhibitory circuits, intracortical facilitation (ICF) has been reported to be significantly reduced in PD, indicating that various facilitatory motor inputs and cortical connections are also defective in PD [37, 78-80]. It has been suggested that this might represent a compensatory response to excessive corticospinal motor output at rest, i.e. steeper SR curve [37]. However, it should be noted that the steeper SR curve itself has also been postulated as a compensatory mechanism against slow recruitment of commands to move by making it easier to recruit activity from a resting state [4].

In summary, there still remain significant uncertainties about the role of cortical excitability changes in PD. Perhaps this is best highlighted by the fact that similar cortical excitability changes have been reported in dystonia which is an example of

hyperkinetic movement disorder which gives rise to unwanted excessive movements rather than paucity of movements in PD. Currently, it remains to be shown whether the changes seen in cortical excitability are primary, compensatory or secondary to effects of dopaminergic medications, but recent evidence from Ni et al. [76] raises the possibility that reduced SICI in PD and dystonia may be underpinned by opposite changes in the subcomponents of SICI. In PD, SICI may be reduced due to increased facilitation (SICF) while in dystonia, it may be reduced due to increased inhibition in the subcomponents of SICI.

One possible way to unravel the significance of cortical excitability changes in the PD pathophysiology would be to examine how changes in cortical excitability relate to clinical and objective measures of clinical features of PD, including bradykinesia and rigidity. Another way would be to examine how changes in cortical excitability vary in different motor states. For example, if intracortical inhibition is in fact important for correct specification and selection of movements, then its behaviour during task performance is likely to reveal how cortical excitability changes contribute to pathophysiology of PD [77].

1.5 CONCLUSIONS

Cortical excitability studies using paired-pulse TMS has been extensively applied in the field of movement disorders and significant abnormalities have been reported in various parameters. However, the significance of the changes in cortical excitability remains incompletely understood, as its relationship with clinical and neurophysiological measures of impairment and disability has not been investigated in

detail. One of the areas of interest is the question of how much of the reported findings are primary or instead compensatory as this can have potential implications for therapeutics. Therefore further research is required to gain further insights into the reported cortical excitability changes in various disorders. It is likely that with deeper understanding of the significance of cortical excitability changes in one disease will spark and translate into further research and insights into other related movement disorders and hence it would seem fitting to concentrate the research efforts on the prototypical basal ganglia disorder, namely PD.

**Chapter 2 DYSFUNCTION OF CORTICAL AND
SPINAL INHIBITORY CIRCUITS UNDERLIE
DEVELOPMENT OF STIFF-MAN SYNDROME**

Abstract

Background: Stiff-man syndrome (SMS) is a rare autoimmune disorder characterised by fluctuating muscular rigidity and spasms. Dysfunction of GABA-ergic intracortical circuits has been reported in SMS and shown to correlate with anti-glutamic acid decarboxylase antibody (anti-GAD Ab) levels in the cerebrospinal fluid, although the role of this GABA-ergic dysfunction in the disease pathophysiology remains to be fully elucidated. Consequently, the present study utilised novel threshold tracking transcranial magnetic stimulation techniques to further explore the pathophysiology of clinical features in SMS.

Methods: Cortical excitability studies were undertaken in 5 SMS patients, using a 90 mm circular coil, and results were compared to 61 normal controls. All patients were clinically staged for disease severity and assessed using exteroceptive reflexes and serum anti-GAD Ab levels.

Results: Short interval intracortical inhibition (SMS, $-1.2 \pm 2.4\%$; controls, $10.3 \pm 0.7\%$; $P < 0.001$) and cortical silent period duration (SMS, 159.1 ± 18.7 ms; controls, 210.2 ± 3.1 ms; $P < 0.001$) were significantly reduced in SMS patients while intracortical facilitation was increased (SMS $-6.3 \pm 0.9\%$; controls -0.4 ± 0.8 ; $P < 0.01$). In contrast, exteroceptive reflexes were abnormal at presentation but absent in all but one patient at the time of this study. There was no correlation between either changes in cortical function or exteroceptive reflexes and clinical disease severity or serum anti-GAD Ab levels.

Conclusions: There is dissociation between measures of cortical hyperexcitability, spinal hyperexcitability, clinical disease severity and anti-GAD Ab levels in SMS. This finding suggests complex mechanisms are likely to underlie the pathophysiology

of clinical features in SMS and have implications for treatment and disease monitoring.

2.1 INTRODUCTION

Stiff-man syndrome (SMS) is an autoimmune disorder characterised by fluctuating muscular rigidity and spasms, most prominent in axial and proximal limb muscles usually in a symmetric manner [92]. Up to 60-80% of the patients harbour serum anti-glutamic acid decarboxylase (GAD) antibody, which have been shown to inhibit GABA synthesis *in vitro* [93]. Although dysfunction of intrinsic cortical inhibitory GABA-ergic circuits has been reported [94-96], the pathophysiological mechanisms underlying the development of clinical features of SMS remain to be fully elucidated.

Cortical function, and in particular cortical excitability, may be assessed by using paired-pulse transcranial magnetic stimulation (TMS) techniques [26, 58]. Paired pulse TMS techniques utilising the constant stimulus method, have reported cortical hyperexcitability in SMS, thereby suggesting an impairment of GABA-ergic inhibitory cortical interneuronal circuits [94, 96]. Of further relevance, spinal reflex myoclonus in SMS has been reported as indicated by the presence of pathological exteroceptive reflexes, reportedly a biomarker of spinal hyperexcitability mediated by propriospinal pathways [92, 95, 97]. Unexpectedly, spinal motor neuronal excitability and the function of local spinal inhibitory circuits (GABA-ergic interneuron mediated reflexes) were reportedly normal [95, 98].

Separately, cortical hyperexcitability has been shown to correlate with anti-GAD antibody levels in the cerebrospinal fluid (CSF) [96], thereby suggesting that anti-GAD antibodies underlie the development of cortical hyperexcitability in SMS. The role of cortical hyperexcitability in development of symptoms of SMS, however,

remains obscure [99]. In an attempt to further clarify the pathophysiology of clinical features of SMS, the present study utilised novel threshold tracking TMS techniques to assess:

- i. whether cortical GABA-ergic dysfunction was a feature of SMS;
- ii. the relationship between cortical hyperexcitability and a measure of spinal hyperexcitability – exteroceptive reflexes;
- iii. the relationship between either cortical or spinal hyperexcitability and clinical features of SMS.

2.2 METHODS

Studies were undertaken in 5 patients with SMS (1 male, 4 females, mean age 57.8 years, age range 26-83) referred to the Movement Disorders Unit at Westmead Hospital, Sydney, Australia. The diagnosis of SMS was based on a typical presentation with back pain, stiffness and stimulus-induced spasm in 4/5 and stiff limb syndrome subsequently generalising in 1 patient, together with supportive investigations including positive exteroceptive reflexes at presentation and positive anti-GAD antibodies in all patients. At the time of testing, all patients exhibited stable disease and their therapy had not been changed for at least 3 months. Regular medications included benzodiazepines that were not discontinued for the study. All patients gave informed consent to the procedures, which were approved by the Sydney Western Area Health Service Human Research Ethics Committee.

2.2.1 Clinical disease severity

All SMS patients were clinically staged using the *distribution-of-stiffness* and *heightened sensitivity* scales [100], and were based on reported symptoms in the preceding week. For heightened sensitivity, 1 point was given for the presence of noise-induced stiffness and cramps, visual stimuli-induced stiffness and cramps, somatosensory-induced stiffness and cramps, voluntary activity induced spasms, emotional upset and “stress”-induced spasms, awakenings due to nocturnal spasms, and untriggered cramps and spasms (maximum score, 7). For distribution of stiffness, 1 point was given for the presence of stiffness in each of the following areas: face, arms, upper trunk, abdomen, lower trunk, and legs (maximum score, 6).

2.2.2 Cortical excitability

Cortical excitability studies were undertaken by applying a 90 mm circular coil to the motor cortex with currents generated by two high-power magnetic stimulators connected via a BiStim (Magstim Co., Whitland, South West Wales, UK). Conditioning and test stimuli could be independently set and delivered through one coil. Paired-pulse threshold tracking TMS was undertaken according to a previously reported technique [26]. Briefly, the motor evoked potential (MEP) amplitude was fixed and changes in the test stimulus intensity required to generate a target response of 0.2 mV ($\pm 20\%$), when preceded by sub-threshold conditioning stimuli, were measured [26]. The MEP response was recorded over the right abductor pollicis brevis (APB). Resting motor threshold (RMT) was defined as the stimulus intensity required to maintain this target MEP response. Initially, the maximum MEP amplitude (mV), MEP onset latency (ms) and cortical silent period (CSP) duration

were recorded with magnetic stimulus intensity set to 140% RMT. Three stimuli were delivered at this level of stimulus intensity. Central motor conduction time (CMCT, ms) was calculated according to the F-wave method [101].

Short interval intracortical inhibition (SICI) and intracortical facilitation (ICF) were measured according to a previously devised protocol [26]. 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, and 7 ms. ICF was measured at ISIs of 10, 15, 20, 25 and 30 ms. Stimuli were delivered sequentially as a series of three channels: channel 1 tracked the stimulus intensity required to produce the unconditioned test response (i.e., RMT); channel 2 monitored the response to the subthreshold conditioning stimulus; and channel 3 tracked the stimulus required to produce the target MEP when conditioned by a stimulus equal in intensity to that on channel 2. Stimuli were delivered every 5-10 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 was measured as the increase in the test stimulus intensity required to evoke the target MEP. Inhibition was calculated off-line as follows [26]:

$$\text{Inhibition} = (\text{Conditioned test stimulus intensity} - \text{RMT}) / \text{RMT} * 100$$

2.2.3 Spinal excitability

In order to assess spinal excitability, we measured exteroceptive reflexes following median nerve stimulation at the wrist, using a modified technique of Meinck and colleagues [95]. Spinal hyperexcitability is reflected by the presence of spinal reflex myoclonus. Briefly, a train of 6 square wave pulses of 0.5ms duration were delivered at 200Hz to the median nerve at the wrist just proximal to the wrist crease. The stimulus intensity was adjusted to 1.5-2.0 times the motor threshold as detected by muscle twitch. Stimulation was repeated 5-6 times, and each stimulation was delivered at greater than 45-second intervals to avoid habituation. Surface electromyographic (EMG) recordings were obtained over the sternocleidomastoid, paraspinals at C7, T4, T12, L4, and rectus femoris muscles using Ag-AgCl electrodes placed 2 cm apart. Recorded EMG activity was rectified and the area under the curve for all reflex EMG activity between 50 and 800 ms after the stimulus was calculated. The latency (ms) to onset was also measured.

2.2.4 Peripheral studies

In the same sitting, the median nerve was stimulated electrically at the wrist using 5-mm Ag-AgCl surface electrodes (ConMed, Utica, USA). The resultant compound muscle action potential (CMAP) response was recorded from the APB and the CMAP onset latency and peak-peak amplitude were measured.

Recordings of MEP and CMAP responses were amplified and filtered (3Hz-3kHz) using a Grass ICP511 A.C. amplifier and sampled at 10 kHz using a 16-bit data acquisition card (National Instruments, Austin, Texas; PCI-MIO-16E-4). Data

acquisition and stimulation delivery (both electric and magnetic) were controlled by QTRAC software (copyright Institute of Neurology, Queen Square, London, UK).

2.2.5 Anti-GAD antibody titre

Anti-GAD antibody levels were measured at the same visit. For detection and quantification of anti-GAD antibodies in serum, GAD65 antibody ELISA kit (RSR Ltd, Cardiff, UK) was used. The results are reported in manufacturer's units per millilitre (normal <10 U/mL). The final concentration of antibodies was obtained after normalisation of the different dilutions to the RSR standard curve in the kit assay.

2.2.6 Statistics

Cortical excitability in SMS patients was compared to 61 healthy controls (31 males, 30 females, mean age 45.8 years, age range 23-83). Student t-test test was used to compare mean differences between SMS patients and controls. Correlations between excitability indices and clinical scales were analysed by Pearson's rank test. A probability (P) value of < 0.05 was considered statistically significant. Results are expressed as mean \pm standard error of the mean.

2.3 RESULTS

2.3.1 Clinical disease severity

The clinical and laboratory characteristics of the 5 SMS patients are outlined in Table 2-1. The mean disease severity score, combining distribution-of-stiffness and

heightened sensitivity scales, was 5.8 (range: 0-12) indicating a mild-to-moderate level of disease activity. All patients tested positive for anti-GAD antibodies, with the mean anti-GAD antibody titre being 1, 248,974 U/mL (range: 7,020 - 4,621,000).

Patient no.	Age/ Sex (years)	Disease duration (Years)	Disease severity	Anti-GAD Ab level (U/ml)	Associated disease	Medication (daily dose)
1	26/F	14	12	4,621,000		diazepam 180mg + clonazepam 8mg + baclofen 75mg
2	83/M	13	0	685,000	Psoriatic arthropathy	diazepam 5mg
3	55/F	15	7	7020	Asthma, IDDM	diazepam 10mg + clonazepam 2mg
4	65/F	23	10	899,000	Asthma, hypothyroidism, IDDM	diazepam 60mg
5	60/F	7	0	32850	IDDM	nil
Mean	57.8	14.4	5.8	1, 248, 974		

Table 2-1 Clinical characteristics of patients. Disease duration refers to the period from symptom onset to date of testing. Disease severity was determined by adding the scores from *distribution of stiffness* (maximum score, 6) and *heightened sensitivity* (maximum score, 7) scales (see Methods). Serum anti-GAD antibody levels (normal <10 U/ml) were measured at the time of cortical excitability studies. Total daily dose of medications was assessed in each patient.

2.3.2 Spinal excitability

Exteroceptive reflexes were positive with evidence of spinal reflex myoclonus in all patients at the time of diagnosis, indicating spinal hyperexcitability (Figure 2-1). However, at the time of assessment of cortical excitability in the present study,

enhanced exteroceptive reflexes were present in only 1 patient, with a latency of 56 ms.

2.3.3 Cortical excitability

Short interval intracortical inhibition represents an increase in the conditioned stimulus intensity required to track a constant target MEP of 0.2 mV, and typically develops between interstimulus intervals of 1-7 ms with two peaks evident, a smaller peak at ISI 1ms and larger peak at 2.5-3 ms [26]. SICI is thought to be mediated by GABA_A-ergic activity [58, 63, 67, 102, 103]. The average SICI, between ISIs of 1-7 ms, was significantly reduced in SMS patients when compared to controls (SMS averaged SICI $-1.2 \pm 2.4\%$; controls $10.3 \pm 0.70\%$, $P < 0.001$, Figure 2-2). In addition, peak SICI at ISI 1ms (SMS $1.6 \pm 1.7\%$; controls $7.4 \pm 0.8\%$; $P < 0.05$, Figure 2-3A) and ISI 2.5-3ms (SMS 2.1 ± 2.9 ; controls 15.3 ± 1.3 ; $P < 0.01$, Figure 2-3B) were also reduced in SMS patients.

Following SICI, a period of facilitation develops between ISIs of 10-30 ms and is reflected by a decrease in the conditioned test stimulus intensity required to evoke a target MEP. Intracortical facilitation is believed to be mediated by glutamate activity [26, 104]. ICF was significantly increased in SMS patients when compared to controls over ISIs 10-30 ms (SMS $-6.3 \pm 0.9\%$; controls -0.4 ± 0.8 ; $P < 0.01$, Figure 2-3C).

Cortical silent period refers to a period of electrical silence in a contracting muscle, and was measured from the onset of the MEP to the return of EMG activity [26, 49].

CSP is believed to be mediated by GABA-ergic activity acting via GABA_B receptors [50, 52]. The cortical silent period duration was significantly reduced in SMS patients when compared to controls (SMS 159.1±18.7 ms; controls 210.1 ±3.1 P<0.001, Figure 2-3D)

In contrast to significant differences in cortical GABA-ergic function, the resting motor threshold (SMS 56.9 ± 4.9%; controls 59.5 ± 1.0%) and MEP amplitude (SMS 17.2 ± 3.9%; controls 24.9 ± 1.8%) were similar between the groups, as was the central motor conduction time (SMS 5.41 ± 0.36 ms; controls 5.29 ± 0.21 ms).

2.3.4 Peripheral studies

The CMAP amplitude (SMS 12.3 ± 2.1 mV; controls 10.2 ± 0.4 mV) and neurophysiological index (SMS 2.8 ± 0.7; controls 2.5 ± 0.1) were similar in SMS patients and controls, excluding peripheral abnormalities as the basis of abnormalities demonstrated in cortical excitability.

2.3.5 Relationship between clinical, immunological, and neurophysiological findings

There was no significant correlation between SICI and the heightened sensitivity (R= 0.31, P = 0.61) or distribution of stiffness (R = 0.31, P = 0.62). There was no significant correlation between SICI (R = 0.22, P = 0.99), ICF (R = -0.5, P = 0.39) or CSP (R = -0.154, P = 0.80) and serum anti-GAD antibody levels. Assessment of CSF anti-GAD antibodies was not undertaken in this study.

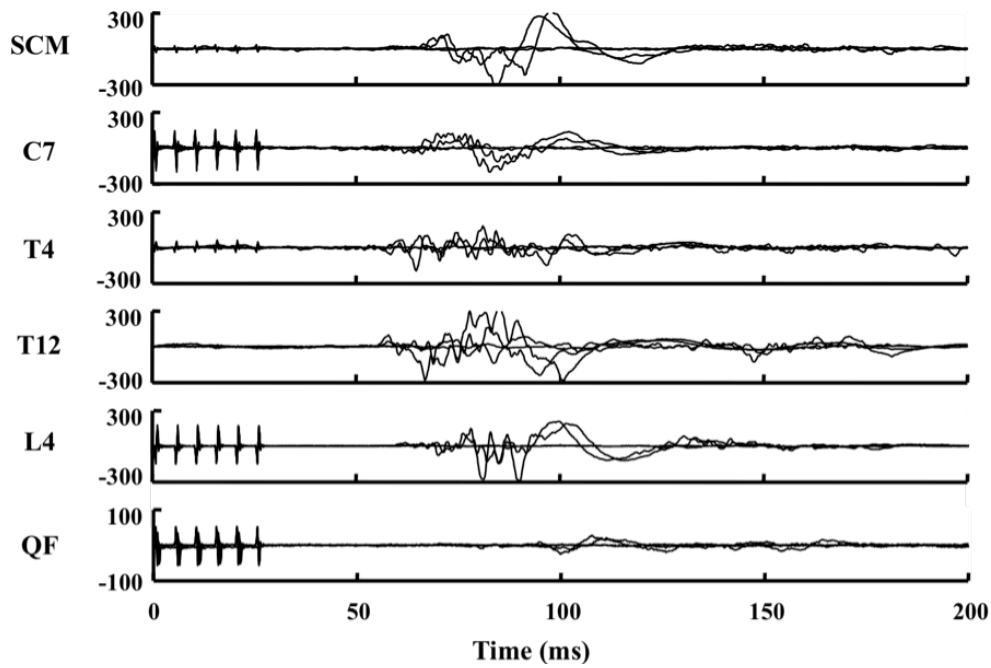


Figure 2-1 A representative exteroceptive reflexes in a patient at the time of diagnosis. Note the onset of pathological EMG activity at a latency of 55 ms, seen first in T12 paraspinal muscles and followed by ordered recruitment of rostral (T4 and C7) and caudal L4 paraspinal muscles, as well as recruitment of the quadriceps femoris (QF) and sternocleidomastoid (SCM) muscles.

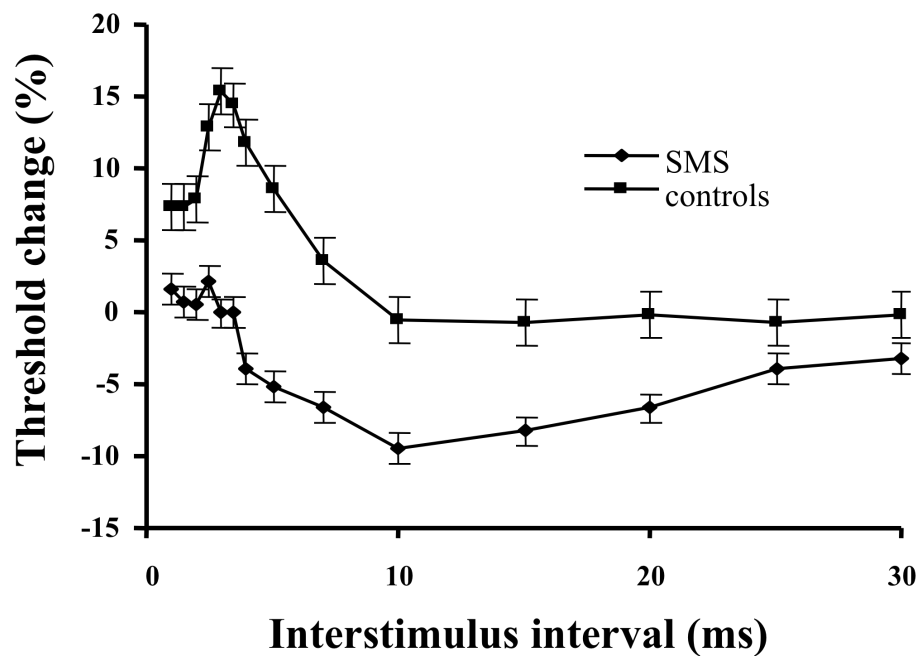


Figure 2-2 Short interval intracortical inhibition (SICI), was markedly reduced in stiff-man syndrome (SMS) patients when compared to normal controls ($P < 0.001$).

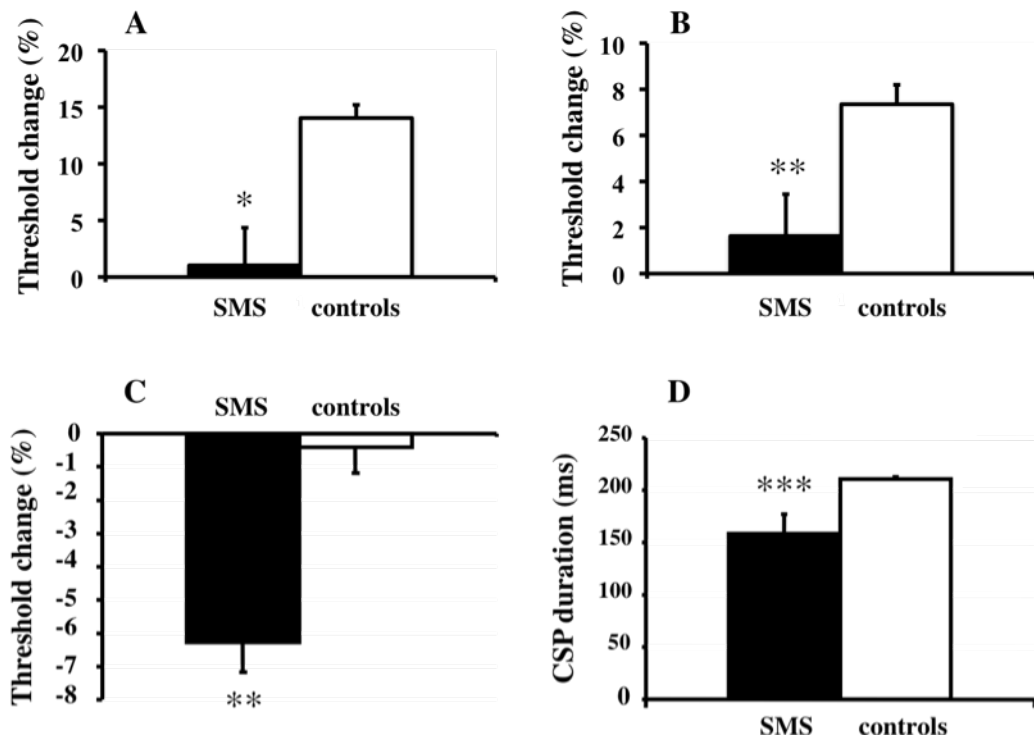


Figure 2-3 There was a significant reduction of SICI in stiff-man syndrome (SMS) patients at both (A) ISI of 1 ms and (B) ISI of 2.5-3ms; * $P < 0.05$; ** $P < 0.01$. (C) Intracortical facilitation (ICF) was significantly increased in SMS patients when compared to controls over ISIs 10-30 ms; ** $P < 0.01$. (D) Cortical silent period (CSP) duration was significantly reduced SMS patients; *** $P < 0.001$.

2.4 DISCUSSION

This is the first study to concurrently assess cortical and spinal neurophysiology, anti-GAD antibodies and clinical features in a cohort of stiff-man syndrome patients. The present study has identified evidence of ongoing cortical GABA-ergic dysfunction in a cohort of chronic stiff-man syndrome patients treated with high doses of benzodiazepines. Specifically, there was a significant reduction in short interval intracortical inhibition and cortical silent period duration with an increase in intracortical facilitation. It is noteworthy that these changes in cortical function persisted despite a relative improvement in spinal hyperexcitability as well as partial symptomatic benefit during treatment with high doses of benzodiazepines. However,

the most notable finding is that there was a dissociation between cortical GABA-ergic dysfunction, spinal hyperexcitability, clinical disease severity and anti-glutamic acid decarboxylase antibody levels. Taken together, these findings suggest that although there is dysfunction of GABA-ergic intracortical circuits, there are complex interrelationships between these factors in the generation of the clinical features of SMS.

2.4.1 Pathophysiology of cortical dysfunction in SMS

It is now accepted that SICI is mediated by cortical inhibitory neurons acting via GABA_A receptors [58, 63, 102, 103, 105]. Evidence for such a mechanism is provided by pharmacological studies revealing that GABA agonists, such as benzodiazepines, enhance SICI [104]. Of further relevance, ICF also appears to be modulated by GABA-ergic processes acting via GABA_A receptors, with ICF being reduced by GABA_A receptor agonists [23, 42, 104]. As such, the finding of persistent reduced SICI and increased ICF in the present cohort of SMS patients, despite benzodiazepine treatment, suggest significant dysfunction of GABA-ergic inhibitory neurons acting via GABA_A receptors and is in keeping with previous studies [94, 106].

In addition to dysfunction of GABA_A circuits, the findings in the present study suggest concomitant dysfunction of GABA_B circuits. Specifically, the cortical silent period is mediated by cortical inhibitory mechanisms acting via GABA_B circuits and local spinal inhibitory processes [50, 52]. Given that CSP duration was significantly reduced in SMS patients, dysfunction of cortical GABA-ergic processes acting via

GABA_B receptors may in part account for this finding, although a contribution from dysfunctional spinal inhibitory processes cannot be discounted [50, 52].

2.4.2 Relationship between cortical dysfunction and anti-GAD antibodies

Only one previous study has examined the relationship between cortical excitability and anti-GAD antibodies [96]. Our study supports their finding of a lack of correlation between serum anti-GAD antibody levels and measures of cortical GABA-ergic dysfunction. In their study, a correlation was found between cortical excitability and CSF anti-GAD antibody levels, which were not assessed in the present study. The lack of a correlation between cortical excitability and serum anti-GAD levels is in keeping with previous reports that serum anti-GAD antibody levels do not reflect CSF levels, due to strong intrathecal production of anti-GAD antibodies by a clonal activation of B cells within the central nervous system in SMS patients [107].

2.4.3 What underlies the generation of symptoms in SMS?

A reduction of GABA synthesis, mediated by anti-GAD antibodies, has been postulated as a likely mechanism underlying the development of clinical features of SMS, including axial and limb rigidity, muscle stiffness and stimulus-induced spasms [92, 93, 107]. Specifically, it was postulated that inhibition of GABA synthesis would result in a reduction or loss of supraspinal inhibition, thereby resulting in hyperexcitability of local spinal circuits and ultimately the clinical features of SMS [92, 95].

Our findings suggest that there may not be a simple causal relationship between cortical GABA-ergic dysfunction and spinal hyperexcitability and associated stimulus-induced muscle spasms in SMS. The absence of a correlation between TMS biomarkers of cortical GABA-ergic dysfunction and measures of stimulus induced spasms, such as heightened sensitivity, would seem to argue against a significant contribution of cortical GABA-ergic dysfunction to the development of stimulus-induced muscle spasms. Of further relevance, the absence of correlation between measures of cortical GABA-ergic dysfunction and spinal hyperexcitability, as reflected by exteroceptive reflexes, suggests that cortical mediated disinhibition of spinal reflexes may not be the predominant mechanism underlying the development of clinical features in SMS [108]. Disinhibition of brainstem circuits directly mediating muscle tone and spasms, along with dysfunction of non-GABA-ergic inhibitory subcortical circuits may potentially contribute to local spinal hyperexcitability, and thereby symptom generation in SMS [97, 109]. From a therapeutic perspective, the findings in the present study may explain the limited efficacy of therapeutic measures aimed at solely modulating GABA-ergic circuits, highlighting the importance of considering novel therapeutic approaches in SMS.

2.4.4 Limitations of the present study

It could be argued that the findings in the present study were confounded by benzodiazepine therapy, an agent that modulates cortical processes [23]. This seems unlikely given that SICI and CSP were reduced, or moved in an opposite direction to that expected with exposure to benzodiazepines [23]. The withdrawal or reduction in benzodiazepine dose for the purposes of this study would have been unethical in the

present SMS patients given the potential to lead to a marked exacerbation of symptoms, potentially resulting in death [110].

2.5 CONCLUSIONS

There is dissociation between measures of cortical hyperexcitability, spinal hyperexcitability, serum anti-GAD antibodies and clinical symptoms in stiff man syndrome. Spinal hyperexcitability and muscle stiffness and spasms are unlikely to be simply due to cortical disinhibition of spinal circuits. This study highlights the current incomplete understanding of the pathophysiology of SMS. More detailed studies of the relationship between cortical excitability and other features of SMS such as associated psychiatric symptoms, as well as studies of the relationship between brainstem excitability and features of SMS, may give further insights into the pathophysiology and treatment of SMS.

**Chapter 3 Hand dominance influences side-to-side
differences in tapping speeds but not SICI in
normal subjects**

ABSTRACT

Background: Cortical processes underlying hand dominance has yet to be fully clarified. There is good evidence that the degree of interhemispheric asymmetry in the size of the cortical hand area correlates highly with the asymmetry in hand performance in normal subjects. However, results from cortical excitability studies using paired-pulse transcranial magnetic stimulation (TMS) have been inconsistent, with a lack of studies which have examined hand dominance objectively and correlated it against cortical excitability.

Objectives: To explore the relationship between hand dominance and side-to-side differences in speed of ballistic hand movements and cortical excitability using the threshold tracking paired-pulse TMS.

Methods: Cortical excitability studies were undertaken in 10 normal subjects. Short interval intracortical inhibition (SICI) was assessed using a threshold tracking paired-pulse TMS with conditioning stimulus intensity set to 70% of resting motor threshold. Motor evoked potentials were recorded over the abductor pollicis brevis. Hand tapping rate was measured for each side with a timed tapping task in which two electronic touch pads positioned 20 cm apart were tapped as fast as possible for 60 seconds. Data were grouped by hand dominance and comparisons were made between the dominant and non-dominant sides. Interhemispheric differences in SICI were correlated with side-to-side differences in timed tapping task.

Results: There was a significant difference in the TR between the dominant and non-dominant sides (mean difference = 28.2 ± 6.4 , $P < 0.01$) but no significant interhemispheric difference in SICI (mean difference = $-1.5 \pm 2.5\%$, $P = 0.57$). Furthermore, there was no significant correlation between the interhemispheric

difference in SICI and the side-to-side difference in hand tapping rate (Spearman correlation coefficient = -0.25, $P > 0.24$).

Conclusion: The present study has identified that there is no significant asymmetry in cortical excitability between the dominant and non dominant cerebral hemispheres in normal subjects despite there being a clear asymmetry in motor performance as tested with an objective hand tapping task. Furthermore, there was no correlation between the asymmetries in motor performance and cortical excitability, suggesting that hand dominance influences side-to-side differences in tapping speeds but not SICI in normal subjects.

3.1 INTRODUCTION

Cortical processes underlying hand dominance has yet to be fully clarified. There is consistent evidence that the degree of side-to-side asymmetry in the size of the hand area in the primary motor cortex correlates highly with the asymmetry in hand motor performance, i.e., handed dominance [111-113]. However, results from cortical excitability studies in normal subjects, comparing the dominant and non-dominant cerebral hemispheres have been inconsistent.

A number of studies have reported significant functional differences in intracortical inhibition and facilitation between the dominant and non-dominant hemispheres within individual subjects [114-117], and additionally, significant differences in cortical excitability between the right handed and left handed subjects have been reported by a number of studies [113]. Specifically, Hammond et al. [114] reported that short interval intracortical inhibition (SICI) and intracortical facilitation (ICF) developed more rapidly with increasing conditioning stimulus intensity in the dominant hemisphere compared with non dominant hemisphere which was interpreted as the intracortical circuits being more “potent” in the dominant hemisphere, and hypothesised that this difference in shaping motor outflow contributes to the asymmetrical dexterity while Civardi et al [117] reported enhanced ICF and to a lesser extent SICI in the dominant hemisphere compared with non-dominant hemisphere in the right handed subjects although the left handed group did not show such hemispheric asymmetries. However, other studies have reported minimal interhemispheric asymmetry in SICI and ICF [118, 119].

Motor threshold has been reported to be lower on the dominant hemisphere compared with the non-dominant hemisphere by a number of groups [19, 28, 120], but it has also been reported as being comparable between the dominant and non-dominant

sides and been replicated with slightly different techniques [117, 121, 122]. Further, cortical silent period has been reported to be shorter in the dominant hand compared with the non-dominant hand of right-handers [117, 122], but Cicinelli et al. reported no significant differences in cortical silent period.

Significantly, almost all of the studies to date have determined the hand dominance based on preferred writing hand or handedness inventories, e.g. Oldfield inventory or Edinburgh Handedness Inventory, and only a few studies have measured the hand function objectively and correlated these with measures of cortical function [27, 111].

Of note, the previous studies examining intracortical facilitation and inhibition have utilized the conventional paired-pulse TMS, which fixes the test stimulus and measures the changes in MEP when the test stimulation is preceded by a subthreshold conditioning stimulus. One of the main limitations of this technique is marked variability in MEP amplitude with consecutive stimuli, making it difficult to discern whether changes in the MEP produced are secondary to a true change in cortical excitability or, alternatively, represent fluctuations related to the delivery of the test stimuli [59, 62]. By setting a tracking target of 0.2 mV, located in a steep portion of the nonlinear SR curve [41], marked variability in MEP amplitudes translate to smaller changes in stimulus intensity, potentially overcoming this limitation and provides the added advantage of speeding up the recording process thereby allowing multiple recordings to be made in quick succession.

In the present study, cortical excitability was measured from both cerebral hemispheres in 10 normal subjects using the threshold tracking paired-pulse transcranial magnetic stimulation (TMS) to examine whether there are significant

functional differences between the dominant and non-dominant hemispheres. Furthermore, hand motor performance was correlated with measures of cortical excitability.

3.2 METHODS

3.2.1 Subjects

Studies were undertaken in 10 normal subjects (5 males and 5 females, median age 65 years, age range 46-72). All subjects underwent review of their medical history including medications followed by detailed neurological examination and cleared of any neurological problems (See Table 1).

All patients gave written informed consent to the research, which was approved by the Sydney Western Area Health District Human Research Ethics Committee.

3.2.2 Handedness

Handedness was rated using the Edinburgh Handedness Inventory and a score was calculated as a continuous variable for each patient from a scale ranging from -100 and 100. A score of 0 indicated ambidexterity while -100 denoted complete left handedness and 100, complete right handedness.

3.2.3 Objective measures

Tapping task

Speed of hand tapping was measured for each side with a timed tapping task in which subjects alternately tapped two electronic touch pads positioned 20 cm apart as fast as possible for 60 seconds as previously described [123]. The number of taps and changes in the rate of tapping between the first and the last 15-second epochs were measured for each hand.

Cortical excitability

Cortical excitability was assessed by measuring the resting motor threshold (RMT), short interval intracortical inhibition (SICI), intracortical facilitation (ICF) and cortical silent period (CSP) as previously described [26]. Briefly, paired-pulse threshold tracking TMS was undertaken by applying a 90 mm circular coil to the motor cortex with currents generated by two high-powered magnetic stimulators connected via a BiStim (Magstim Co., Whitland, South West Wales, UK) [26]. The MEP response was recorded over the right abductor pollicis brevis (APB). RMT was defined as the stimulus intensity required to maintain the target MEP response of 0.2 mV. Cortical excitability was determined by fixing the motor evoked potential (MEP) amplitude at 0.2 mV ($\pm 20\%$) (target response) and measuring the changes in the test stimulus intensity required to generate this target response, when preceded by a sub-threshold conditioning stimuli at various interstimulus intervals (ISIs) [26]. Sub-threshold conditioning stimuli was set at 70% RMT. SICI was measured at increasing ISIs as follows: 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, and 7 ms, and ICF at ISIs of 10, 15, 20, 25 and 30 ms.

short interval intracortical inhibition was measured as the increase in the test stimulus intensity required to evoke the target MEP, and calculated off-line [26]. Intracortical facilitation was measured as the decrease in the conditioned test stimulus intensity required to evoke a target MEP.

Cortical silent period refers to a period of electrical silence in a contracting muscle, and was measured from the onset of the MEP to the return of EMG activity with magnetic stimulus intensity set to 140% RMT [26, 49].

3.2.4 STATISTICS

Analysis of variance (ANOVA) was used to compare mean differences between the dominant hand and non dominant hand. Correlations between excitability indices and objective motor performance (hand tapping rate) was analysed by Spearman's rank test. A probability (P) value of < 0.05 was considered statistically significant. Results are expressed as mean \pm standard error of the mean.

3.3 RESULTS

Participant demographics are presented in Table 3-1.

Subjects	Age	Sex	Handedness	EHI score
1	65	F	Left	-100
2	72	F	Ambidextrous	0
3	70	M	Right	100
4	46	F	Right	100
5	66	F	Right	100
6	65	M	Right	100
7	63	M	Right	100
8	66	M	Right	93.75
9	61	F	Right	100
10	64	M	Right	100

Table 3-1 Subject demographics

3.3.1 Cortical Excitability

Motor Threshold

RMT was comparable between the dominant and non-dominant sides (dominant = $58.69 \pm 3.0\%$; non dominant = $59.23 \pm 2.9\%$).

Motor evoked potential amplitude

MEP amplitude was comparable (dominant = 2.29 ± 0.76 ; non-dominant = 2.36 ± 0.60) as was MEP latency (dominant = 23.27 ± 0.49 ; non-dominant = 23.28 ± 0.48).

Cortical silent period

The CSP duration was also comparable between the DH and NDH (dominant = 223.4 ± 10.14 ; non-dominant = 227.1 ± 9.55 ; $P = 0.80$, Figure 3-1).

Paired stimulation

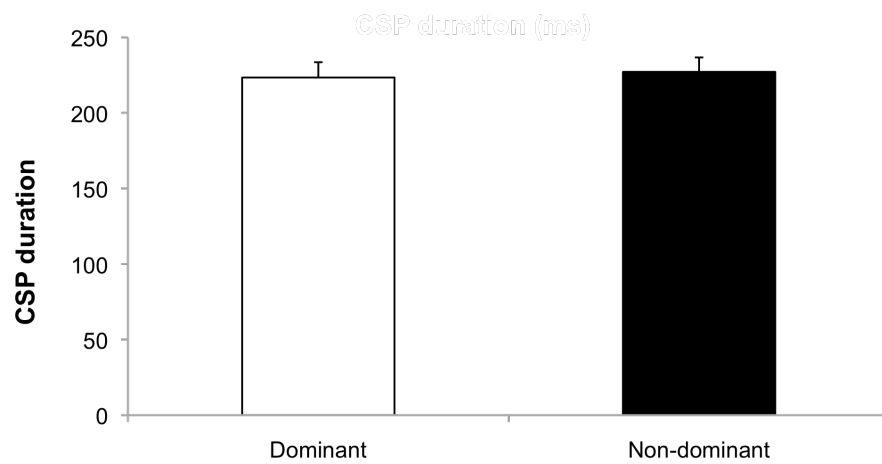


Figure 3-1 CSP duration between the dominant and non dominant hemispheres.

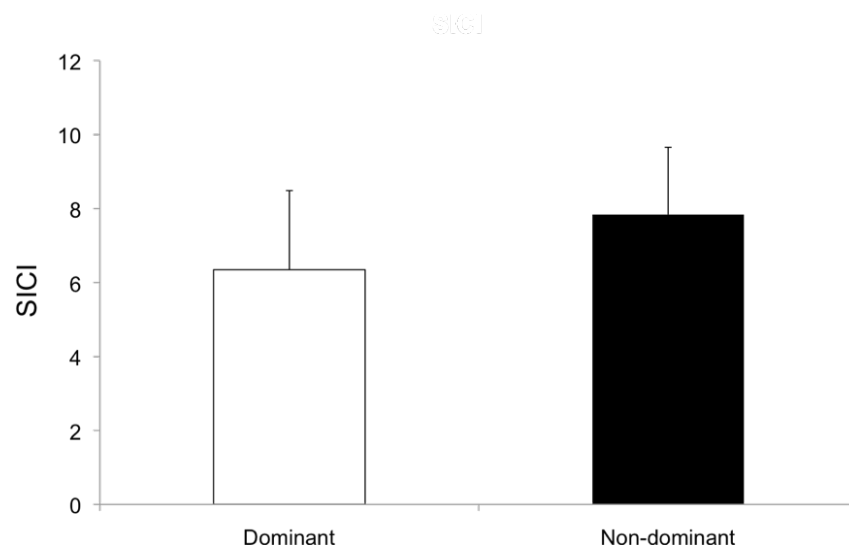


Figure 3-2 SICI between the dominant and non dominant hemispheres.

Averaged SICI from ISIs of 1-7ms was $6.35 \pm 2.1\%$ on the dominant hand and $7.84 \pm 1.8\%$ on the non dominant hand (Figure 3-2). These were somewhat lower when compared with our normative data in 61 patients obtained from the right hand ($10.3 \pm 0.7\%$). ICF was $-1.87 \pm 1.2\%$ on the dominant hand and $-2.07 \pm 2.74\%$ on the non dominant hand compared with -0.4 ± 0.8 from our normative data. CSP was $223.4 \pm$

10.1ms on the dominant hand while it was 227.1 ± 9.6 ms on the non dominant hand, compared with 210.2 ± 3.1 ms from our normative data.

3.3.2 Objective hand function

Timed tapping task

There was a significant difference in tapping rate between the dominant and non-dominant hands (dominant side 254.5 ± 6.7 /minute; non-dominant side 226.3 ± 5.3 ($P = 0.006$)).

Relationship between cortical excitability studies and objective hand function

There was no correlation between cortical excitability parameters and hand function as measured by hand tapping speed or EHI. Given there was significant variability in the cortical excitability parameters between individuals, the side-to-side difference in SICI and ICF were correlated with side-side difference in hand tapping speed. However, the degree of asymmetry in TR between the dominant and non-dominant hands did not correlate with the degree of asymmetry in measures of cortical excitability, i.e., SICI, ICF and CSP.

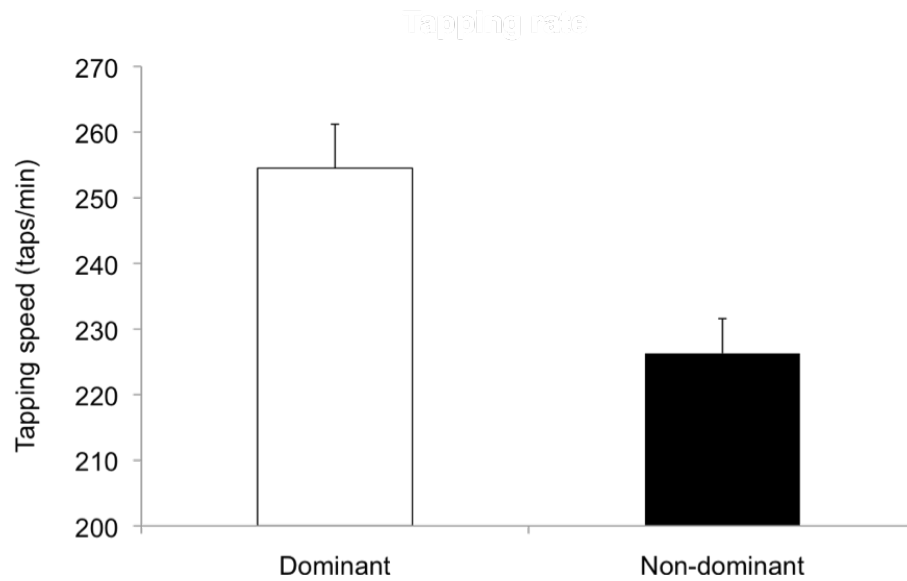


Figure 3-3 Tapping speed between the dominant and non dominant hands.

3.4 DISCUSSION

To our knowledge, the present study is the first to apply threshold tracking paired-pulse TMS to compare the cortical excitability between the two hemispheres in normal subjects. The present study has identified that there is no significant asymmetry in cortical excitability between the dominant and non-dominant hemispheres in normal subjects despite the clear side-to-side difference in motor performance as measured with an objective hand-tapping task. SICI and ICF were comparable between the two hemispheres, suggesting absence of asymmetry in GABAergic function as both SICI and ICF are mediated by cortical inhibitory neurons acting via GABA_A receptors.

Of interest, a number of studies have examined the cortical excitability of both cerebral hemispheres with TMS but the results have been inconsistent. The results from the present study are in line with previous studies that have reported no

functional asymmetry between the motor cortices and suggest the presence of other influences such as ipsilateral cortical activation and transcallosal inhibition underlying hand dominance. The fact that this finding has been replicated with two different but validated TMS techniques lends further support for the validity of the findings from the present study.

The functional differences of the cerebral hemispheres in man can be explained with using two models. One model hypothesises that hand dominance is a consequence of “intrinsic specialisation” such as asymmetry in the size of the motor cortex and motor threshold between the dominant and non-dominant motor cortices [111]. The other model assumes that both motor cortices have identical intrinsic function and the asymmetry hand dominance results from asymmetry in inhibitory interaction between the two motor cortices such as transcallosal inhibition or asymmetry of ipsilateral motor cortex activation [124]. It is noteworthy that a number of studies have examined the cortical excitability of both cerebral hemispheres with TMS but again the results have been inconsistent. The results from the present study provide further evidence that rather than there being “intrinsic specialisation,” influences such as ipsilateral cortical activation and transcallosal inhibition likely underlie hand dominance.

In conclusion, this study provides evidence that cortical excitability is symmetrical in healthy normal controls and as such hand dominance influences side-to-side difference in tapping speed but not SICI.

**Chapter 4 Interhemispheric differences in cortical
excitability in Parkinson's disease**

ABSTRACT

Objective: To investigate whether there are interhemispheric differences in cortical excitability in Parkinson's disease (PD), and whether these correlate with clinical and objective measures of bradykinesia and rigidity.

Background: The pathophysiological mechanisms linking the dopaminergic deficits and motor manifestations are not well understood. Transcranial magnetic stimulation techniques have suggested abnormalities of cortical excitability in PD, but it remains unclear as to whether these abnormalities are a focal phenomena reflecting the asymmetrical motor and nigrostriatal deficit, or a generalised process.

Methods: Cortical excitability studies were undertaken using threshold tracking paired-pulse TMS on the more and less affected sides in 12 PD subjects in the OFF-medication state and compared with 10 age-matched control subjects as previously described. The results were correlated with clinical as well as objective measures of bradykinesia and rigidity by also assessing MDS-UPDRS, timed hand tapping task and angular impulse score using a torque motor. Results were compared between more and less affected hemispheres in PD subjects and between dominant and non-dominant hemispheres and to normal controls.

Results: ICF was statistically significantly reduced in the PD subjects compared with the age matched control subjects ($F(41) = 3.678$, $*P=0.02$), but there were no significant side-to-side differences between the more and less affected sides. However, there were significant interhemispheric differences in SICI (mean difference -4.06 ± 1.72 %, $P < 0.04$) and CSP (mean difference -12.8 ± 4.5 ms, $P < 0.02$) although both SICI and CSP were not significantly different to controls. There was a significant correlation between interhemispheric differences in SICI and side-to-side differences in both clinical ($\rho = -0.59$, $P < 0.05$) and objective measures ($\rho = -$

0.80, $P < 0.002$) of bradykinesia, while there was no such correlation in normal controls.

Conclusion: The results suggest that cortical dysfunction is an asymmetrical process in PD, reflecting the asymmetry in motor and nigrostriatal deficit. Furthermore, the significant correlation between interhemispheric differences in SICI and side-side differences in akinesia suggest that asymmetry in cortical excitability is a pathophysiological feature of PD and contribute to asymmetrical motor impairment, although it remains unclear to what extent these changes are primary or compensatory.

4.1 INTRODUCTION

Parkinson's disease (PD) is characterized by progressive asymmetric loss of dopaminergic neurons in the nigrostriatal pathways with corresponding asymmetric bradykinesia, rigidity and tremor. However, the pathophysiological mechanisms linking the dopaminergic deficit and motor impairments are poorly understood.

The motor cortex is the primary target for the output neurons of the basal ganglia, and therefore the deficits in movement control that occur in the basal ganglia as part of PD should ultimately be reflected in the activity of the cortical neurons. Indeed, transcranial magnetic stimulation (TMS) techniques have revealed relatively consistent abnormalities of cortical excitability in PD as indicated by reduction in short interval intracortical inhibition (SICI) and cortical silent period (CSP), which normalise with dopaminergic treatment and deep brain stimulation, suggesting a role for cortical dysfunction in disease pathophysiology. However, the significance of these findings remains uncertain, as a relationship has yet to be demonstrated between the cortical excitability changes and impairments in motor function. It has already been shown that the changes in cortical excitability do not arise simply because of abnormal muscle tone, i.e. rigidity that characterises PD as demonstrated by normal motor thresholds (MTs) and magnetic evoked potentials (MEPs) [5, 7, 30-33] and therefore if cortical excitability changes can be shown to be a focal phenomenon, this would provide a further link between the asymmetrical nigrostriatal pathology and corresponding clinical manifestations and support the role of cortical excitability changes in the disease pathophysiology.

There have been a small number of studies, which examined the interhemispheric differences in cortical excitability in PD patients [6, 28, 78] but the findings have been inconsistent. A single-pulse TMS study reported significantly reduced MT and

shortened CSP duration on the more affected hemisphere [28] but another study reported no significant side-to-side differences in MEP and CSP [6] although this study showed significant correlation between maximal CSP duration and Unified Parkinson's Disease Rating Scale (UPDRS) scores on the more affected side as well as correlation between some less well validated parameters (MEP-Int50 and CSP-Int50) and UPDRS on the less affected side. This finding may suggest evolution of TMS parameters from a less to more symptomatic state [6]. Further, a paired-pulse TMS study using the constant stimulus technique in PD subjects with early disease reported no significant side-to-side differences in SICI and ICF between the more and less affected hemispheres although this study found significant reduction in SICI and ICF [78].

Before cortical excitability changes can be reliably interpreted in PD, the influence of hand dominance on cortical excitability needs to be clarified as results from cortical excitability studies comparing the dominant and non-dominant cerebral hemispheres in normal subjects have been inconsistent. A number of studies have reported significant differences in SICI and ICF between the dominant and non-dominant hemispheres of individual subjects [114-117] and similarly, significant differences in cortical excitability have been reported between the right handed and left handed subjects [113]. However, others have reported conflicting findings with minimal interhemispheric asymmetry in SICI and ICF [118, 119, 121, 125] although it has to be noted that the influence of handedness was only assessed in one of these studies in which all of the subjects were right handed [121].

Of note, these previous studies have utilized the conventional paired pulse TMS, which fixes the test stimulus and measures the changes in MEP when the test stimulation is preceded by a subthreshold conditioning stimulus. One of the main

limitations of this technique is the marked variability in MEP amplitude with consecutive stimuli such that multiple stimuli need to be delivered at each interstimulus interval, which likely makes the technique less reliable in PD as the patients may change from one state to another in the time required to complete a study, particularly in those with motor fluctuations.

The present study assessed the cortical excitability of the more-affected versus less-affected cerebral hemispheres in 12 PD subjects using the threshold tracking paired pulse TMS and these were compared with those of 10 age-matched healthy control subjects in order to determine whether the changes in cortical excitability are asymmetrical. In addition, clinical and objective measures of PD severity were measured and correlated with the cortical excitability parameters in order to examine whether a relationship can be found between these measures.

4.2 METHODS

4.2.1 Subjects

Studies were undertaken in a convenience sample of 12 non-tremor-dominant PD patients referred to the Movement Disorders Unit, Westmead Hospital, Sydney Australia (10 males, 2 females, median age 63 years, age range 46-82) and the mean disease duration was 4.5 years (median 4.5 years), Table 4-1. The diagnosis of PD was based on the United Kingdom Parkinson's Disease Society Brain Bank clinical diagnostic criteria for PD [126]. More affected side was determined on the basis of clinical examination using Part 3 of Movement Disorder Society - Unified Parkinson's Disease Rating Scale (MDS-UPDRS) [127]. In patients with symmetrical signs, the side of onset was designated as the more affected side. Motor

cortex contralateral to more affected upper limb was designated as the more affected cortex.

Studies were performed in a practically defined off state in the morning after an overnight withdrawal of medications, at least 12 hours after the last dose. All subjects gave informed consent to the procedures, which were approved by the Sydney Western Area Health Service Human Research Ethics Committee.

Each subject underwent TMS, clinical assessments, and objective measures of bradykinesia and rigidity. All assessments were performed on both sides in a single study visit over approximately 2 hours.

Patient no.	Age (years) / Sex	Disease duration (years)	MDS-UPDRS ON	MDS-UPDRS OFF	H&Y stage	More affected side	LD dose (mg/day)
1	56/M	6	12	4	1	L	450
2	58/F	8	19	4	2	R	1000
3	72/M	3	40	22	2	R	450
4	63/F	1	4	0	1	R	0
5	70/M	5	12	4	2	L	200
6	60/M	2	17	9	1	L	450
7	81/M	5	12	8	2	L	400
8	68/M	8	24	11	3	L	750
9	47/M	4	24	15	2	L	600
10	50/M	4	28	7	2	L	750
11	70/M	5	17	10	2	L	700
12	74/M	3	10	8	1	L	600
Mean	64.1	4.5	21.75	10	1.75	-	529

Table 4-1 Disease duration refers to the period from symptom onset to date of assessment. MDS-UPDRS refers to part 3 of Movement Disorder Society - Unified Parkinson's Disease Rating Scale score ON-medication and OFF-medication. H&Y refers to Hoehn and Yahr. Total daily dose of medications was assessed in each patient.

4.2.2 Clinical staging of PD

In 9 patients the left side was the more affected side and in 3 the right side. Each subject was assessed clinically using Part 3 of MDS-UPDRS. Upper limb bradykinesia scores were calculated for each subject based on the sum of three 4-point measures of finger tapping, hand movements and pronation-supination movements of hands, with a score of 0 indicating absence of bradykinesia, and a score of 12, severe bradykinesia. Rigidity was assessed at the wrists, with 0 being no rigidity and 4, severe rigidity.

4.2.3 Experimental set up

Objective measures – Rigidity

Objective rigidity was measured according to the method described by Fung et al. [128]. Briefly, subjects were seated comfortably in a dental chair, with their forearms resting in a splint and their hands semi-pronated in a manipulandum. Motion of the wrist was restricted to a horizontal plane, negating the effects of gravity.

The manipulandum was mounted on the vertically orientated shaft of a printed circuit torque motor (PMI Motion Technologies JR16M4CH-1, Commack, NY, USA) so that the wrist joint was overlying the axis of rotation. Torque output was directly proportional to the current supplied to the motor. During rigidity measurements, resistive torque was assessed by monitoring and converting this current to a proportional voltage. A precision potentiometer mounted to the lower end of the motor shaft signalled angular position.

The torque motor was operated as a position servo to generate sinusoidal perturbations of 60° peak-to-peak amplitude about the 0° position, i.e., $\pm 30^\circ$. Ten non-contiguous 5-sec epochs were recorded for each objective rigidity measurement with the total recording time taking approximately 2 minutes. During each 5-sec epoch, a 3-sec interval of oscillation at 1.0 Hz was either preceded or followed by a contiguous 2-sec interval of oscillation at 1.5 Hz. The number of epochs beginning with each frequency (1.0 Hz or 1.5 Hz) was equal and the order of presentation of epochs varied in a pseudorandom fashion. Resistive torque was rectified and integrated with respect to time, yielding the scalar equivalent of angular impulse referred to as angular impulse score [128].

Objective measures - Bradykinesia

Bradykinesia was measured for each hand with a timed tapping task as described by Nutt et al. [129]. The subjects were seated on a chair in front of a table on which was positioned two electronic touch pads 20 cm apart. Subjects were instructed to tap the two touch pads alternately as fast as possible for 60 seconds and the number of taps and changes in the rate of tapping between the first and the last 15 second epochs were measured for each hand.

TMS procedure

Cortical excitability was assessed by measuring the resting motor threshold (RMT), magnetic evoked potential (MEP) amplitude, short interval intracortical inhibition (SICI), intracortical facilitation (ICF) and cortical silent period (CSP).

Cortical excitability studies were undertaken by applying a 90 mm circular coil to the motor cortex with currents generated by two high-power magnetic stimulators connected via a BiStim (Magstim Co., Whitland, South West Wales, UK). Conditioning and test stimuli could be independently set and delivered through one coil. Paired-pulse threshold tracking TMS was undertaken according to a previously reported technique [26]. Briefly, the motor evoked potential (MEP) amplitude was fixed and changes in the test stimulus intensity required to generate a target response of 0.2 mV ($\pm 20\%$), when preceded by a sub-threshold conditioning stimuli at various interstimulus intervals (ISIs), were measured [26]. The MEP response was recorded over the right abductor pollicis brevis (APB). RMT was defined as the stimulus intensity required to maintain this target MEP response (0.2 mV). SICI was determined by using sub-threshold conditioning stimuli (70% RMT) at increasing ISIs as follows: 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, and 7 ms. ICF was measured at ISIs of 10, 15, 20, 25 and 30 ms.

Stimuli were delivered sequentially as a series of three channels: channel 1 tracked the stimulus intensity required to produce the unconditioned test response (i.e., RMT); channel 2 monitored the response to the subthreshold conditioning stimulus; and channel 3 tracked the stimulus required to produce the target MEP when conditioned by a stimulus equal in intensity to that on channel 2. The EMG activity was monitored continuously to ensure the absence of voluntary background activity affecting MEP amplitude. Response from channel 2 was subtracted from channel 3 to eliminate contribution from conditioning stimulus. Stimuli were delivered every 5 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 was measured as the increase in the test stimulus intensity required to evoke the target MEP. Inhibition was calculated off-line as follows [26]:

$$\text{Inhibition} = (\text{Conditioned test stimulus intensity} - \text{RMT})/\text{RMT} * 100$$

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

Cortical silent period refers to a period of electrical silence in a contracting muscle, and was measured from the onset of the MEP to the return of EMG activity with magnetic stimulus intensity set to 140% RMT [26, 49].

4.2.4 STATISTICAL ANALYSIS

Cortical excitability was compared to 10 age-matched control subjects (5 males, 5 females, median age 65 years, age range 46-72) using one-way ANOVA. Paired samples t-test was used to compare the side-to-side differences in both PD and age matched control subjects. Correlations between excitability indices and objectives measures of bradykinesia and rigidity were analysed using Spearman's rank correlation coefficient. Results were expressed as mean \pm standard error of the mean and a probability (P) value of less than 0.05 was considered significant. For the correlation studies between cortical excitability changes and motor function, because there is a large inter-subject variability, side-to-side differences in cortical excitability were correlated with side-to-side differences in motor function.

4.3 RESULTS

4.3.1 Clinical measures of disease of severity (modified MDS-UPDRS)

The average MDS-UPDRS score in the OFF state was 18.3 (range = 4 - 40), consistent with mild-moderate disease. Mean upper limb MDS-UPDRS bradykinesia score was 3.9 ± 0.8 on the more affected side and 2.3 ± 0.6 on the less affected side, with the mean difference being 1.7 ± 0.5 , (**P < 0.01, Figure 4-1A) indicating significant asymmetry of motor impairment between the more and less affected sides. Furthermore, there was a significant difference in MDS-UPDRS rigidity scores between the more and less affected sides (more-affected side = 1.4 ± 0.3 ; less-affected = 0.8 ± 0.3 , mean difference = 0.7 ± 0.2 , **P < 0.01, Figure 4-1B).

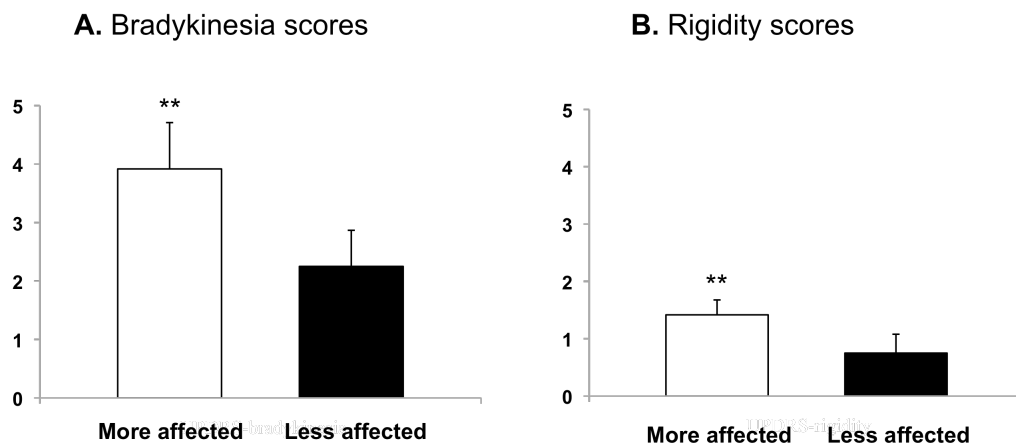


Figure 4-1 There were significant differences in mean upper limb MDS-UPDRS subscores for (A) bradykinesia; **P < 0.01 and (B) rigidity; **P < 0.01

4.3.2 Objective bradykinesia - hand tapping speed

In the control subjects, there was a significant difference in tapping speed as analysed by hand dominance (dominant side = 254.5 ± 7.0 /minute; non-dominant side = 226.3 ± 5.5 , ** P < 0.002, Figure 4-2A).

In the PD subjects, there was a significant difference in hand tapping speeds between the more and less affected sides (more-affected side = 168.1 ± 11.3 /minute; less-affected side = 196.3 ± 12.0 ** $P < 0.01$, Figure 4-2B).

4.3.3 Objective rigidity

In age-matched control group, there was no difference in rigidity between the dominant and non-dominant hands (dominant side = 0.24 ± 0.02 Nm; non dominant side = 0.20 ± 0.02 Nm, $P = 0.07$).

Interestingly, PD subjects also did not show significant differences in objective rigidity between the more and less affected sides (more affected side = 0.22 ± 0.02 Nm; less affected side = 0.23 ± 0.02 Nm, $P = 0.41$).

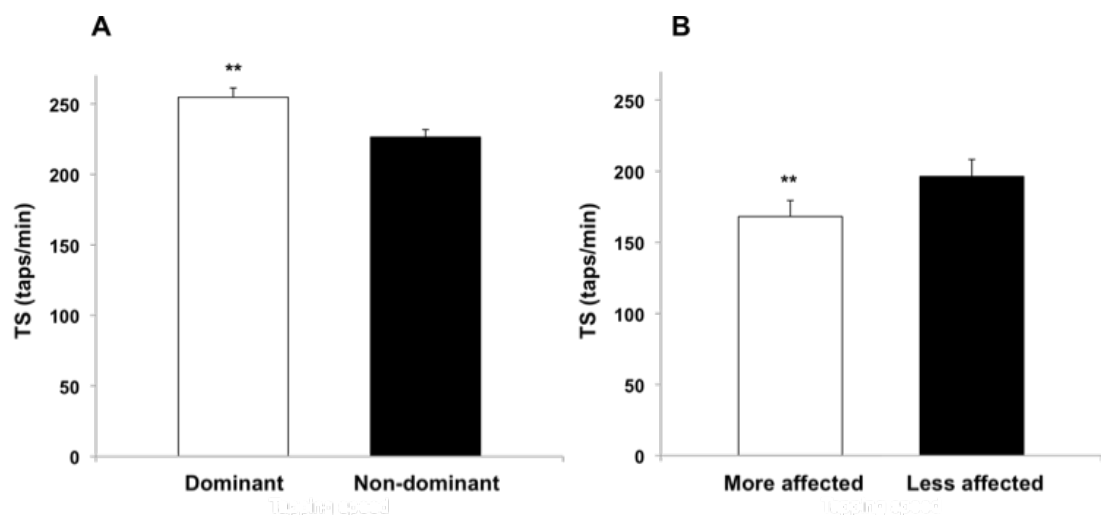


Figure 4-2 Comparison of average hand tapping speeds (TS). (A) There was a significant difference in TS between the dominant and non-dominant sides in age-matched normal control subjects; ** $P < 0.01$; (B) Similarly, there was a significant difference in TS between the more affected and less affected sides in PD subjects; ** $P < 0.01$

4.3.4 Cortical excitability measurements

Motor thresholds and magnetic evoked potentials

In the age-matched controls, RMT was comparable between the dominant and non-dominant sides (dominant = $58.69 \pm 3.0\%$; non dominant = $59.23 \pm 2.9\%$). MEP amplitude was comparable (dominant = 2.29 ± 0.76 ; non-dominant = 2.36 ± 0.60) as was MEP latency (dominant = 23.27 ± 0.49 ; non-dominant = 23.28 ± 0.48).

In the PD subjects, the RMT (more-affected = $48.3 \pm 2.2\%$; less-affected = $49.2 \pm 2.1\%$, $P = 0.66$) and MEP amplitude (more affected side = $1.18 \pm 0.25\text{mV}$; less affected side = 1.13 ± 0.14 , $P = 0.83$) were similar between the more and less-affected sides suggesting that side-to-side differences did not lead to significant differences in these measures of cortical excitability.

However, there were significant group differences in RMT ($F(43) = 5.069$, $P = 0.005$) although no such differences were found in MEP amplitude ($F(42) = 2.468$, $P = 0.08$).

Cortical silent period duration

In the age-matched controls, CSP duration was comparable between the dominant and non-dominant sides (dominant = 223.4 ± 10.7 ms; non-dominant = 227.5 ± 10.7 ms, $P = 0.3$, Figure 4-3A). However, in PD subjects, CSP duration was significantly reduced on the more affected side compared with the less affected side (more affected = 201.4 ± 10 ms, less-affected = 214.3 ms; mean difference -12.8 ± 4.5 ms, $*P < 0.02$, Figure 4-3B).

There was no significant difference in CSP duration between the PD and age-matched control groups ($F(42) = 1.259$, $P = 0.30$).

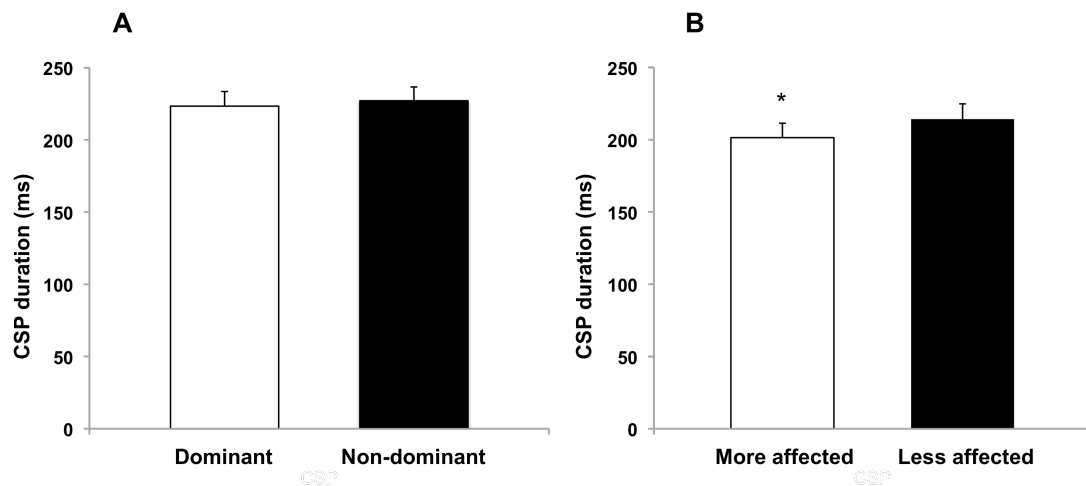


Figure 4-3 Cortical silent period (CSP) duration, as measured from the onset of the motor evoked potential to the beginning of electromyography (EMG) activity, was significantly reduced in PD subjects; * $P < 0.05$.

SICI

In the age matched control subjects, SICI was comparable between the dominant and non-dominant sides (dominant = 6.35 ± 2.24 %; non-dominant = 7.84 ± 1.91 %, $P = 0.57$, Figure 4-4A).

In the PD group, there was a significant side-to-side difference in SICI (more-affected = 9.01 ± 2.32 %; less-affected = 13.07 ± 2.27 %, mean difference = -4.06 ± 1.72 %, * $P < 0.04$, Figure 4-4B). However, no statistically significant difference was detected between the PD and age-matched control subjects ($F(43) = 1.721$ $P = 0.178$).

ICF

In the age-matched controls, there was no significant difference in ICF between the dominant and non-dominant sides (dominant side = -0.91 ± 2.17 %; non-dominant side = -2.74 ± 2.07 %, mean difference = 1.83 ± 2.60 %, $P = 0.50$, Figure 4-5A).

Likewise, in the PD subjects there was no significant statistical difference between the more and less affected sides (more affected side = 4.22 ± 1.23 ; less affected = 3.15 ± 1.35 , mean difference = 1.07 ± 1.35 , $P = 0.44$, Figure 4-5B).

However, ICF was statistically significantly reduced in the PD subjects compared with the age matched control subjects ($F(41) = 3.678$, $*P = 0.02$).

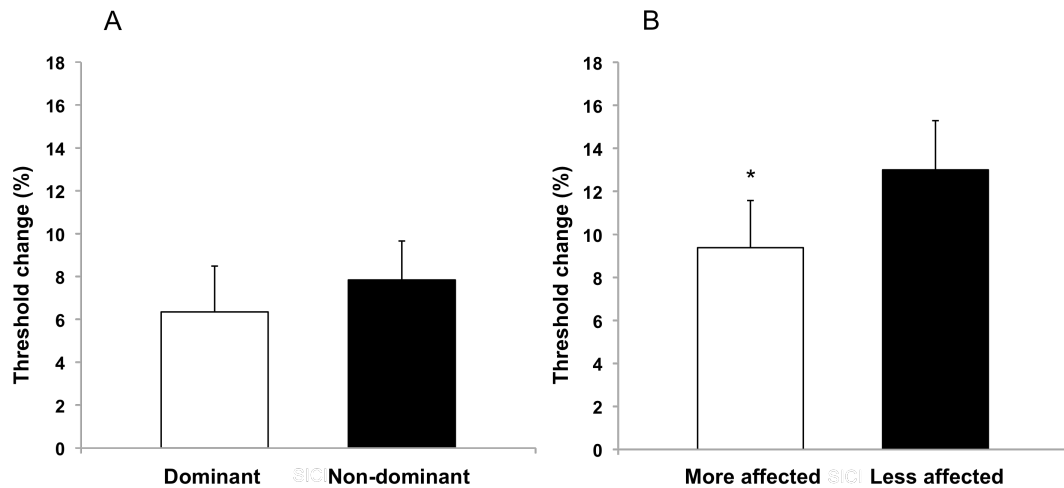


Figure 4-4 Short interval intracortical inhibition (SICI) was comparable between the PD and normal control (NC) subjects (Not shown). SICI was comparable between the dominant and non-dominant sides in normal control subjects (A), but significantly reduced on the more affected side relative to the less affected side in PD patients (B); $*P < 0.05$

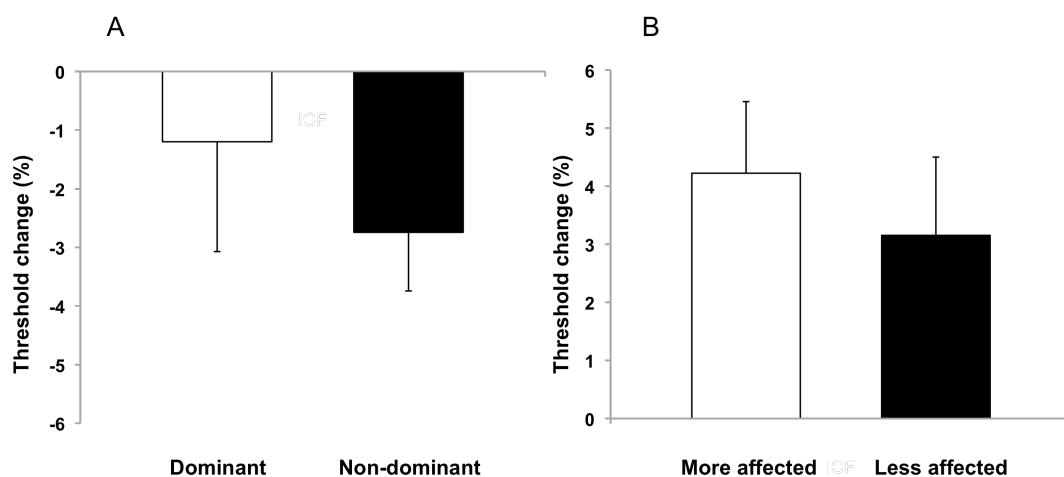


Figure 4-5 ICF was significantly reduced in the PD compared with NC subjects (Not shown), but there was no significant difference between the dominant and non-dominant sides in NC (A) or between the more and less affected sides in PD subjects (B).

4.3.5 Relationship between clinical measures of disease severity and cortical excitability in PD subjects

In PD subjects, measures of bradykinesia did not correlate with absolute SICI values from the corresponding hemisphere, likely owing to large variability in SICI between subjects. However, there was a significant correlation between interhemispheric differences in SICI (more affected side – less affected side) and side-to-side differences in MDS-UPDRS bradykinesia ($\rho = -0.59$, $*P < 0.05$, Figure 4-6), with the correlation being stronger with the hand tapping speeds ($\rho = -0.80$, $**P < 0.002$, Figure 4-7). In contrast, there was no such correlation in age-matched control subjects despite there also being significant side-to-side difference in hand tapping speed ($\rho = -0.25$, $P < 0.49$).

The side-to-side differences in hand tapping speed did not correlate with interhemispheric differences in CSP ($\rho = -0.284$, $P = 0.37$) or ICF ($\rho = 0.172$, $P = 0.59$) in PD subjects. Similarly, there was no significant correlation between hand tapping speed and side-to-side difference in CSP duration ($\rho = -0.201$, $P = 0.60$) in the age matched control group.

In terms of rigidity, there was no correlation between side-to-side differences in SICI and angular impulse scores ($\rho = 0.495$, $P = 0.102$) or MDS-UPDRS rigidity scores ($\rho = 0.004$, $P = 0.99$). However the correlation between side-to-side difference in ICF and objective rigidity approached statistical significance ($\rho = -0.519$, $P = 0.084$) in PD subjects.

4.4 DISCUSSION

The present study has identified significant cortical dysfunction in PD. Specifically, there was a significant reduction in ICF in PD subjects compared with age matched control subjects. Additionally, there was a significant reduction in SICI and CSP duration on the more affected side compared with the less affected side in PD subjects, although there was no difference between PD and age-matched normal control subjects overall. It is notable that in PD, the side-to-side difference in SICI correlated with side-to-side difference in clinical and objective measures of bradykinesia, while there was no such side-to-side difference in SICI in the age-matched normal control subjects despite there being a clear side-to-side difference in hand tapping speeds between the dominant and non-dominant hands. Taken together our findings suggest that asymmetry in cortical function (SICI and CSP) is a pathophysiological feature of PD and is reflective of asymmetry in motor impairment, although it remains to be determined whether this represents a primary abnormality underlying asymmetry in bradykinesia or instead is compensatory.

To our knowledge, the present study is the first to apply the threshold tracking paired-pulse TMS to examine interhemispheric differences in cortical excitability in PD and age-matched normal subjects. The threshold tracking technique has the advantage of overcoming marked variability in MEP amplitudes from stimulus to stimulus seen with conventional paired stimulation TMS. With this technique, the target MEP is set in a steep portion of the nonlinear SR curve (i.e. 0.2mV) and changes in stimulus intensity required to elicit this target MEP are measured, thereby avoiding the marked variability in MEP amplitudes and measuring changes in stimulus intensity [26, 57] that are less prone to random variation. This speeds up the recording process and allows studies to be completed in a relatively short period of time, making it

particularly suited to subjects who have difficulty tolerating prolonged recording sessions or fluctuating motor function such as those with PD.

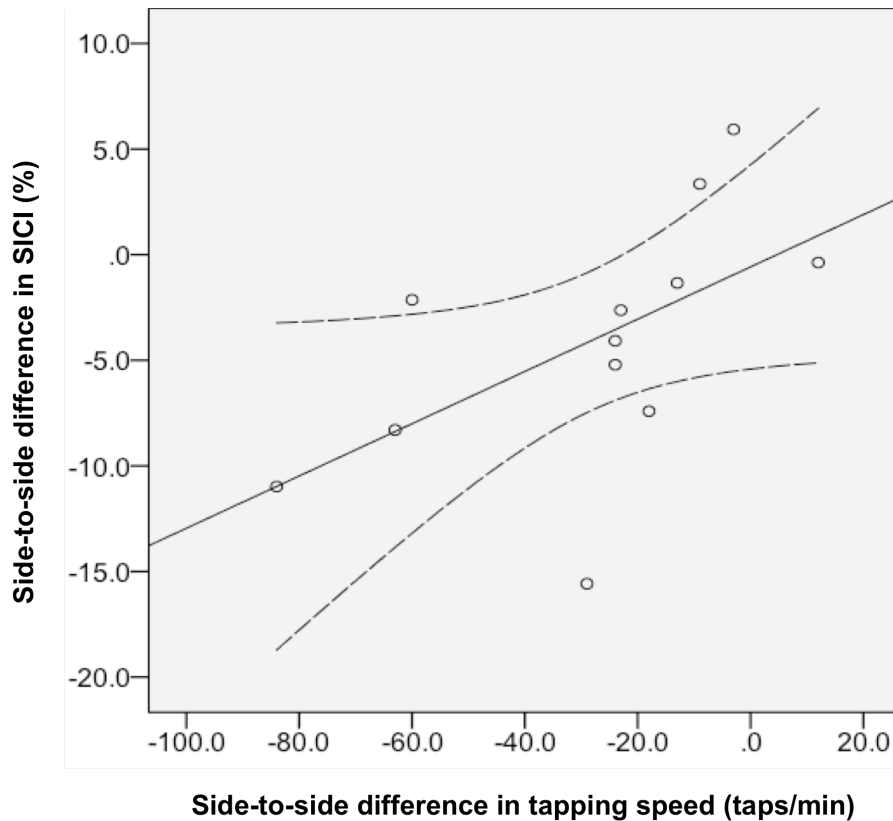


Figure 4-6 There was a significant correlation between interhemispheric differences in SICI (more affected side – less affected side) and side-to-side differences in MDS-UPDRS bradykinesia scores; $\rho = -0.59$; $*P < 0.05$.

To date, cortical excitability studies in PD patients using the paired pulse TMS have revealed mixed results. Several studies have reported reduced ICF in PD subjects [31, 78-80], but others have failed to find significant reduction [7, 76]. Similarly, SICI and CSP duration have been reported to be either normal or reduced, but it is likely that cortical excitability changes depend on the clinical state in which the recordings are made. For instance, SICI and CSP duration have both been reported to be reduced in the OFF-medication state and to normalise with acute as well as chronic

dopaminergic therapy [7, 28, 45, 130, 131], with some studies even reporting lengthening in CSP duration in patients who are on high doses of dopaminergic therapy [7, 132].

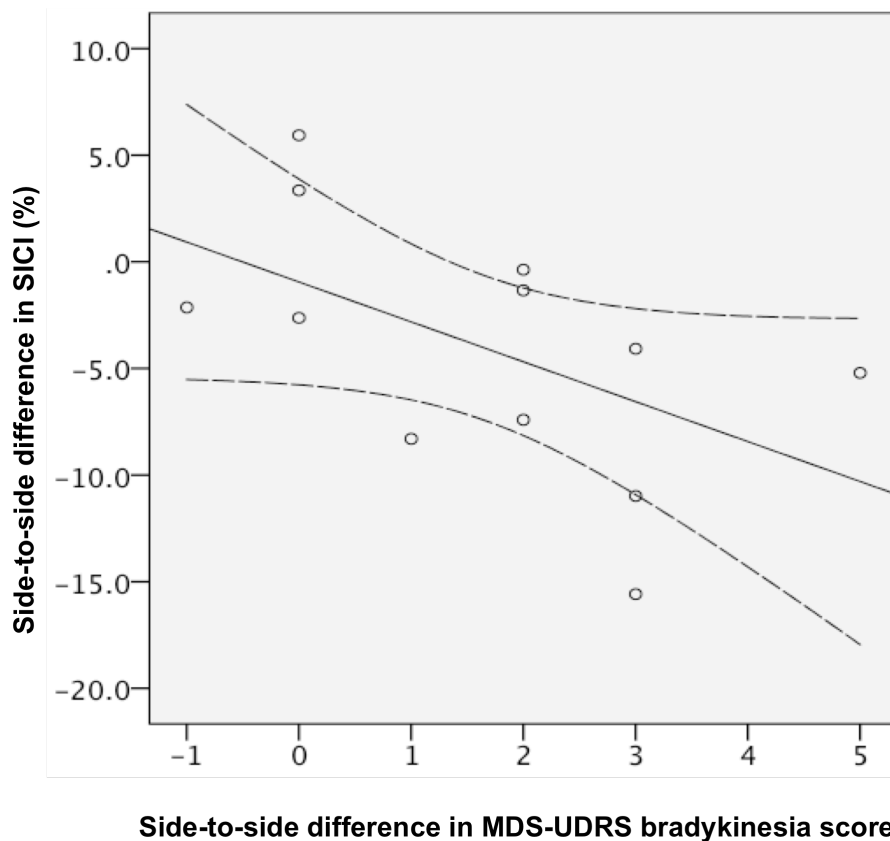


Figure 4-7 There was highly significant correlation between interhemispheric differences in SICI and corresponding side-to-side differences in hand tapping speeds; $\rho = -0.80$; $**P < 0.002$.

Our finding of significantly reduced SICI on the more affected side compared with the less affected side within PD subjects is in contrast to previous studies [6, 78]. However, the fact that the magnitude of asymmetry in SICI correlated with corresponding asymmetry in objective bradykinesia in the present study supports the validity of our findings and the apparent discrepancy between the results of ours and others' studies could be explained by differences in TMS technique and study population.

We cannot completely discount the fact that the side-to-side asymmetry in SICI in PD patients in the present study was due to the underlying rigidity as minor increases in muscle tone has a significant influence on cortical excitability [28], with the side-to-side differences in SICI merely reflecting the differences in muscle tone. However, in contrast to this previous study [28] in our cohort of PD patients, MTs and MEPs were comparable between the more and less affected sides suggesting that differences in tone did not have significant influence on the side-to-side differences in SICI. Furthermore, there was no correlation between corresponding side-to-side differences in clinical and objective measures of rigidity and SICI.

What is the significance of the cortical excitability changes in PD subjects?

In the present study, ICF was significantly reduced in PD subjects compared with healthy age-matched control subjects despite the fact that the PD subjects had relatively mild disease. Previously, it has been demonstrated that deficits in muscle activation [81] and movement scaling [82] are pathophysiological mechanisms underpinning bradykinesia. Furthermore, it has been shown that MT is normal at rest, but increases during voluntary contraction, resulting in a fewer motor neurons being recruited for an intended movement, i.e. *relative failure of voluntary facilitation* may be a possible mechanism of bradykinesia in PD [34, 37]. The reduced ICF in PD subjects could be due to reduced facilitatory inputs involved in ICF and underlie these two key deficits in PD bradykinesia although the fact that there was lack of correlation between ICF and hand tapping speed suggests that other as yet unclarified mechanisms are also likely to be involved.

The fact that there was no significant side-to-side difference in ICF in both physiological state and PD suggests that ICF has no direct influence on hand dominance or asymmetry in bradykinesia, but our finding of significantly reduced SICI on the more affected side relative to the less affected side in PD subjects, suggests that it could contribute to greater reduction in movement speed and amplitude on the more affected side by allowing the “unhelpful” competing motor circuits to be active during an intended movement thereby interrupting the focus of neuronal activity onto the appropriate pathways [56]. However, the fact that there was no significant difference in SICI and CSP between the PD and healthy age-matched control groups in the present study suggests that it is unlikely to be the primary mechanism underlying bradykinesia although it could still contribute to the asymmetry in motor function in PD. Instead the asymmetry in SICI could represent a compensatory phenomenon in which intracortical inhibition is reduced in order to facilitate voluntary movement production by countering deficits in muscle activation and movement scaling as well as *relative failure of voluntary facilitation*. If indeed relatively reduced SICI on the more affected side is a compensatory phenomenon, it might be a process whereby inhibitory pathways are down-regulated in an attempt to reverse the reduced facilitation (ICF) that is thought to underlie bradykinesia. The fact that SICI and CSP are reduced on the more affected side relative to the less affected side in PD but still normal when compared to age-matched control subjects would be consistent with the notion that reduced SICI appears first on the more affected side and later spreads to involve the less affected side, and suggests that this is a late and inadequate compensatory phenomenon.

It is well accepted that SICI is mediated by cortical inhibitory neurons acting via GABA_A receptors [58, 63, 102, 133, 134], as evidenced by pharmacological studies revealing that GABA agonists, benzodiazepines, enhance SICI [23]. Therefore the finding of significantly reduced SICI on the more affected hemisphere relative to the less affected side in the present cohort of PD subjects suggest relative dysfunction of GABA-ergic inhibitory neurons on the more affected hemisphere acting via GABA_A receptors. In addition to dysfunction of GABA_A circuits, the findings from this study suggest concomitant dysfunction of GABA_B circuits. Specifically, the cortical silent period is mediated by cortical inhibitory mechanisms acting via GABA_B circuits and local spinal inhibitory processes [50, 52]. Given that CSP duration was also significantly reduced on the more affected side relative to the less affected side, relative dysfunction of cortical GABA-ergic processes acting via GABA_B receptors may in part account for this finding [50, 52].

4.4.1 Limitations of this study

As patients with persistent moderate or severe tremor were excluded from this study, a question arises as to whether this resulted in selection of an atypical PD cohort. This is unlikely because all patients fulfilled the UK clinical criteria for IPD [126] and approximately 25% of pathologically proven cases do not experience tremor [135]. A further question is whether the findings of this study can be extended to subjects who have tremor or dyskinesia.

4.5 CONCLUSION

The present study has identified significant side-to-side differences in cortical excitability between the more and less affected sides in PD patients but not in age matched controls. This was despite the fact that both groups had similar side-to-side differences in hand tapping speed, suggesting that asymmetry in cortical excitability might be a pathophysiological feature of PD. However, it remains to be determined whether this represents a primary abnormality contributing to asymmetrical bradykinesia or instead is compensatory.

**Chapter 5 Effects of levodopa on cortical
excitability in Parkinson's disease**

ABSTRACT

Cortical excitability studies were undertaken using threshold tracking paired-pulse TMS in 19 PD subjects before and after their usual morning levodopa medication and the results were compared with 10 age-matched control subjects. All patients underwent assessment of their MDS-UPDRS, timed hand tapping task and angular impulse score as described in Chapter 4 in the OFF and ON-medication states. Each set of assessments was completed within 2 hours.

There was a significant improvement in the hand tapping speed (TS) after medications in line with the UPDRS results ($TS_{\text{OFF}} = 184.3 \pm 12.2$, $TS_{\text{ON}} = 202.3 \pm 11.7$, $TS_{\text{DIFF}} = -18.0 \pm 4.6$, $P = 0.001$). ICF was reduced in the PD patients compared with the controls with the difference being greater and approaching statistical significance in the OFF than ON-medication state (one-way ANOVA $P = 0.12$), but there was no significant difference in ICF between the OFF and ON-medication states. There was no significant difference in SICI between the PD and control subjects (one-way ANOVA $P = 0.54$) nor was there difference between OFF and ON-medication states. There was a correlation between SICI and decrement in hand tapping speed in the OFF-medication state ($\rho = -0.59$, $P = 0.01$, Figure 5-11A). In addition, there was correlation between ICF and decrement in hand tapping speed in the OFF-medication state ($\rho = -0.47$, $P < 0.05$, Figure 5-12). There was no significant correlation between SICI, ICF and CSP with TS and objective rigidity. The findings suggest that reduced SICI is a compensatory rather than primary pathophysiological change and is involved in modifying the rate of decrement in repetitive movements that characterise parkinsonian bradykinesia.

5.1 INTRODUCTION

Cortical excitability studies in PD using paired-pulse transcranial magnetic stimulation (TMS) have provided reasonably firm evidence that short interval intracortical inhibition (SICI) is reduced [7, 11, 30, 32, 33, 74] but intracortical facilitation (ICF) has been variably reported [31, 78-80] [7, 76], making it difficult to draw firm conclusions about cortical excitability changes and their role in PD pathophysiology.

In Chapter 4 (Side-to-side study), cortical excitability studies undertaken in a cohort of PD patients in the off-medication state and age-matched healthy control subjects demonstrated significantly reduced ICF and relatively reduced SICI on the more affected side compared with the less affected side in PD patients. Notably, there was significant correlation between interhemispheric asymmetry in SICI and side-to-side asymmetry in hand tapping speed in PD patients but not in age-matched healthy control subjects despite the fact that the control subjects also demonstrated significant side-to-side asymmetry in hand tapping speed on the basis of hand dominance. The findings from Chapter 3 are consistent with the notion that reduced ICF and relatively reduced SICI on the more affected side are pathophysiological features of PD.

Studies on effects of levodopa therapy on cortical excitability provide not only further information about the cortical excitability changes in PD but also insights into the significance of cortical excitability changes in PD. A number of previous studies have demonstrated that levodopa therapy restores the reduced SICI [7, 30, 74], perhaps via its direct or indirect effects on disordered intracortical excitability [136], but the influence of levodopa therapy on ICF has been inconsistent and has yet to be

fully clarified. For example, one study reported reduced SICI and ICF in the off-medication state, but only SICI and not ICF was restored in the on-medication state [79] while another study reported normal SICI and reduced ICF in the off-medication state with restoration of the reduced ICF with levodopa therapy [80]. The restoration of intracortical inhibition (SICI) and facilitation (ICF) under effective levodopa therapy is assumed to be due to changes in the basal ganglia where the drugs exert their effect, but whether the cortical excitability changes represent a primary or secondary compensatory phenomenon and furthermore whether these are beneficial or harmful remains to be resolved.

The primary objective of the present study was to examine the effect of levodopa therapy on cortical excitability using the threshold tracking paired-pulse TMS and the relationship between cortical excitability and motor function in patients with PD. The effects of levodopa therapy can be divided into short duration response (SDR) and long duration response (LDR) in which the SDR is the transient improvement in motor function lasting minutes to hours that occurs after each dose of levodopa and the LDR, an anti-parkinsonian action that decays over hours-days. Hence the present study examined the cortical excitability and motor function associated with the SDR and LDR of levodopa therapy in order to gain further insights into the significance of cortical excitability changes in PD. Specifically, the SDR was assessed by examining the motor function and cortical excitability in the OFF-medication state and 1 hour after levodopa while LDR was assessed by examining the time course of changes in motor function and cortical excitability during prolonged withdrawal of levodopa.

Importantly, the present study sought to investigate whether a single dose of levodopa therapy exerts an influence on cortical excitability and if so, whether a relationship exists between the changes in cortical excitability and changes in objective and

clinical measures of motor function. To date, threshold tracking paired-pulse TMS has not been utilised in the study of cortical excitability in PD patients and hence the pattern of cortical excitability changes obtained from the present study would provide further insights into the significance of these changes.

5.2 METHODS

5.2.1 Experiment 1 – Short duration response to levodopa

Subjects

Studies were undertaken in a convenience sample of 19 non-tremor-dominant PD patients recruited from the Movement Disorders Unit, Westmead Hospital, Sydney Australia (14 males, 5 females, median age 61.7 years (age range 46-74), See Table 5-1). All patients satisfied the United Kingdom Parkinson's Disease Society Brain Bank clinical diagnostic criteria for PD [126]. The accuracy of the PD diagnosis was proven by subsequent long-term clinical follow-up and positive response to levodopa treatment. Studies were performed in a practically defined off state in the morning after overnight withdrawal of medications, at least 12 hours after the last dose and then repeated on the same morning 1 hour after patients were administered their usual first dose of levodopa therapy. All patients gave informed consent to the procedures, which were approved by the Sydney Western Area Health Service Human Research Ethics Committee.

Patient no.	Age/ Sex	Disease duration (Years)	Dominant hand	More-affected side	H & Y stage	MDS-UPDRS OFF	Medication (daily dose)
1	54/M	6	Left	Left	1	12	LD 450mg + Pramipaxole 0.375mg
2	46/M	3	Right	Left	2	24	LD 600mg + Entacapone + Pramipaxole ER 0.375mg
3	71/F	21	Right	Left	3	33	LD 1000mg
4	56/F	8	Right	Right	2	19	LD 1000mg + Pramipaxole 1.5mg
5	67/M	8	Right	Left	2	24	LD 750mg
6	49/M	3	Right	Left	1	28	LD 750mg
7	59/M	9	Right	Right	4	35	LD1000mg
8	65/F	11	Right	Left	2	12	LD 500mg + Entacapone + Pramipaxole 4mg + Apomorphine 0.6mg mane
9	74/M	3	Right	Left	2	10	LD 600mg
10	58/F	2	Left	Left	1	17	LD 450mg
11	70/F	11	Right	Left	2	27	LD 400mg
12	48/M	6	Ambidextrous	Right	1	7	LD 600mg + Pramipaxole 1.5mg
13	73/M	7	Right	Right	2	32	LD 1000mg + Pramipaxole 4.5mg
14	74/M	3	Right	Left	1	33	LD 300mg
15	65/M	9	Right	Right	2	8	LD 1050mg + Pramipaxole 0.25mg
16	72/M	6	Right	Right	1	21	LD 1125mg
17	51/M	6	Righjt	Left	1	8	LD 200mg + Pramipaxole 4.5mg
18	72/M	3	Right	Right	1	40	LD 450mg
19	49/M	5	Right	Right	1	6	LD 550mg + Rasagiline 1mg

Table 5-1 Patient characteristics. LD denotes levodopa and doses indicate total daily doses. Hand dominance was determined using the Edinburgh Handedness Index.

Experimental set up

Each subject underwent a set of assessments before and after their usual morning levodopa medication in the following order.

- i. Clinical assessment of PD
- ii. Objective assessment of bradykinesia and rigidity
- iii. Cortical excitability with threshold tacking paired-pulse TMS

All neurophysiological assessments were made on the more-affected side as assessed clinically and in those with symmetrical disease, the side that first manifested symptoms was considered as the more affected side. Each set of assessments was completed within 2 hours.

Clinical Assessment of PD

Each subject was clinically assessed using Part III (motor section assessment) of Movement Disorder Society - Unified Parkinson's Disease Rating Scale (MDS-UPDRS) [127]. Upper limb bradykinesia scores were calculated for each subject based on the sum of three 4-point measures of finger tapping, hand movements and pronation-supination movements of hands, with a score of 0 indicating absence of bradykinesia, and a score of 12, severe bradykinesia. Rigidity was assessed at the wrist, with 0 being no rigidity and 4 being severe rigidity.

Objective assessments

Rigidity

Objective rigidity was measured using a torque motor as described by Fung et al. [128]. Briefly, subjects were seated comfortably in a dental chair, with their forearms resting in a splint and their hands semi-pronated in a manipulandum and resistive torque was measured using a torque motor (PMI Motion Technologies JR16M4CH-1, Commack, NY, USA), which was operated as a position servo to generate sinusoidal perturbations of 60° peak-to-peak amplitude about the 0° position. Ten non-contiguous 5-sec epochs were recorded for each objective rigidity measurement and during each 5-sec epoch, a 3-sec interval of oscillation at 1.0 Hz was either preceded or followed by a contiguous 2-sec interval of oscillation at 1.5 Hz. The number of epochs beginning with each frequency was equal and the order of presentation of epochs varied in a pseudorandom fashion. Resistive torque was rectified and integrated with respect to time, yielding the scalar equivalent of angular impulse referred to as angular impulse score [128].

Bradykinesia

Objective bradykinesia was measured with a timed tapping task as described in Chapter 3. The subjects tapped two touch pads positioned 20 cm apart with one hand as fast as possible for 60 seconds and the total number of taps, i.e., tapping speed (TS), was recorded before and after levodopa. The rate of change in hand tapping speed was calculated for each patient by working out the slope of change in hand tapping frequency. As a negative slope indicated decrement in hand tapping frequency, this was converted to a positive value and referred to as *decrement*.

Cortical excitability

Cortical excitability was assessed by measuring the resting motor threshold (RMT), magnetic evoked potential (MEP) amplitude, short interval intracortical inhibition (SICI), intracortical facilitation (ICF) and cortical silent period (CSP).

Briefly, threshold tracking TMS was adapted to the paired-pulse paradigm to investigate SICI in which a subthreshold (70% resting motor threshold, RMT) conditioning stimulus preceded a suprathreshold test stimulus. The interstimulus intervals (ISIs) between the conditioning and test stimuli were 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 7 ms. Intracortical facilitation was assessed at ISIs 10,15,20,25 and 30 ms. For the purposes of threshold tracking, resting motor threshold (RMT) was defined as the stimulus intensity required to produce a 0.2-mV (peak-to-peak) magnetic evoked potential (MEP) amplitude. Stimuli were delivered sequentially as a series of three channels recorded the following: channel 1 tracked the stimulus intensity required to produce the unconditioned test response (i.e., RMT); channel 2 monitored the MEP response to the subthreshold conditioning stimulus; and channel 3 tracked the stimulus required to produce the target MEP when conditioned by a stimulus equal in intensity to that on channel 2. Stimuli were delivered every 5 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 was measured as the increase in the test stimulus intensity required to evoke the target MEP. Facilitation was measured as the decrease in the conditioned test stimulus intensity required to evoke the target MEP.

Cortical silent period refers to a period of electrical silence in a contracting muscle, and was measured from the onset of the MEP to the return of EMG activity with magnetic stimulus intensity set to 140% RMT [26, 49].

5.2.2 Experiment 2 – Long duration response to levodopa

Long duration response of levodopa was examined in 3 patients with early PD (Table 5-2). Baseline motor and cortical excitability assessments were made after an overnight withdrawal of levodopa after which patients were given their usual morning dose of levodopa and measurements were made periodically over days until there was a sustained decline in motor function. On Day 1, measurements were made at 30-minute intervals for the first 4 hours, then 60-minute intervals for the next 4 hours, and from Day 2, measurements were made twice daily approximately 6 hours apart, e.g. 8am and 4pm, until there was a sustained decline in motor function. Cortical excitability was compared between the more and less affected cerebral hemispheres as well as being correlated with hand tapping speed. Because of the need to acquire data in a timely manner in order to accommodate making repeated measurements every 30 minutes, a modified TMS protocol was applied in which the interstimulus intervals (ISIs) between the conditioning and test stimuli were selected at 1, 2.5 and 3 ms for SICI and ICF had to be omitted. Further, bradykinesia was measured with hand tapping speed but objective rigidity was not measured. All experimental techniques were otherwise identical to Experiment 1.

Patient No.	Age	Sex	Disease duration	Dominant hand	More affected side	Presenting symptom	Treatment
1	55	M	6 years	Right	Right	akinesia	Levodopa/benserazide 100/25 1½ qid
2	55	F	2 years	Right	Left	Tremor	Levodopa/carbidopa 100/25 1½ tds
3	64	M	2 years	Right	Right	akinesia	Levodopa/benserazide 100/25 1 qid

Table 5-2 Patient characteristics for long duration response to levodopa.

5.3 STATISTICAL ANALYSIS

Cortical excitability in PD patients was compared between the OFF and ON-medication states using the paired samples t-test and these results were compared to 71 healthy controls (36 males, 35 females, mean age 48.3 years, age range 23-83) using one-way ANOVA. Correlations between excitability indices and objective measures of bradykinesia and rigidity were analysed using Spearman's rank correlation coefficient. Results were expressed as mean \pm standard error of the mean and a probability (P) value of less than 0.05 was considered significant.

5.4 RESULTS

5.4.1 Experiment 1

Clinical Staging of Parkinson's Disease

There was a significant difference in MDS-UPDRS Part III scores between the OFF-medication and ON-medication states ($UPDRS_{OFF} = 20.2 \pm 2.5$, $UPDRS_{ON} = 10.8 \pm 2.0$, $UPDRS_{DIFF} = 9.4 \pm 1.4$, $P < 0.0001$, Figure 5-1A), indicating a significant medication effect. Specifically, bradykinesia and rigidity subscales of MDS-UPDRS

showed highly significant differences between OFF and ON-medication states (UPDRS-brady_{OFF} = 6.2 ± 1.0, UPDRS-brady_{ON} = 3.5 ± 0.7, UPDRS-brady_{DIFF} = 2.7 ± 0.6, P = 0.001, Figure 5-1B; UPDRS-rigid_{OFF} = 1.8 ± 0.3, UPDRS-rigid_{ON} = 0.9 ± 0.2, P = 0.001, Figure 5-1C).

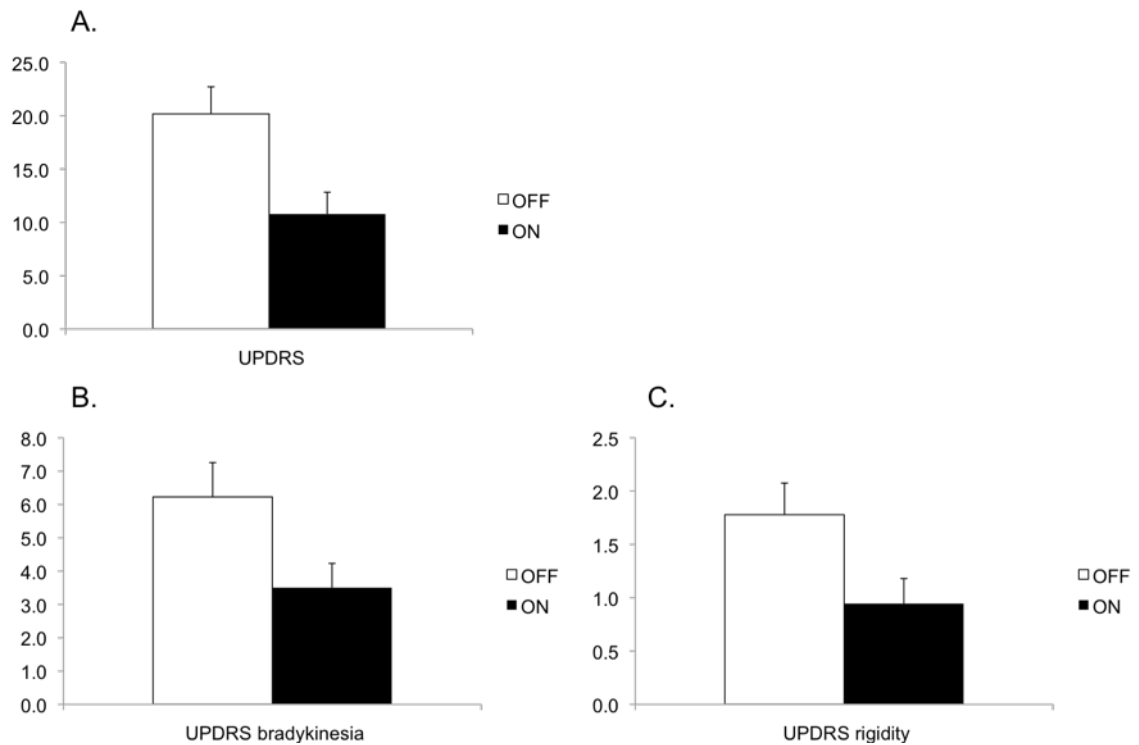


Figure 5-1 Mean MDS-UPDRS scores before and 1 hour after levodopa (A); mean MDS-UPDRS scores for bradykinesia(B) and rigidity(C).

Objective motor assessment

A. Bradykinesia

There was a significant improvement in the hand tapping speed (TS) after medications in line with the UPDRS results (TS_{OFF} = 184.3 ± 12.2, TS_{ON} = 202.3 ± 11.7, TS_{DIFF} = -18.0 ± 4.6, P = 0.001, Figure 5-2), confirming that tapping speed is a reliable measure of bradykinesia as utilized in previous published papers [129]. In the OFF-

medication state, there was greater decrement in hand tapping speed than in the ON-medication state although this was not statistically significant ($\text{Decrement}_{\text{OFF}} = 8.7 \times 10^{-3} \pm 1.4 \times 10^{-3}$, $\text{Decrement}_{\text{ON}} = 5.1 \times 10^{-3} \pm 2.8 \times 10^{-3}$, Mean difference = $3.6 \times 10^{-3} \pm 2.1 \times 10^{-3}$, $P = 0.11$, Figure 5-3).

B. Rigidity

There was no significant improvement in the objective rigidity scores in the ON-medication state despite the fact that there was a significant improvement in UPDRS rigidity scores ($\text{Rigidity}_{\text{OFF}} = 0.242 \pm 0.016$ Nm/sec, $\text{Rigidity}_{\text{ON}} = 0.241 \pm 0.020$ Nm/sec, $\text{Rigidity}_{\text{DIFF}} = 0.001 \pm 0.013$ Nm/sec, $P = 0.92$, Figure 5-3).

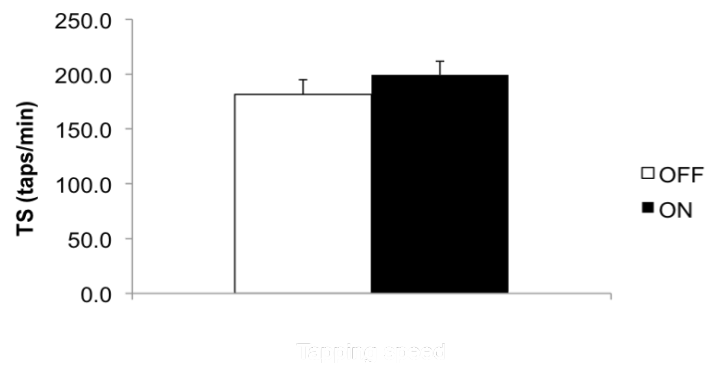


Figure 5-2 Hand tapping speed before and 1 hour after medication

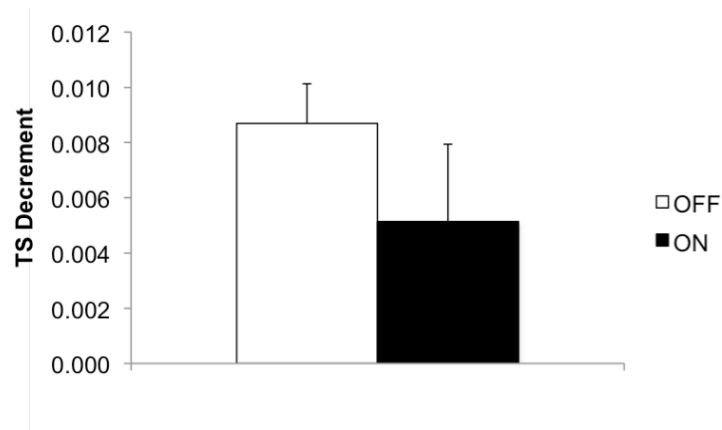


Figure 5-3 Decrement in hand tapping speed 1 hour after levodopa

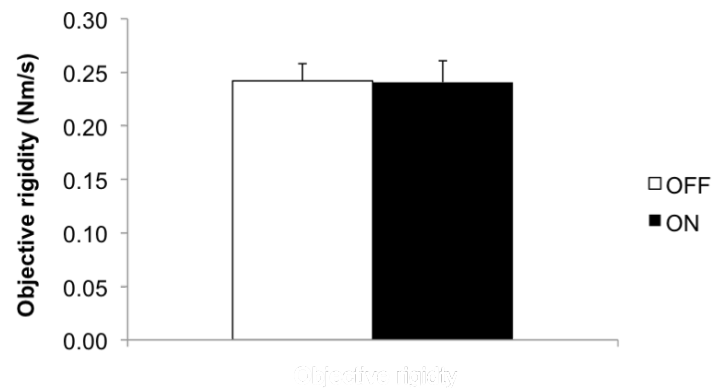


Figure 5-4 Objective rigidity before and 1 hour after medication

Cortical excitability measures

A. MT and MEPs

Resting motor threshold ($RMT_{\text{OFF}} = 49.6 \pm 2.1\%$, $RMT_{\text{ON}} = 50.2 \pm 1.9\%$, $RMT_{\text{DIFF}} = -0.7 \pm 0.8$, $P = 0.41$, Figure 5-5A) and MEP amplitudes ($MEP_{\text{OFF}} = 4.7 \pm 0.4$ mV, $MEP_{\text{ON}} = 4.9 \pm 0.4$ mV, $MEP_{\text{DIFF}} = -0.2 \pm 0.3$ mV, $P = 0.58$, Figure 5-5B) were comparable between the OFF-medication and ON-medication states. One-way ANOVA showed that there was a significant reduction in both MTs ($P = 0.004$) and MEPs ($P < 0.0001$) in the PD patients compared with healthy control subjects.

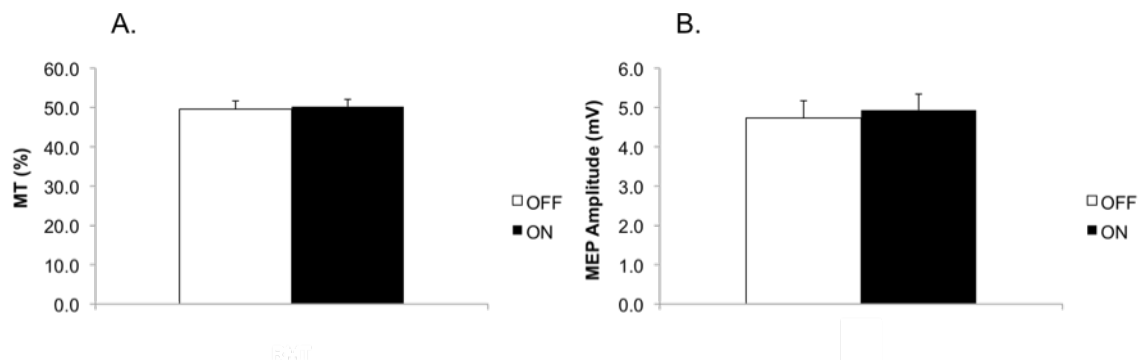


Figure 5-5 Motor threshold (MT) and motor evoked potential (MEP) amplitude before and 1 hour after levodopa

B. CSP

The cortical silent period was similar between the OFF-medication and ON-medication states ($CSP_{OFF} = 202.35 \pm 10.06$ ms, $CSP_{ON} = 201.23 \pm 8.04$ ms, $CSP_{DIFF} = 1.12 \pm 9.62$ ms, $P = 0.91$, Figure 5-6). When compared with the control group, there was no significant difference ($CSP_{OFF} = 202.4 \pm 10.1$ ms, $CSP_{ON} = 201.2 \pm 8.0$, $CSP_{NC} = 211.9 \pm 3.1$ ms, one-way ANOVA $P = 0.28$).

C. SICI

There was no significant difference in SICI between the PD and control subjects ($SICI_{OFF} = 8.26 \pm 1.64$ %, $SICI_{ON} = 8.65 \pm 1.55$ %, $SICI_{NC} = 9.83 \pm 0.69$ %, one-way ANOVA $P = 0.54$) nor was there difference between OFF and ON-medication states ($SICI_{OFF} = 8.26 \pm 1.64$ %, $SICI_{ON} = 8.65 \pm 1.55$ %, $SICI_{DIFF} = -0.39$ %, $P = 0.76$, Figure 5-7).

D. ICF

ICF was reduced in the PD patients compared with the controls with the difference being greater and approaching statistical significance in the OFF than ON-medication state ($ICF_{OFF} = 2.20 \pm 1.39 \%$, $ICF_{ON} = 1.68 \pm 1.21 \%$, $ICF_{NC} = -0.53 \pm 0.72 \%$, one-way ANOVA $P = 0.12$, Figure 5-8), but there was no significant difference in ICF between the OFF and ON-medication states ($ICF_{OFF} = 2.20 \pm 1.39 \%$, $ICF_{ON} = 1.68 \pm 1.21 \%$, $ICF_{DIFF} = 0.52 \pm 1.45 \%$, $P = 0.72$, Figure 5-8).

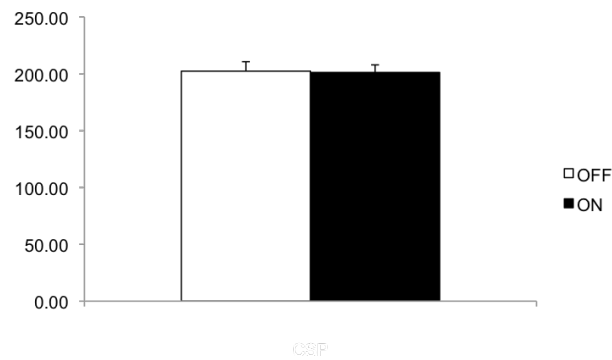


Figure 5-6 CSP before and 1 hour after levodopa.

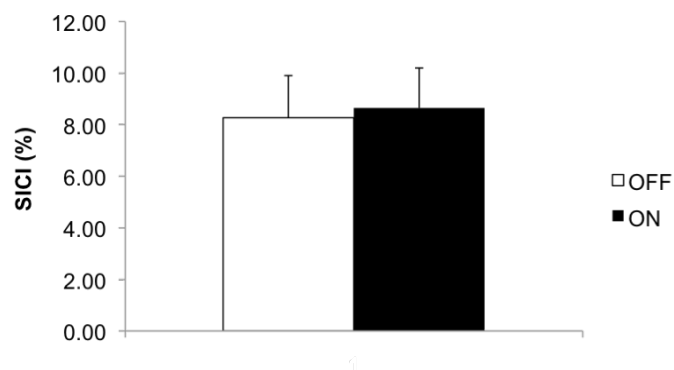


Figure 5-7 SICI before and 1 hour after levodopa.

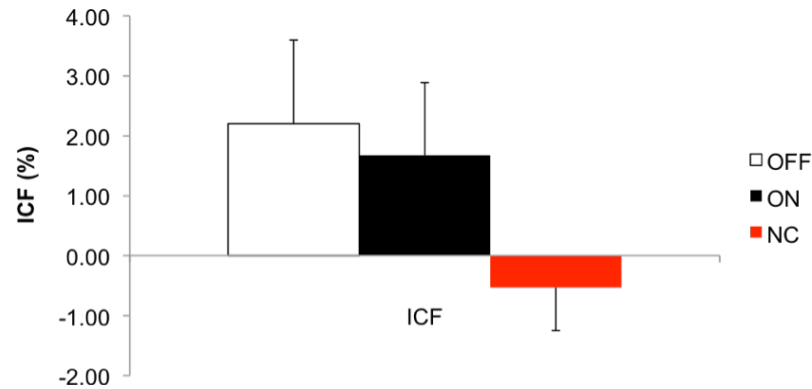


Figure 5-8 ICF before and 1 hour after levodopa

Correlation between clinical and objective measures of motor function

In the OFF-medication state, the correlation between TS_{OFF} and total $UPDRS_{OFF}$ approached statistical significance ($\rho = -0.46$, $P = 0.06$, Figure 5-9A) although there was no significant correlation between TS_{OFF} and bradykinesia sub-scale of MDS-UPDRS (i.e. $UPDRS\text{-}brady_{OFF}$) ($\rho = -0.35$, $P = 0.17$, Figure 5-9B). In the ON-medication state, there was a significant correlation between TS_{ON} and $UPDRS_{ON}$ ($\rho = -0.64$, $P = 0.006$, Figure 5-10A) as well as with $UPDRS\text{-}brady_{ON}$ ($\rho = -0.72$, $P = 0.001$, Figure 5-10B). On the other hand, objective rigidity scores did not correlate with any of the clinical measures of disease severity (MDS-UPDRS), including the bradykinesia and rigidity subscales.

Correlation between motor function and cortical excitability

There was a correlation between SICI and decrement in hand tapping speed (*decrement*) in the OFF-medication state ($\rho = -0.59$, $P = 0.01$, Figure 5-11A) and the same correlation approached statistical significance in the ON-medications state ($\rho = -0.43$, $P < 0.08$, Figure 5-11B). In addition, there was correlation between ICF and

decrement in the OFF-medication state ($\rho = -0.47$, $P < 0.05$, Figure 5-12), but not in the ON-medication state. There was no significant correlation between SICI, ICF and CSP with TS and objective rigidity.

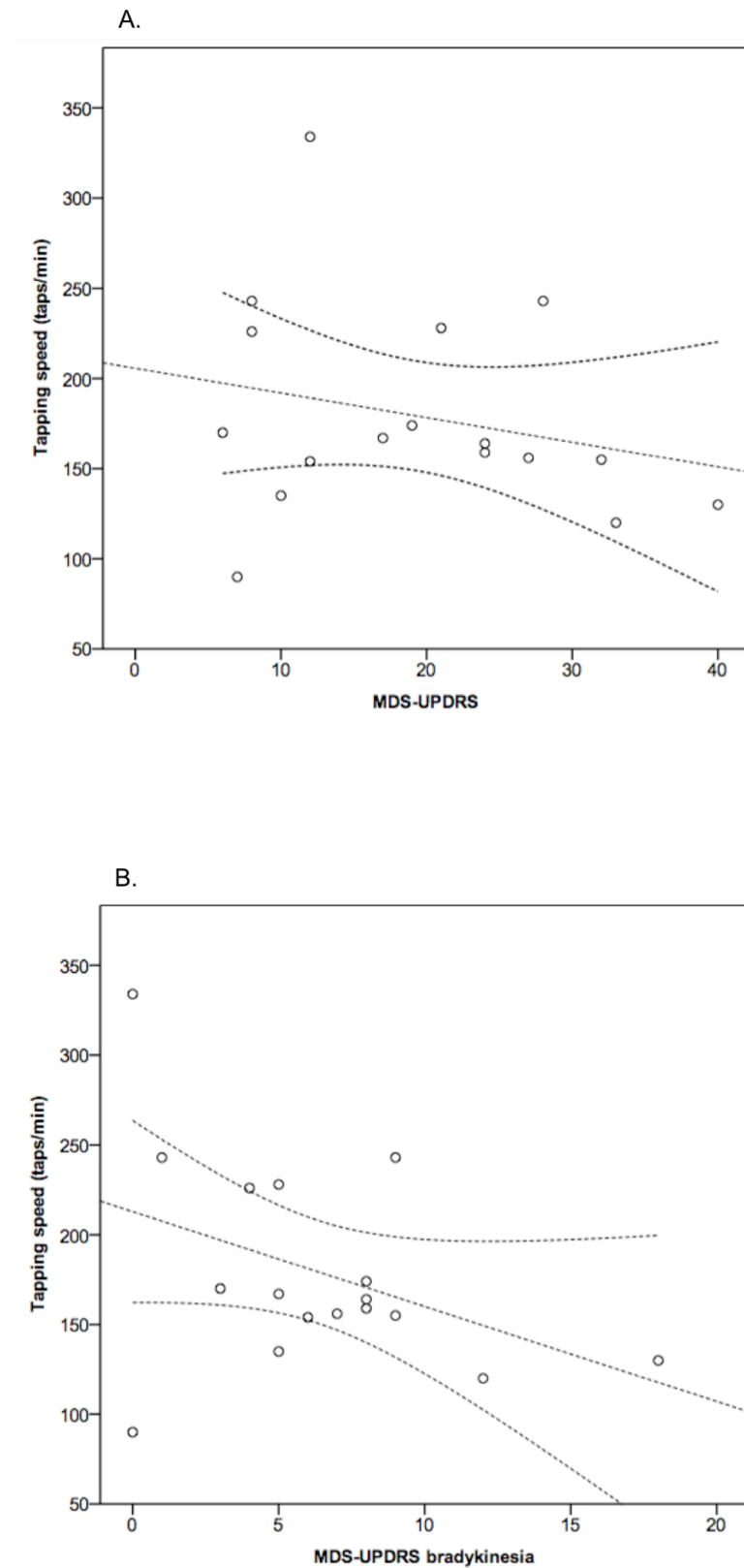


Figure 5-9 Correlation between hand tapping speed and (A) MDS-UPDRS ($P = 0.06$) and (B) bradykinesia subscale of MDS-UPDRS ($P = 0.17$) in the OFF-medication state.

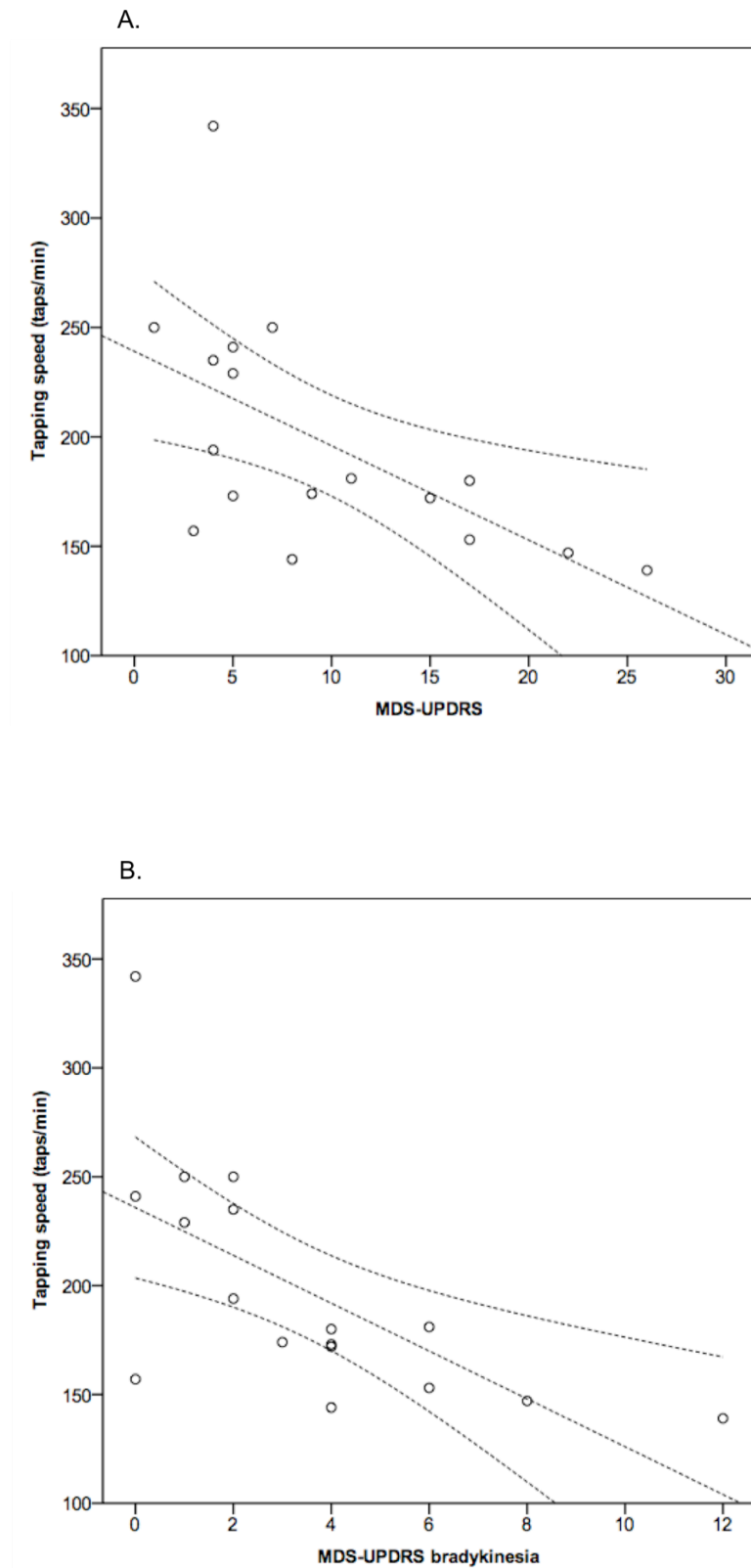


Figure 5-10 Correlation between hand tapping speed and (A) MDS-UPDRS ($P = 0.006$) and (B) bradykinesia subscale of MDS-UPDRS ($P = 0.001$) in the ON-medication state.

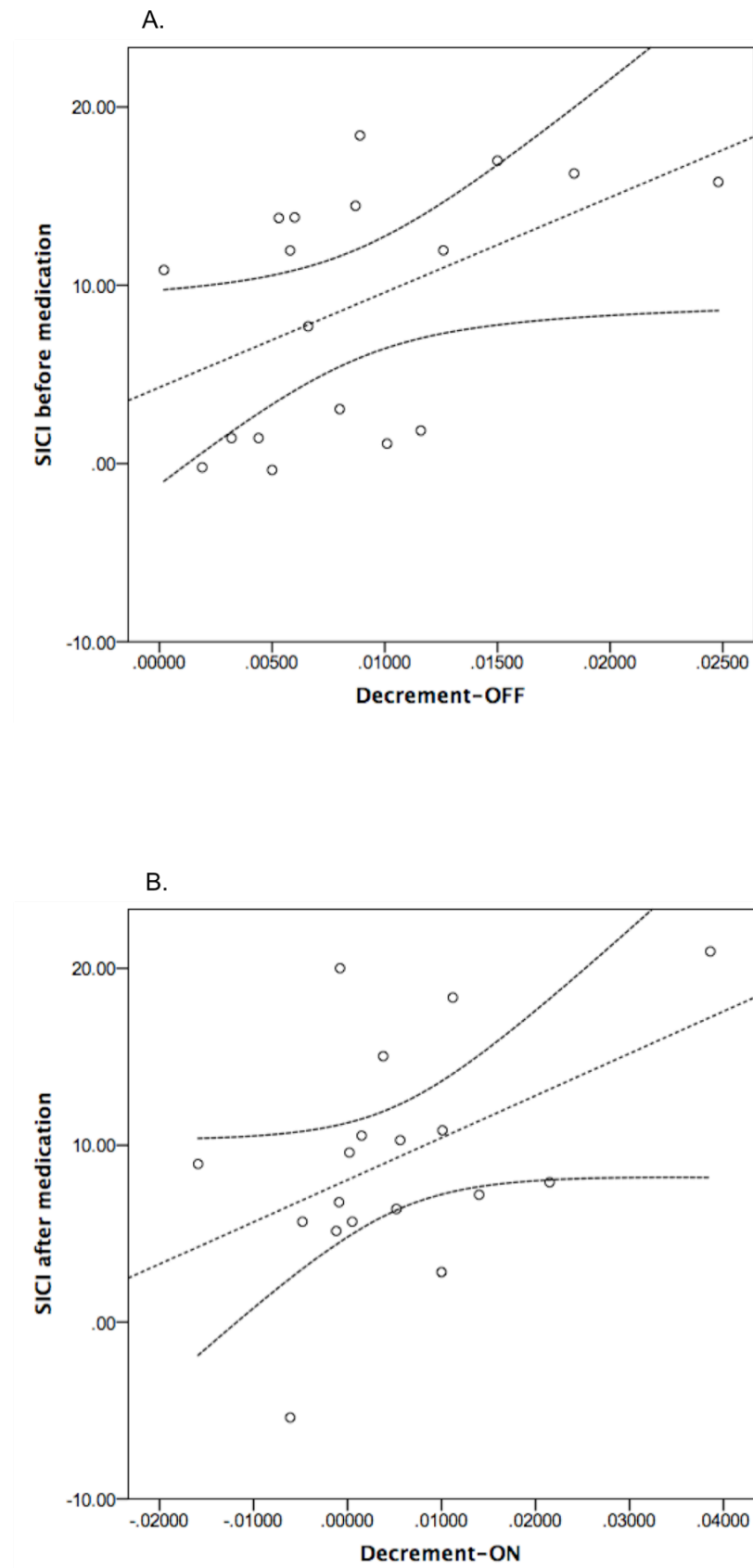


Figure 5-11 Correlation between SICI and (A) decrement in hand tapping speed in the OFF-medication state ($P = 0.01$) and (B) ON-medication state ($P < 0.08$).

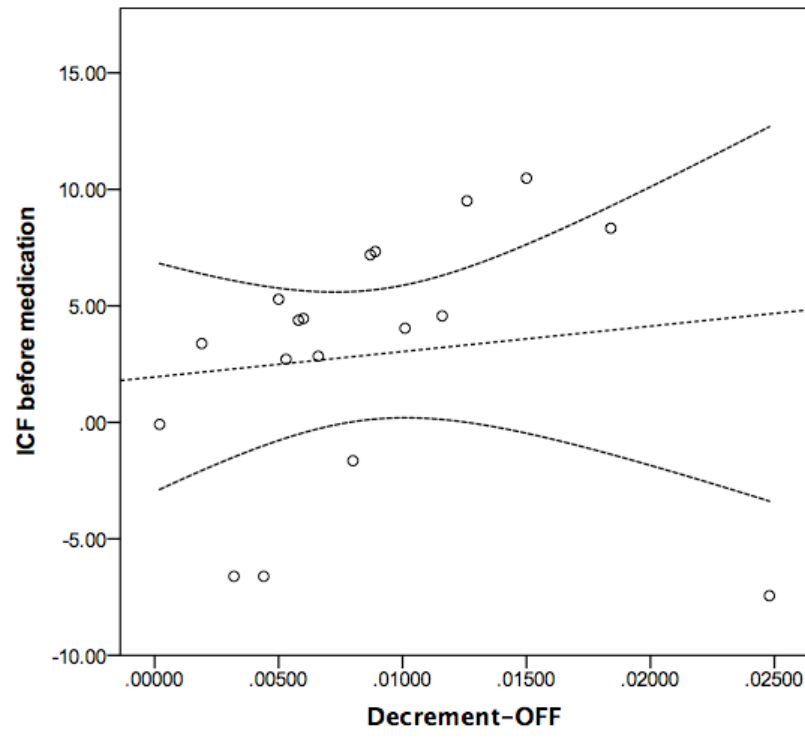


Figure 5-12 Correlation between ICF and decrement in hand tapping speed in the OFF-medication state ($P < 0.05$)

5.4.2 Experiment 2

Assessments were made until day 5 in subject 1, day 26 in subject 2 and day 8 in subject 3. Side-to-side asymmetry and changes in TS reflected asymmetry in akinesia rather than handedness in all three patients.

Objective motor assessment - hand tapping speed

Subject 1

The TS increased transiently for approximately 3.5 hours after levodopa on the more-affected side with the expected wearing off of the SDR whereas the TS returned to the baseline after approximately 8 hours on the less-affected side. Thereafter, the TS

remained stable for 24 hours before decreasing below that of the baseline on the more-affected side while it remained stable on the less-affected side for the duration of the assessment (Figure 5-13).

Subject 2

The TS increased rapidly after LD and thereafter remained elevated and did not return to the baseline until day 24 on the more-affected side although there was a gradual decline over time (Figure 5-14).

Subject 3

There was a transient rise in TS after levodopa. Thereafter the TS gradually reached a plateau after 24 hours on the more-affected side and after 78 hours on the less-affected side (Figure 5-15).

Cortical Excitability - SICI

Subject 1

On the more affected side, SICI transiently increased after levodopa, peaking at 1.25-hours and staying elevated until 3.5-hours following which it progressively decreased over time following the variations in TS (Figure 5-16A). On the less-affected side, SICI appeared to increase after levodopa but the difference was smaller compared with the more-affected side (Figure 5-16B). Comparison between the more and less-affected sides showed significantly reduced SICI on the more-affected side (mean difference = 7.23 ± 2.37 %, $P < 0.05$ – paired samples t test, Figure 16C).

Subject 2

There was a transient increase in SICI after levodopa on both the more and less-affected sides, which peaked over 1-3 hours and appeared to match the increase in TS (Figure 5-17A,B). Thereafter, there were marked fluctuations in SICI although there appeared to be a trend for SICI to remain relatively stable over time. Comparison between the more and less-affected sides showed no significant difference in SICI.

Subject 3

On the more-affected side SICI increased and peaked over 2 hours after levodopa before returning to the baseline over the next 1.5 hours. Thereafter SICI remained relatively stable over time although at times there were marked fluctuations (Figure 5-18A). On the less-affected side, there was no particular pattern of change (Figure 5-18B). There was no significant side-to-side difference in SICI.

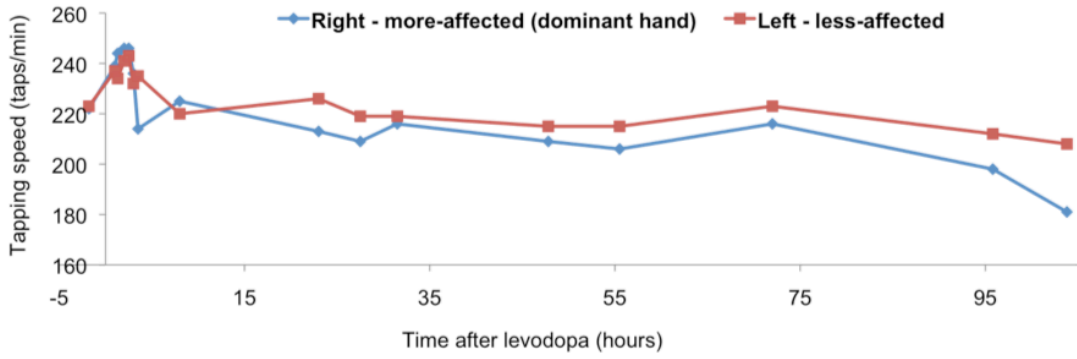


Figure 5-13 Time profile of changes in hand tapping speed for the more and less affected sides – Patient 1.

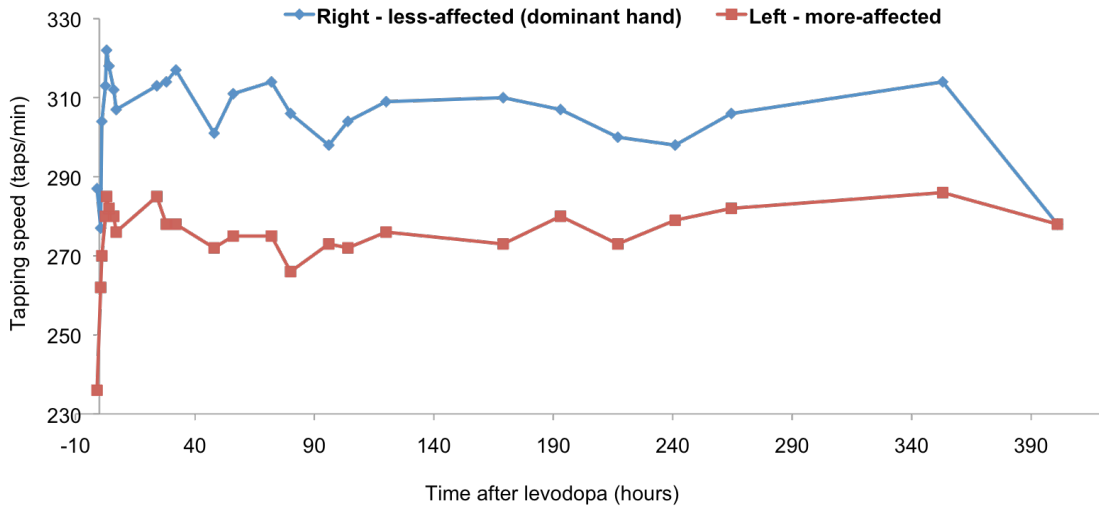


Figure 5-14 Time profile of changes in hand tapping speed for the more and less affected sides – Patient 2.

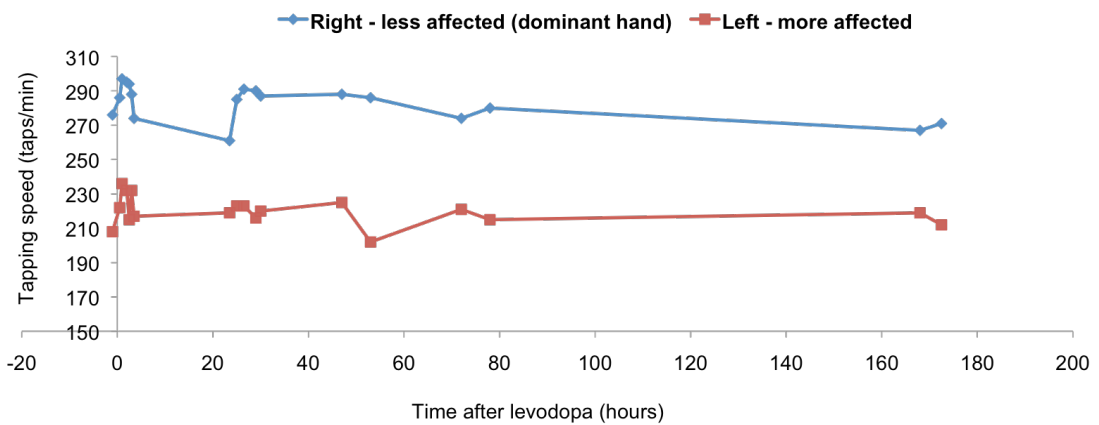


Figure 5-15 Time profile of changes in hand tapping speed for the more and less affected sides – Patient 3.

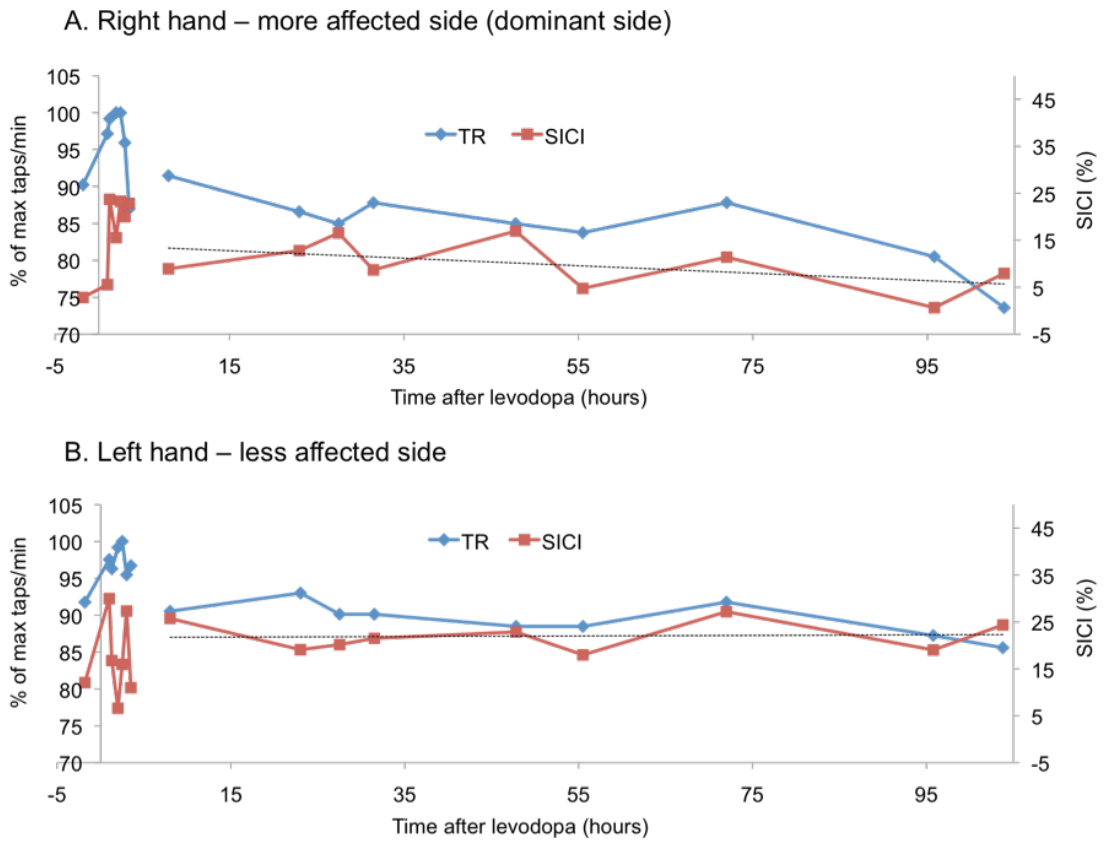


Figure 5-16 Time profile of changes in SICI and hand tapping speed in Patient 1 – more affected side (A), less affected side (B). Note tapping rate was converted into a percentage of the maximum tapping rate and plotted against time. The graph is broken into 2 segments; the short segment reflects the SDR and the long segment reflects the LDR

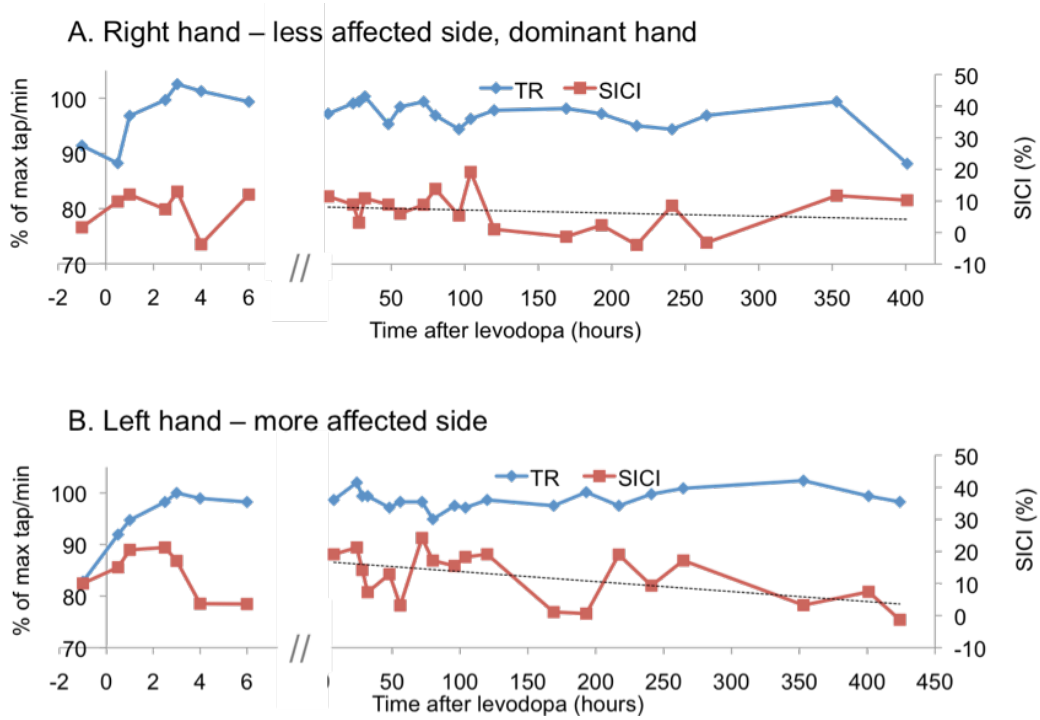


Figure 5-17 Time course of changes in Tapping rate and SICI in Patient 2 – more affected side (A), less affected side (B).

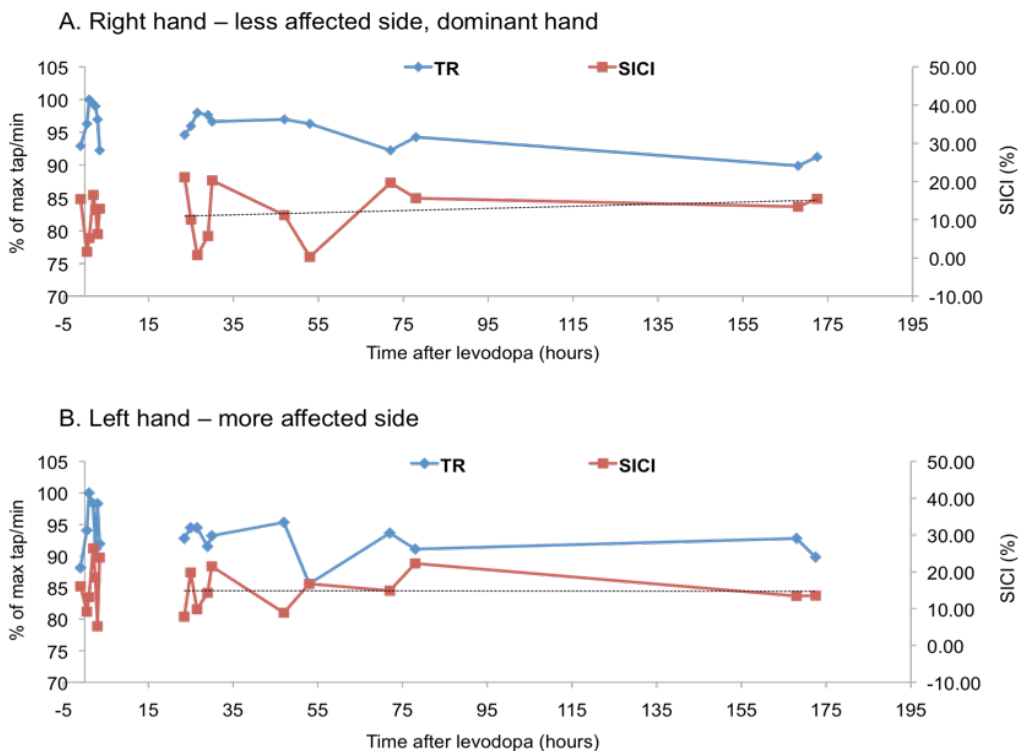


Figure 5-18 Figure 5-19 Time profile of changes in SICI and hand tapping speed in Patient 3 – more affected side (A), less affected side (B).

5.5 DISCUSSION

5.5.1 Experiment 1 - SDR

The key finding from Experiment 1 was that in PD patients, both ICF and SICI correlated significantly with decrement in hand tapping speed in the OFF-medication state and the correlation between SICI and decrement in hand speed approached statistical significance in the ON-medication state ($P < 0.08$). ICF tended to be reduced in the OFF-medication state and subsequently increased towards that of control subjects in the ON-medication state when measured 1-hour after the dose, but there was no significant reduction in SICI in the OFF-medication state nor was there significant effect in the ON-medication state despite there being a clear improvement in clinical and objective measures of bradykinesia.

In the side-to-side study (Chapter 4), patients with PD had relatively reduced SICI on the more affected side compared with the less affected side although there was lack of overall difference in SICI between PD and healthy age-matched control subjects. Given that SICI has been reported as being reduced in PD in a number of studies [7, 11, 30, 32, 33, 74], it is possible that the normal SICI seen in the present study and Chapter 4 could have been due to the patients having relatively mild disease, however a relationship has never been demonstrated between SICI and measures of disease severity in previous cortical excitability studies in PD. Rather, the results from the present study support the alternative finding of normal SICI in PD [31, 72, 73] particularly as these result were replicated using a different technique (threshold tracking paired-pulse TMS) to previous studies, and shed further light on the role of

SICI in the pathophysiology of bradykinesia in PD although the results cannot be generalised to those with advanced PD.

The fact that there was *positive* correlation between SICI and decrement in hand tapping speed implies that SICI tends to be normal in patients with relatively fast decrement in hand tapping speed and reduced in those with slow decrement. Given that parkinsonian bradykinesia is characterized by decrement in speed and amplitude of repetitive movements, the findings from the present study point to reduced SICI being a compensatory phenomenon in which SICI is reduced in order to facilitate voluntary movement production by countering deficits in muscle activation and movement scaling as well as *relative failure of voluntary facilitation* [37], but failure of compensatory decrease in SICI in those with greater decrement. Further, if SICI indeed were a primary phenomenon involved in the pathophysiology of bradykinesia, it would have been expected to match and possibly precede the improvement in motor function after a single dose of levodopa but no such change in SICI was observed, arguing against this possibility. The results support the finding of reduced ICF as well as relatively reduced but overall normal SICI on the more affected side in the side-to-side study (Chapter 4) as being compensatory changes.

A possible explanation for the lack of changes in SICI despite significant improvement in hand tapping speed after a single dose of medication is that alterations in SICI reflect long-term changes in cortical function and hence a single dose of levodopa may not influence the longstanding changes. This would be supported by the fact that the study on Stiff-man syndrome (Chapter 2, [137]) revealed markedly reduced SICI even on high doses of benzodiazepines and in the

absence of any symptoms or evidence of exaggerated exteroceptive reflexes that characterise the condition. Another possibility is that the magnitude of change in hand tapping speed was too small in the present study for differences in SICI to become apparent.

In terms of RMTs and MEPs, there was a significant difference between PD patients and healthy control subjects, which needs further explanation. PD patients had lower RMTs and MEPs, which could be explained by PD patients having increased rigidity akin to maintaining a weak voluntary contraction during assessment of activated motor threshold, but the fact that there was no medication effect on these measures despite significant clinical improvement in rigidity would argue against this possibility and having a significant influence on the results of the present study.

5.5.2 Experiment 2 - LDR

The key finding from Experiment 2 was that there was a relatively consistent transient increase in SICI after a single dose of LD, matching the improvement in hand tapping speed despite the fact that only three patients were assessed. Following this, prolonged assessment revealed mixed findings with Patients 2 and 3 displaying marked fluctuations in SICI over the course of days but Subject 1 displaying progressive reduction in SICI following the variations in TS, reflective of the hand tapping speed. The results suggest that there are short-term alterations in SICI after each dose of LD although it may be difficult to capture this from “one-off” recording given that changes were seen 1-2 hours after LD as well as the fact that there can be marked fluctuations from one recording to the next.

Hence the lack of changes in SICI in Experiment 1 in which SICI was assessed 1-hour after levodopa may have been due to the fact that changes in SICI take time to develop as suggested in Experiment 2, which demonstrated relatively consistent increase in SICI after levodopa on the more affected side but the time course of this change differed amongst patients and usually peaked over 1-3 hours. Therefore it is possible that Experiment 1 failed to capture significant changes in SICI simply because of the timing of the assessment.

Interestingly, there were mixed changes in SICI over the course of days, with Patients 2 and 3 demonstrating fluctuations although overall there appeared to be a trend for SICI to progressively decrease over time. The changes in SICI were more prominent on the more affected side compared with the less affected side in agreement with the results from Chapter 3, which demonstrated significant relative reduction in SICI on the more affected side compared with the less affected side. The results from the present study suggest that SICI changes over minutes-hours after levodopa reflective of SDR of levodopa and that it may follow rather than precede the motor improvement as improvement in the hand tapping speed was not associated with changes in SICI in Experiment 1. Overall, the results from the present study point to reduced SICI being a compensatory change.

5.5.3 Limitations

Experiment 2 was limited by the small number of patients and findings need to be replicated in a larger number of patients but this was a difficult study to perform on two accounts. Firstly it is a time consuming and technically challenging study, as patients need to be assessed over a number of days and each block of assessments

needs to be completed within 30 minutes. Secondly, patients need to be willing to stop their medications for a prolonged period of time and make themselves available for several days in a row, which essentially excludes those who are in employment, i.e. relatively young patients with PD. In addition, time course of changes in ICF after levodopa was not addressed in this study and future studies assessing the time course of changes in ICF would provide further insights into the significance of cortical excitability changes in PD.

5.6 CONCLUSION

The findings suggest that reduced SICI is a secondary compensatory rather than a primary pathophysiological change and is involved in modifying the rate of decrement in repetitive movements that characterise parkinsonian bradykinesia.

**Chapter 6 CORTICAL EXCITABILITY IN
PATIENTS WITH PARKINSON'S DISEASE ON
LEVODOPA CARBIDOPA INTESTINAL GEL
THERAPY**

Abstract

This study assessed whether there are corresponding changes in cortical excitability with changes in motor function and plasma levels of levodopa in advanced PD patients. Six patients who have been established on levodopa carbidopa intestinal gel (LCIG) therapy for more than 12 months underwent assessment of their cortical excitability and clinical objective measures of bradykinesia and rigidity as well as plasma levodopa levels. Studies were performed under two conditions, i.e. with the LCIG pump OFF then ON and serial measurements being made every 30 minutes. As expected there was rapid decline in plasma levodopa levels but there were no significant reduction in hand tapping rate. SICI was significantly reduced in PD patients and progressively became more significant at each subsequent time points. There was a statistically significant correlation between mean SICI and mean levodopa concentration, but there was no correlation between mean SICI and mean hand tapping speed or between mean levodopa concentration and mean hand tapping speed. The results from this study suggest that SICI is reduced in patients with severe PD and in addition SICI positively correlates with plasma levodopa levels. Taken together, the findings suggest that SICI is influenced by the disease severity as well as plasma levodopa levels.

6.1 INTRODUCTION

Short interval intracortical inhibition (SICI) has been extensively investigated in both the OFF and ON-medication states in PD but the findings have been somewhat inconsistent. A number of groups have reported reduced SICI in the untreated or OFF-medication state particularly in those with more advanced disease, and further that the reduced SICI normalises with treatment with dopaminergic therapy [7, 30, 74, 78, 138] whilst other studies have failed to detect any changes in SICI [31, 72] or any improvement with dopaminergic therapy. This inconsistency is most likely due to the fact that the studies have been undertaken using different TMS techniques as well as non-uniform patient populations with varying disease stage and severity. In Chapter 4 in which cortical excitability was compared between the more and less clinically affected sides, ICF was significantly reduced in PD patients compared with normal control subjects while SICI was significantly reduced on the more affected side although still normal when compared with normal control subjects. Of importance, the side-to-side asymmetry in SICI correlated with corresponding side-to-side asymmetry in hand tapping speed, thereby suggesting a possible role of reduced SICI in the pathophysiology of asymmetry in bradykinesia. In Chapter 5, which examined the effect of levodopa therapy on cortical excitability, the findings from the first study were extended. Specifically, there was significant correlation between SICI and decrement in hand tapping in the OFF-medication state with the same correlation approaching significance in the ON-medication state. Of note SICI was not significantly reduced in PD patients in the OFF-medication state and oral levodopa did not alter the SICI when measured after 1 hour despite the fact that there was significant improvement in motor function as measured by MDS-UPDRS and timed hand tapping speeds. There are two plausible explanations for the lack of differences

in SICI between the PD and normal control subjects. The first is that the cohort of PD subjects in our studies had relatively mild disease and therefore the magnitude of changes in SICI was difficult to detect, particularly as it has been suggested to be more pronounced in those with more severe disease [138] although there is some evidence for reduced SICI in PD patients with relatively mild disease [78]. The second explanation is that levodopa does not exert acute changes in cortical excitability although there are previous reports of alteration in cortical excitability with both acute and chronic dopaminergic therapies [7, 28, 45, 130, 131].

An interesting phenomenon in the therapeutics of PD is the long duration response (LDR). The lack of fluctuation in patients with early PD treated with levodopa is attributed to the LDR, an antiparkinsonian action that decays over hours to days [129]. It has been suggested that disease progression with wearing-off fluctuations appear when the LDR begins to shorten thereby uncovering the short duration response (SDR), which is dependent on plasma levodopa concentration [123, 129]. In the advanced stages of PD, the LDR becomes progressively reduced and patients become increasingly dependent on the SDR for motor symptom control and therefore the pharmacokinetics of levodopa becomes a critical factor for stable motor control as fluctuations in plasma concentrations of levodopa are accompanied by fluctuations in motor performance [139, 140]. One of the strategies against motor fluctuations is to extend the response to each dose of levodopa with catechol-O-methyl transferase (COMT) inhibitors and monoamine oxidase-B (MAO-B) inhibitors, whereby elimination half-life of levodopa is increased, thus prolonging the availability of levodopa to the brain [141, 142], i.e. extending the SDR. However, it has been demonstrated that the main hurdle in achieving a stable plasma level of levodopa in

PD is delayed gastric emptying, which interferes with the delivery of oral levodopa to the jejunum, where it is absorbed into the blood stream. Importantly, PD subjects with motor fluctuations have gastric emptying half-times which are more than double those of PD subjects without motor fluctuations [143], but steady plasma levels of levodopa can be achieved with duodenal administration of LD with corresponding reduction in motor fluctuations [144]. Levodopa carbidopa intestinal gel (LCIG) therapy delivers levodopa directly to the proximal jejunum via a percutaneous gastrojejunostomy tube using a portable infusion pump and the pharmacokinetic studies have demonstrated that steady plasma levodopa levels similar to those achieved with IV levodopa administration can be achieved [145, 146]. Indeed LCIG therapy has been proven to be an effective therapy for advanced PD patients experiencing motor fluctuations and dyskinesia and the results from some centres have been in the order of those seen with deep brain stimulation [147, 148]. Given that LCIG therapy can provide stable and predictable plasma concentrations of levodopa, it offers a unique opportunity in which to gain further insights into cortical excitability changes in advanced PD. Specifically, it provides the ability to assess the relationship between cortical excitability and plasma levodopa concentration and further, the relationship between these measures and motor function, which would help to gain further insights into the significance of cortical excitability changes in PD pathophysiology. Therefore, the present study assessed the time course of changes in SICI and motor function in relation to plasma levodopa levels in advanced PD patients who have been stabilised on LCIG therapy. The patients were first assessed with the LCIG pump ON, following which LCIG was switched OFF and serial measurements were made to document the time profile of changes in plasma levodopa level, cortical excitability changes and motor function.

6.2 HYPOTHESES

- i. There are corresponding changes in cortical excitability with changes in motor function and plasma levels of levodopa in advanced PD patients.
- ii. The relationship between motor function and cortical excitability are more prominent in PD subjects with advanced disease, i.e. those who have lost the LDR.
- iii. Changes in cortical excitability precede changes in motor function.

6.3 METHODS

6.3.1 Subjects

Studies were undertaken in 6 non-tremor dominant advanced PD patients treated with LCIG therapy recruited from the Movement Disorders Unit at Westmead Hospital, Sydney Australia (6 males, median age 64 years (age range 59-67), See Table 6-1. All patients satisfied the United Kingdom Parkinson's Disease Society Brain Bank clinical diagnostic criteria for PD [126]. The accuracy of the PD diagnosis was proven by long-term clinical follow-up and positive response to levodopa treatment.

Patient no.	Sex	Age	Disease duration (Years)	Dominant hand	More affected side	UPDRS-OFF	H&Y stage	LGIC dose	Time on LCIG therapy
1	M	64	15	R	L	24	2.5	CR 4.0 mL/h 6am-10pm; MD 1.5 mL	1 Year
2	M	59	12	L	L	46	4	CR 5.7 mL/h 6am-10pm; MD 5.7 mL	1 Year
3	M	62	23	R	L	10	2	CR 2.4 mL/h 6am-10pm MD 8.0 mL	1 Year
4	M	66	32	R	L	8	2.5	CR 4.8 mL/h 6am-10pm; MD 11 mL	2 Years
5	M	67	11	L	R	73	4	CR 6.3 mL/h 6am-10pm; MD 5 mL	2 Years
6	M	66	16	R	L	13	2	CR 4.3 mL/h 6am-10pm MD 13mL	3 years

Table 6-1 Patient characteristics.

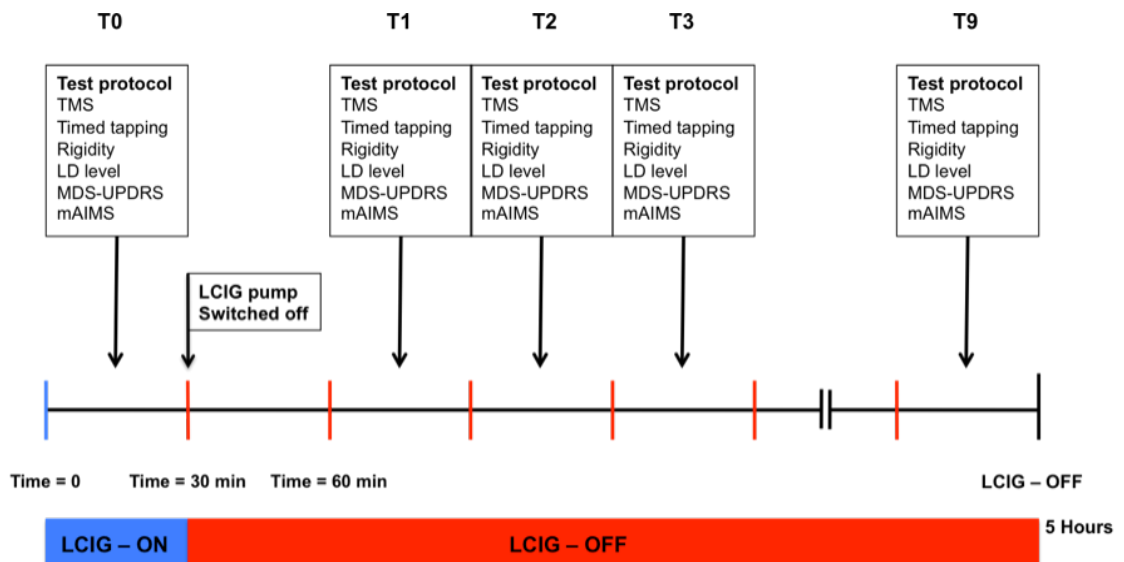


Figure 6-1 Experimental procedures. LCIG – levodopa carbidopa intestinal gel; LD – levodopa; mAIMS – modified Abnormal Involuntary Movement Scale.

6.3.2 Experimental procedure

Studies were performed under two conditions:

LCIG pump *ON* then

LCIG pump *OFF*

Each patient underwent a set of clinical and neurophysiological assessments every 30 minutes (i.e. 30 minute blocks), comprising of the following measures:

TMS – Bilateral cerebral cortex

Timed hand tapping – (Bilateral)

Objective rigidity – (Unilateral)

Plasma levodopa level

MDS-UPDRS (rigidity/bradykinesia 3.3-3.8) and modified AIMS (items 5-7)

The first set of assessments was made with the LCIG pump ON, following which the LCIG pump was switched OFF and serial assessments were made every 30 minutes for 4 hours or until patients could no longer tolerate being in the OFF-medication state and parkinsonian (Fig. 6-1). All patients gave informed consent to the procedures, which were approved by the Sydney Western Area Health Service Human Research Ethics Committee.

6.3.3 Clinical assessment of disease severity

Each subject was assessed clinically using the Part III (motor section assessment) of Movement Disorder Society - Unified Parkinson's Disease Rating Scale (MDS-UPDRS), which was adapted to the study by examining only the upper limb bradykinesia and rigidity [127]. Upper limb bradykinesia scores were calculated for each subject based on the sum of three 4-point measures of finger tapping, hand movements and pronation-supination movements of hands, with a score of 0 indicating absence of bradykinesia, and a score of 12, severe bradykinesia. Rigidity was assessed at the wrists, with 0 being no rigidity and 4 being severe rigidity.

Modified AIMS (mAIMS), a validated 5-point scale was used to assess dyskinesia from three body regions, including upper and lower extremities and trunk. Each body region was rated on a 5 point anchored scale:

0 – None

1 – Minimal

2 – Mild

3 – Moderate

4 – Severe

This resulted in a maximum score of 12 for the three body regions. A score of 3 was taken as being minimal dyskinesia and 9 as moderate dyskinesia.

6.3.4 Cortical excitability

Cortical excitability was assessed by measuring the resting motor threshold (RMT), magnetic evoked potential (MEP) amplitude and short interval intracortical inhibition (SICI). Intracortical facilitation (ICF) was not assessed for the purpose of this study.

Briefly, threshold tracking TMS was adapted to the paired-pulse paradigm to investigate SICI in which a subthreshold (70% resting motor threshold, RMT) conditioning stimulus preceded a suprathreshold test stimulus. The interstimulus intervals (ISIs) between the conditioning and test stimuli were limited to 1, 1.5, 2, 2.5, 3 ms in order to assess the SICI in a timely manner and allow each set of recordings to be completed within a 30 minute block. For the purposes of threshold tracking, resting motor threshold (RMT) was defined as the stimulus intensity required to produce a 0.2-mV (peak-to-peak) magnetic evoked potential (MEP) amplitude. Stimuli were delivered sequentially as a series of three channels: channel 1 tracked the stimulus intensity required to produce the unconditioned test response (i.e., RMT); channel 2 monitored the response to the subthreshold conditioning stimulus; and channel 3 tracked the stimulus required to produce the target MEP when conditioned by a stimulus equal in intensity to that on channel 2. Stimuli were delivered every 5 seconds and the computer advanced to the next ISI only when tracking was stable. SICI was measured as the increase in the test stimulus intensity required to evoke the target MEP.

6.3.5 Hand tapping speed

Objective bradykinesia was measured with a timed tapping task as described in chapter 3. The subjects were asked to tap two touch pads positioned 20 cm apart as fast as possible for 60 seconds with one hand at a time and tapping speed (TS, taps/minute) was recorded for each hand.

6.3.6 Plasma levodopa concentration

Proteins in plasma were precipitated with TCA (5%) and plasma cleared by centrifugation at $10,000 \times g_{av}$ for 5 minutes and filtration of supernatant through a $0.22\mu\text{M}$ filter. The supernatant was then diluted 1 in 10 with water and HPLC analysis of levodopa performed on a LC-10A HPLC system (Shimadzu). System operation was automated by Class LC-10 software. Chromatography was on a Zorbax ODS column (250 mm x 4.6 mm) with a Pelliguard guard column (LC-18). The mobile phase was a gradient of solvent A (10 mM sodium phosphate buffer pH 2.5 with 100 mM sodium perchlorate) and solvent B (80% v/v methanol) at a flow rate of 1 ml/ min. The following gradient was used: isocratic elution with 84% A for 12 minutes then to 80% A over 8 minutes; elution at 80% A for 3 minutes before changing to 50% A in 3 minutes; isocratic elution at 50% A for a further 3 minutes then re-equilibration with 100% A for 10 minutes. The eluate was monitored by UV (Shimadzu, SPD-M10A) and fluorescence detectors (Shimadzu, RF-10AXL). The fluorescence detector was set at an excitation wavelength of 280 nm and an emission wavelength of 320 nm. The elution position of levodopa was defined on the basis of a purified standard. Confirmation of peak identity was obtained by spiking plasma samples with purified levodopa and observing an increase in the area under the curve

of the identified peak. A standard curve was constructed that showed a linear relationship between the area under the curve of the levodopa peak on the HPLC chromatogram and the concentration of standard injected. Levodopa concentration in the samples was then quantified from the standard curve. An additional standard was run every third sample.

6.3.7 Data analysis

All assessments from individual patients were grouped into 30-minute blocks according to the elapsed time from the start of the initial assessment with pump ON at time zero. Accordingly, “T0” was designated to indicate the time period from 0 to 29 minutes and all assessments made in this time period was grouped into T0. As the LCIG pump was switched off after this period and the next set of measurements were made 30 minutes after switching off, “T1” denoted the time period between 60 minutes and 89 minutes from time zero. Accordingly, “T2” denoted the time period between 90 and 119 minutes and “T4”, the time period between 150 and 179 minutes from time zero (Figure 6-1). Due to the small number of patients, both the right and left sided assessments were pooled together for the analysis.

6.4 STATISTICAL ANALYSIS

Repeated measures ANOVA analysis was the most suitable statistical method for the study, but this could not be used because each set of measurements could not be completed within the allocated 30 minute blocks due to patient related and technical factors. Therefore differences in group means at each time point were compared with each other as well as with 71 healthy control subjects (36 males, 35 females, mean

age 48.3 years, age range 23-83) using a one-way ANOVA. Multiple comparisons were conducted with Tukey's post-hoc analysis. Correlations between excitability parameters and clinical and objective measures of bradykinesia and rigidity were analysed by Spearman's rank test. A probability (P) value of less than 0.05 was considered statistically significant. Results are expressed as mean \pm standard error of the mean.

6.5 RESULTS

Three out of six (3/6) patients had mild-moderate dyskinesia at T0 (mean mAIMS = 5.3, range 1-8) but this only precluded one patient from undergoing the TMS studies at T0 because of excessive EMG artefact. Only two out of six (2/6) patients were able to continue with the data collection beyond 150 minutes from when the LCIG pump was switched off, i.e. T4 (180 minutes from time zero) because of inability to tolerate being OFF-medication and parkinsonian although neither the MDS-UPDRS nor hand tapping speeds changed significantly. However, the two patients who were able to tolerate being OFF-medication continued up to T8 and T9.

6.5.1 Clinical measures of disease of severity (modified MDS-UPDRS)

There were no significant differences in group means in MDS-UPDRS scores between different time points ($F_{6,47} = 0.875$, $P = 0.521$, Figure 6-2A). Further, there were no significant differences in group means in the bradykinesia subscale of MDS-UPDRS, ($F_{6,47} = 0.776$, $P = 0.593$, Figure 6-2B) as well as the rigidity subscale ($F_{6,47} = 0.901$, $P = 0.502$, Fig. 6-2B).

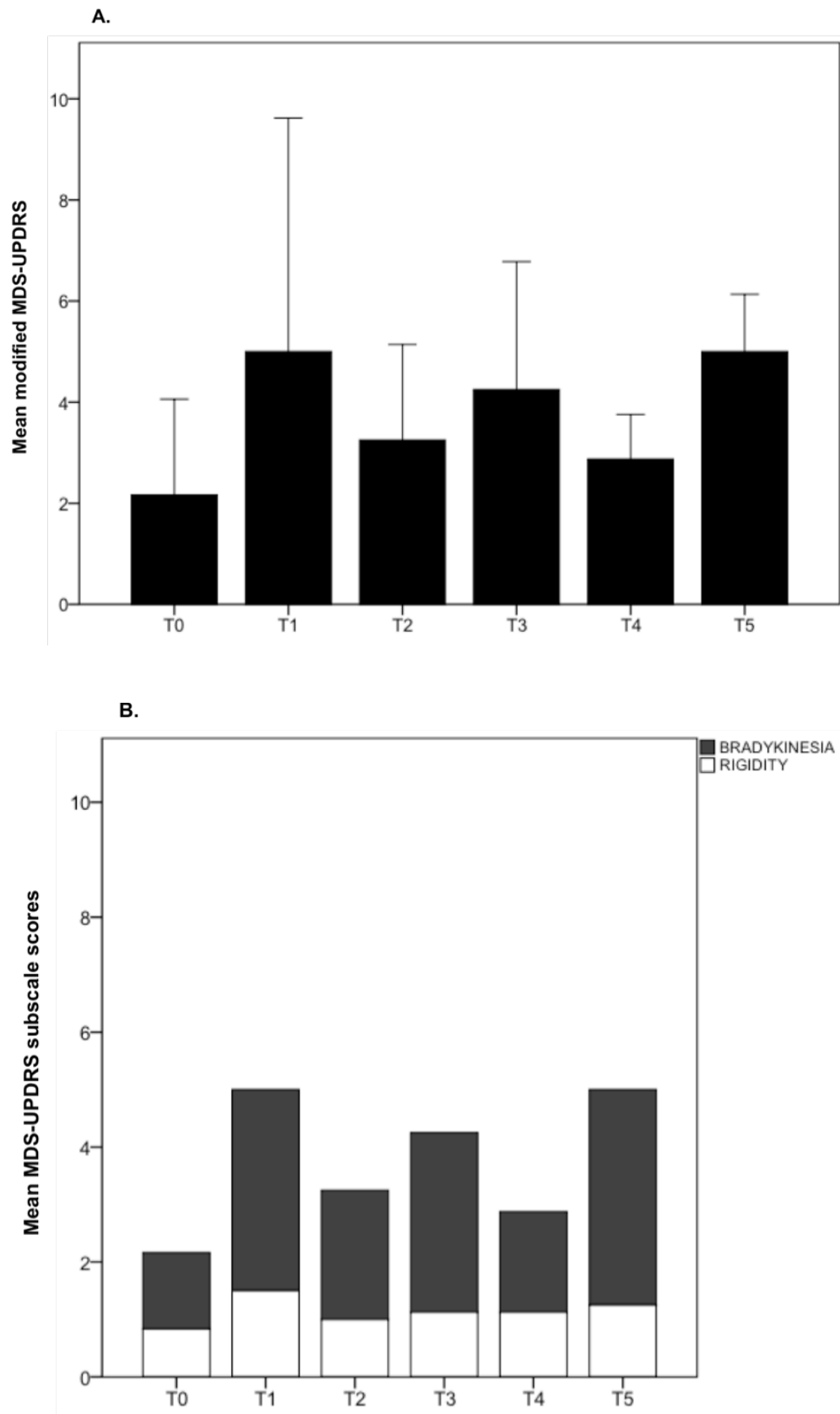


Figure 6-2 Clinical measures of disease severity. (A) means MDS-UPDRS scores at different time points; (B) mean MDS-UPDRS bradykinesia and rigidity scores.

6.5.2 Modified AIMS

Mean mAIMS was 6.3 (range 1-8) at T0 consistent with mild-moderate dyskinesia in the pump ON state while it was 3.8 (range 0-8) at T1 and 3 (range 0-7) at T2 consistent with minimal-mild dyskinesia. Patient 4 who had moderate dyskinesia at T0 with a mAIMS score of 8, had a progressive reduction in hand tapping speed over time despite improvement in dyskinesia, likely suggesting that dyskinesia did not significantly influence the hand tapping speed.

6.5.3 Objective bradykinesia - hand tapping speed

There were no significant differences in group means in hand tapping speeds across different time points ($F_{5,49} = 0.438$, $P = 0.820$, Figure 6-3) despite statistically significant reduction in LD concentration from T0 to T4 (Figure 6-4).

6.5.4 Plasma Levodopa Concentration

There was progressive reduction in levodopa concentrations from T0 to T5 with statistically significant differences in mean levodopa concentrations across time points ($F_{5,23} = 5.339$, $P = 0.002$). Levodopa concentrations were significantly higher at T0 compared to those measured at T3 (4.47 ± 1.10 , $P = 0.014$), T4 (3.26 ± 0.74 , $P = 0.004$) and T5 (3.04 ± 0.84 , $P = 0.006$); see Figure 6-4.

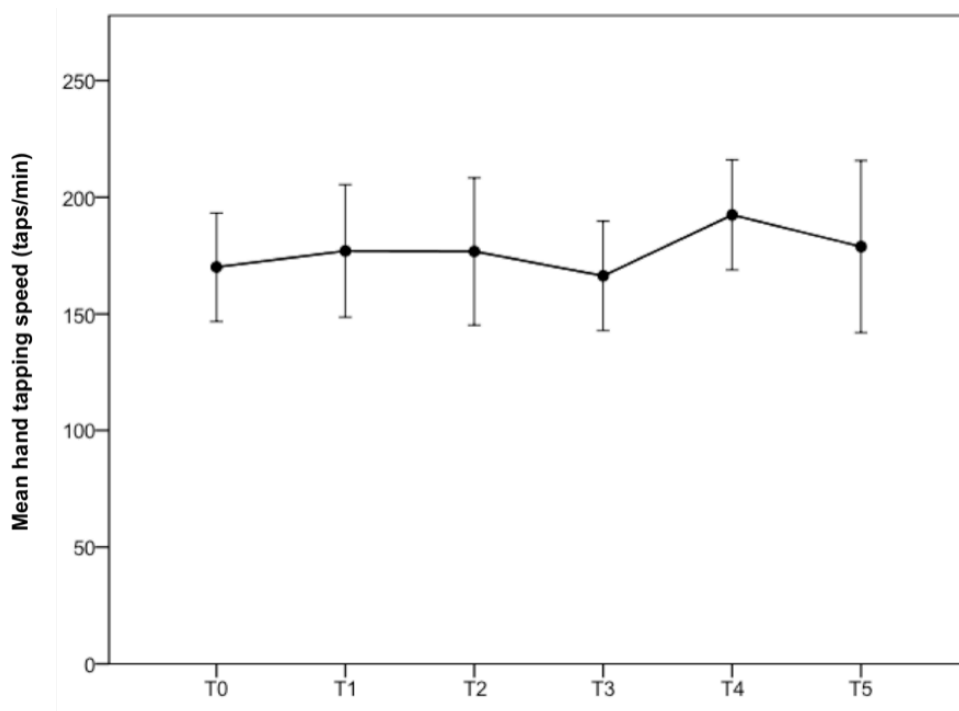


Figure 6-3 Time course of changes in hand tapping speed.

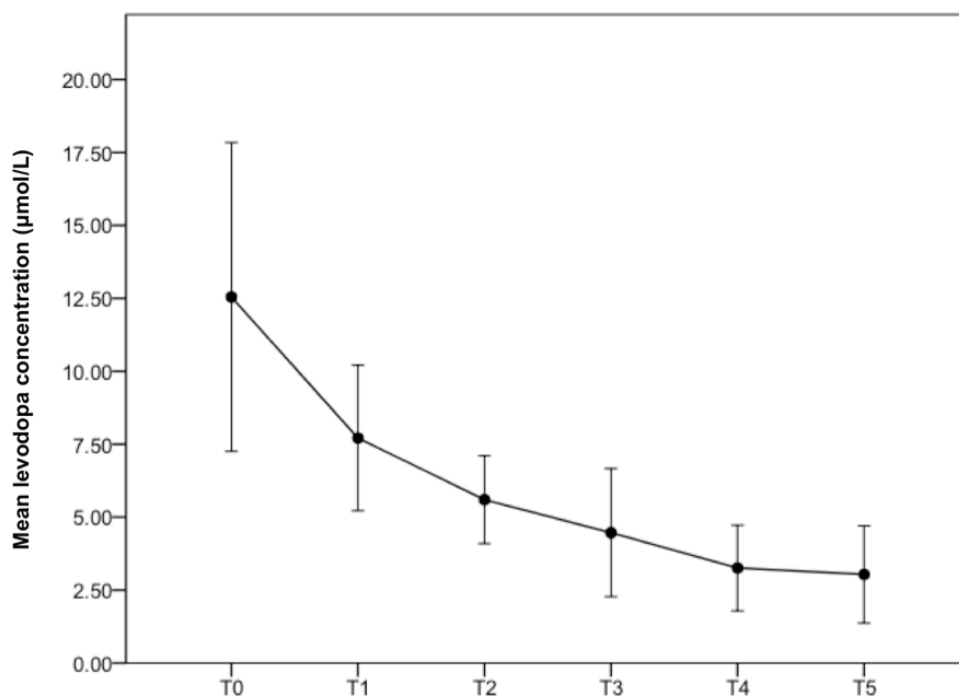


Figure 6-4 Time course of changes in levodopa level.

6.5.5 Cortical excitability measures

Motor thresholds

There were no significant differences in mean RMT between different time points in PD patients ($F_{4,37} = 0.401$, $P = 0.806$, Figure 5A). However, there were highly significant differences in RMT between PD patients and normal control subjects at baseline ($F_{5,97} = 14.372$, $P < 0.000$, Figure 5B).

Cortical silent period duration

There was no significant difference in CSP duration between PD patients and normal control subjects ($F_{5,94} = 0.895$, $P = 0.488$, Figure 6A). However, there appeared to be a trend for reduction in CSP duration from T0 (223.0 ± 19.3 ms) to T4 (181.2 ± 14.3 ms) within PD patients although this was not statistically significant ($F_{4,33} = 1.022$, $P = 0.411$, Figure 6B).

SICI

There were statistically significant differences in SICI between PD patients and normal control subjects ($F_{5,96} = 10.415$, $P < 0.000$, Figure 7A). SICI was significantly reduced in PD patients and progressively became more significant at each subsequent time points, i.e. SICI at T2 (2.07 ± 2.02 %, $P = 0.001$), T3 (2.40 ± 2.12 %, $P = 0.002$) and T4 (-2.55 ± 2.42 %, $P < 0.000$) were significantly reduced compared with normal control subjects (10.29 ± 0.71 %), Figure 7B.

Within PD patients, there were statistically significant differences in SICI between different time points ($F_{4,37} = 2.668$, $P = 0.032$). Specifically, SICI at T4 (-2.55 ± 2.42

%) was significantly reduced compared to T0 (7.39 ± 1.97 %, $P = 0.029$) and approached statistical significance with respect to T1 (6.30 ± 2.14 %, $P = 0.079$). There were no statistically significant differences in SICI between other time points, i.e. T1, T2 and T3 compared with T0 (Figure 6-7B).

When the dataset was further analysed to look for differences between the more and less affected sides, there was no statistically significant difference in SICI ($F_{1,41} = 0.623$, $P = 0.434$).

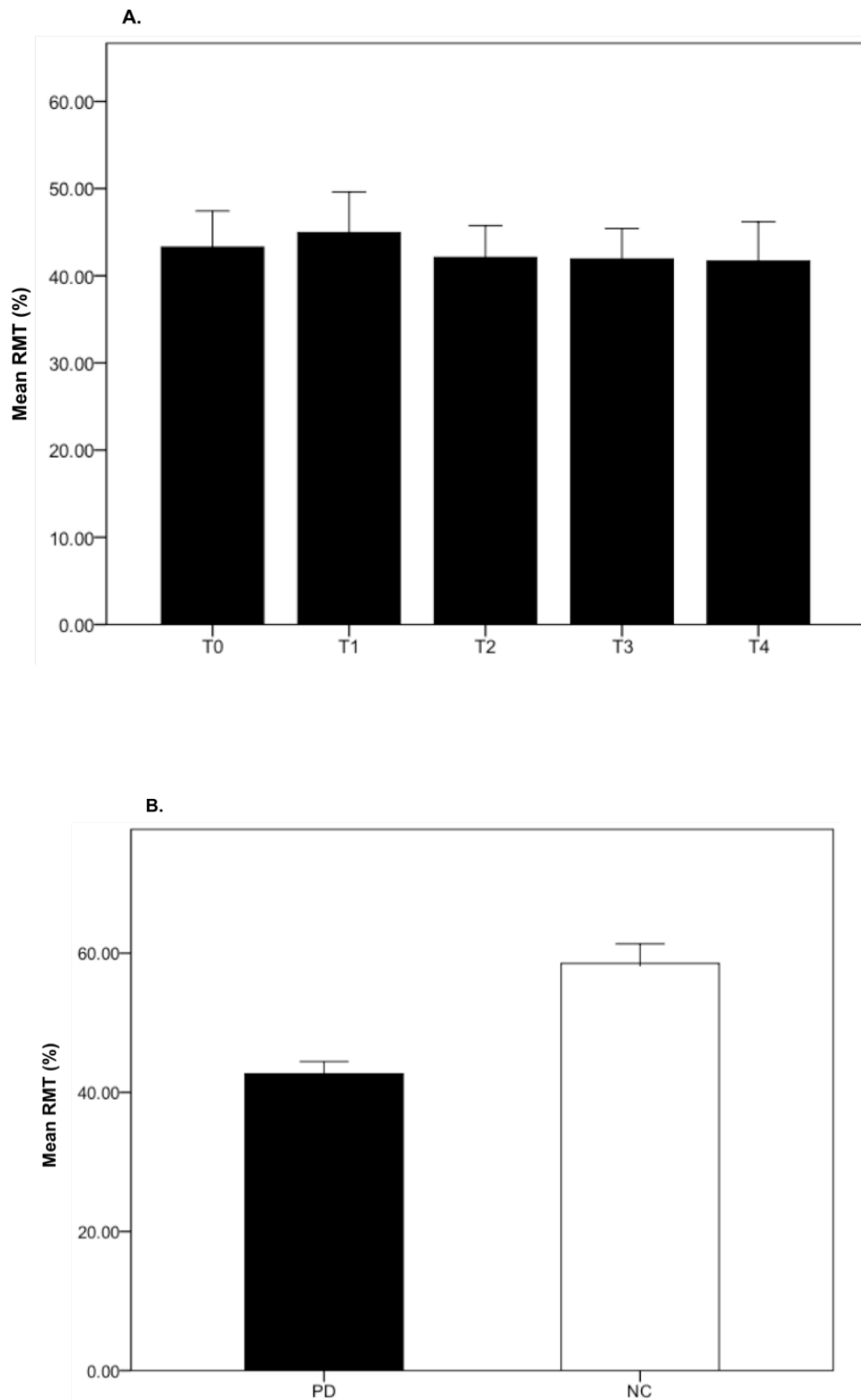


Figure 6-5 Resting motor threshold (RMT). Time course of changes in mean RMT (A); Comparison of RMT between PD and NC subjects (B).

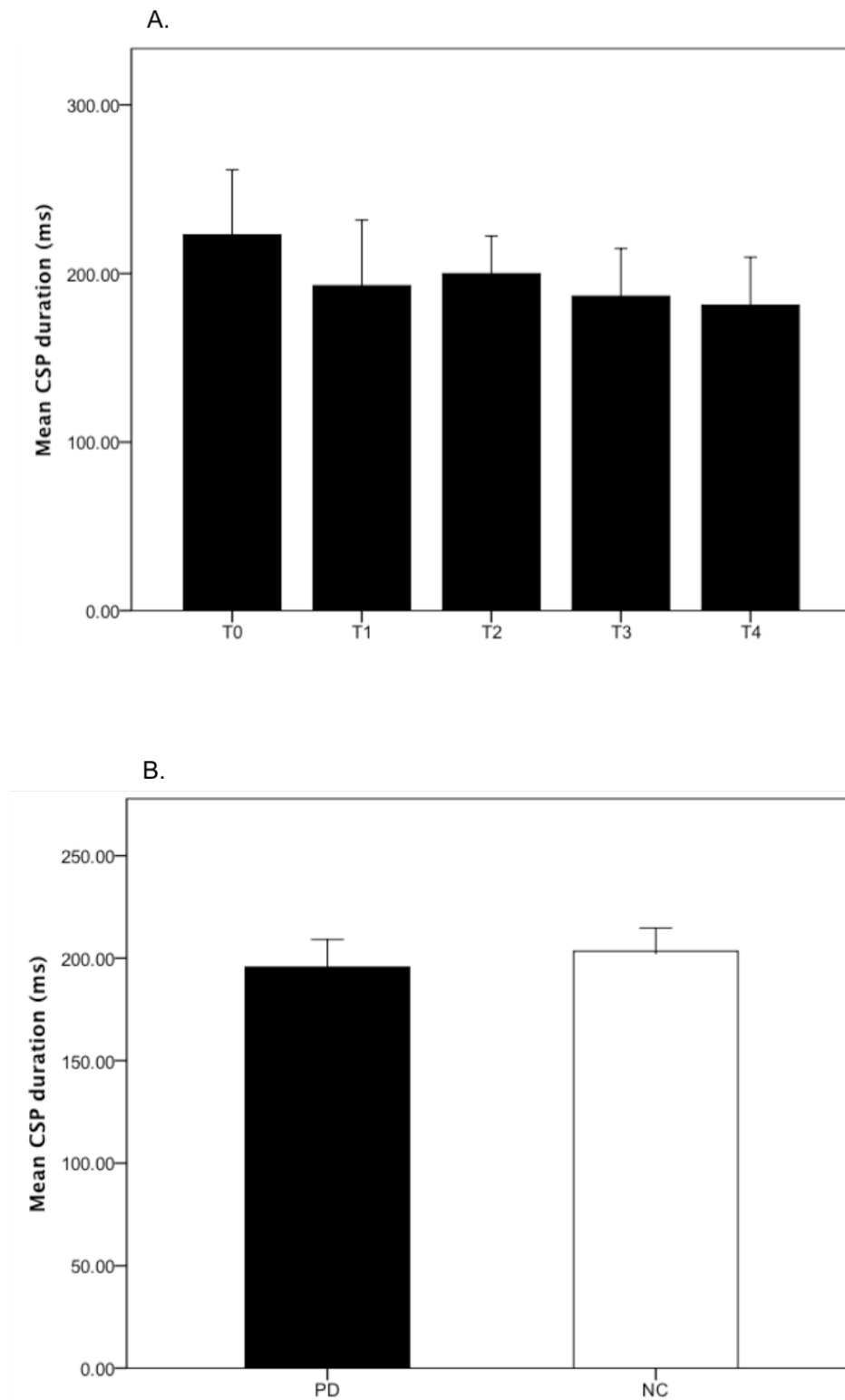


Figure 6-6 Cortical silent period (CSP) duration. Time course of changes in mean CSP duration (A); Comparison of CSP duration between PD and NC subjects (B).

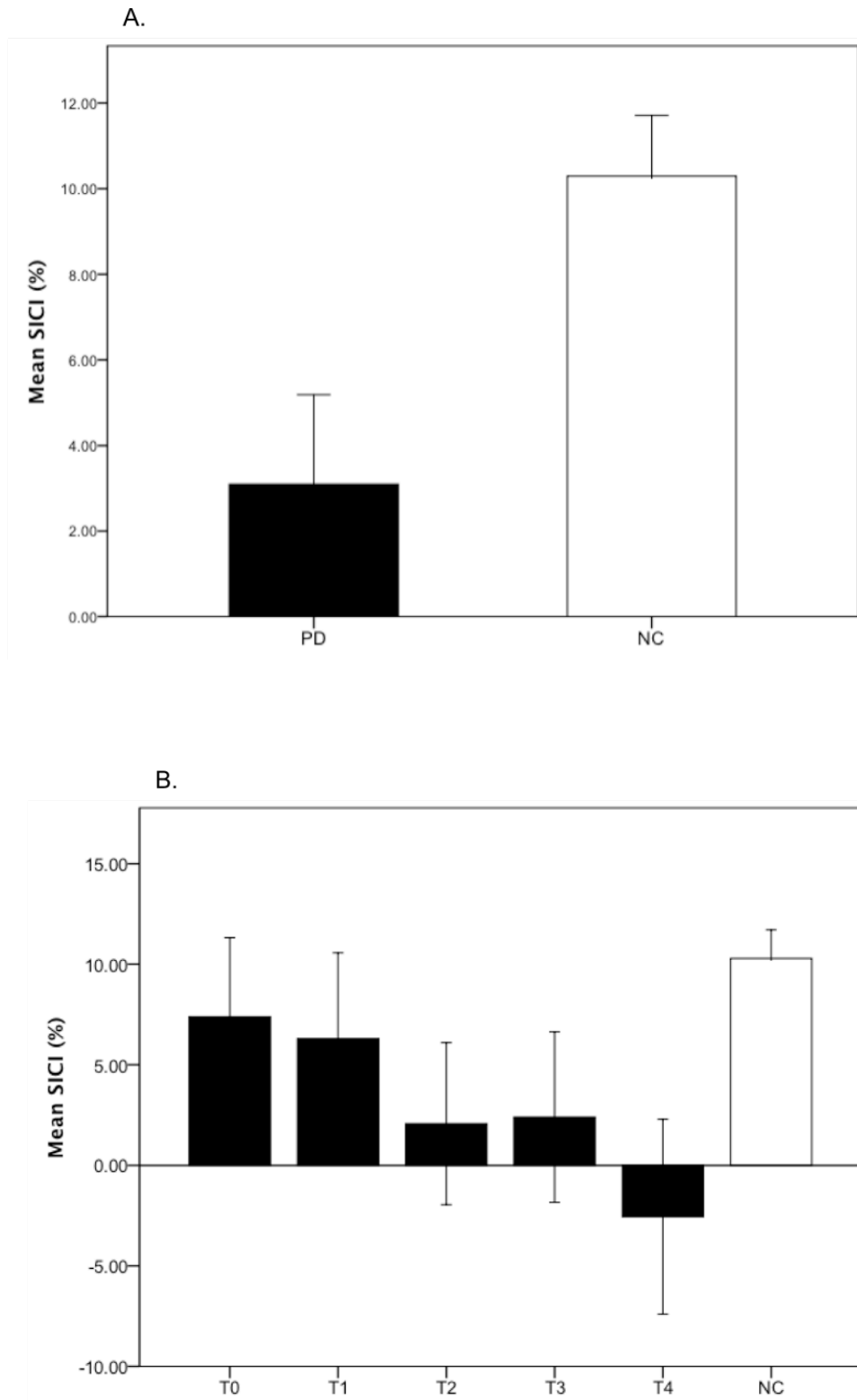


Figure 6-7 Short interval intracortical inhibition (SICI). Time course of changes in mean SICI (A); Comparison of SICI between PD and NC subjects (B).

6.5.6 Relationship between Cortical excitability and hand tapping speed and plasma levodopa concentration

For correlation analysis, right and left sided data were combined and averaged before being analysed as a group.

SICI

There was a statistically significant correlation between mean SICI and mean levodopa concentration ($r = 0.900$, $P = 0.037$, Figure 6-8). However, there was no correlation between mean SICI and mean hand tapping speed ($r = -0.5$, $P = 0.391$) or between mean levodopa concentration and mean hand tapping speed ($r = -0.543$, $P = 0.266$).

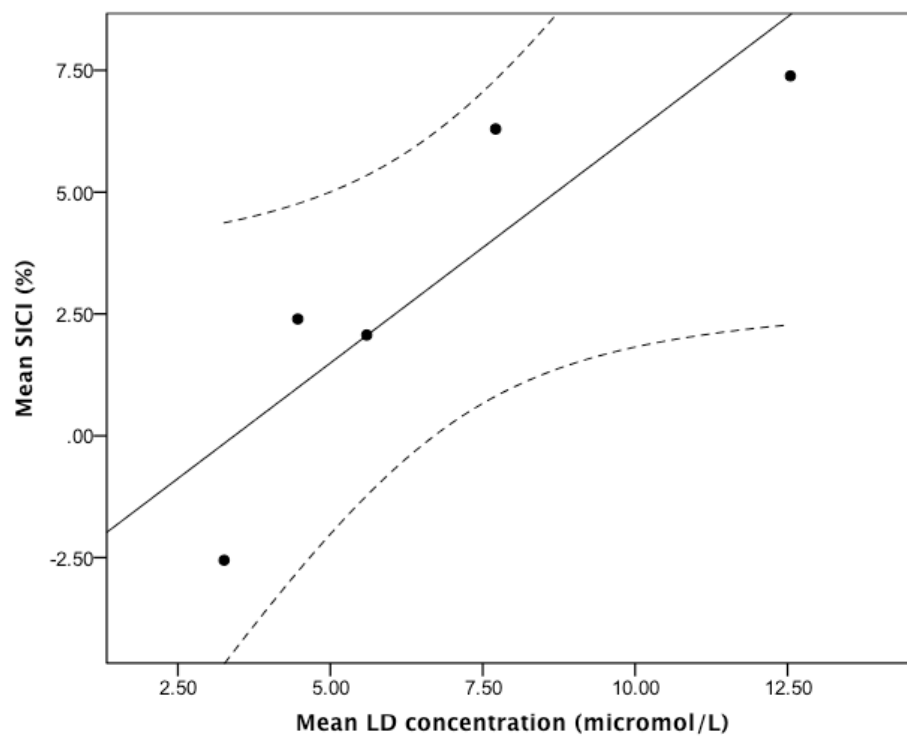


Figure 6-8 Correlation between mean SICI and mean levodopa concentration.

CSP

There was no correlation between CSP duration and levodopa concentration ($r = 0.543$, $P = 0.27$) nor was there correlation between CSP duration and TS ($r = -0.371$, $P = 0.47$).

Clinical measures

In terms of clinical measures, there was no correlation between levodopa concentration and MDS-UPDRS ($r = -0.377$, $P = 0.461$). Similarly, there was no correlation between LD concentration and MDS-UPDRS subscales, bradykinesia ($r = -0.486$, $P = 0.329$) and rigidity ($r = -0.406$, $P = 0.425$).

6.6 DISCUSSION

The key finding from the present study was that SICI correlated with mean levodopa concentration but not with hand tapping speed in subjects with advanced PD treated with LCIG therapy. In other words, there was a progressive reduction in levodopa concentration over time as expected when the LCIG pump was switched OFF and this was matched by progressive reduction in SICI. Further, SICI was significantly reduced in PD patients compared to normal control subjects in the OFF-LCIG phase with the difference becoming significant at T4 compared with T0, suggesting progressive reduction in SICI in the OFF-LCIG phase. However, the present study failed to detect a corresponding reduction in hand tapping speed over the same time interval despite the patients having been off LCIG therapy for more than 2 hours and the mean plasma LD concentration decreasing by more than 70% although one out of

the six patients showed a progressive reduction in hand tapping speed which plateaued at about 60 minutes after the LCIG pump was switched OFF.

The pattern of changes in SICI seen in the present study needs some discussion. The progressive reduction in SICI which developed during the OFF-LCIG phase did not appear until T2 or T3, i.e. 60-120 minutes after the LCIG pump was switched off and the greatest reduction was seen at T4, suggesting that changes in SICI take substantial time to develop, perhaps following rather than preceding change in motor function. The implication is that changes in SICI may not be detectable if subjects are assessed approximately 60 minutes after levodopa although disease severity also has some influence on whether reduced SICI can be detected or not [138]. This is in agreement with the findings from the study in Chapter 5 in which there was no significant increase in SICI when measured 60 minutes after patients' usual morning dose of levodopa and the increases being seen only 60 - 180 minutes after levodopa. The fact that reduced SICI was consistently detected in the PD patients with more advanced disease in the present study despite only examining six patients compared with significantly higher numbers of patients in the preceding experiments (Chapters 4-5) would support the hypothesis that the finding of reduced SICI is dependent on disease severity and would be in agreement with the findings of Hanajima and Ugawa [138]

It has been suggested previously that the finding of an reduced SICI in PD patients was dependent on the intensity of the conditioning stimulus and one particular study reported that SICI became reduced only when the CSI was increased above 80% of RMT, consistent with the idea that reduced SICI arises from changes in the

excitability of pathways that mediate short interval intracortical facilitation (SICF) [5, 76]. The fact that the present study detected significantly reduced SICI with the CSI set at 70% RMT rather than above 80% in a group of PD patients with advanced disease raises the possibility that the threshold of intracortical facilitatory pathways becomes progressively decreased with disease progression although the other possibility that the threshold of inhibitory pathways becomes increased cannot be completely excluded.

It is noteworthy that there was no progressive reduction in hand tapping speed when the LCIG pump was switched OFF despite the fact that all of the patients had advanced disease and could not tolerate being in the OFF-medication state. There was no apparent reduction in hand tapping speed in the majority of patients at T4 even when there had been a significant reduction in the levodopa concentration. This is incompatible with previous pharmacokinetic studies, which demonstrated that motor function mirrors the plasma levels of levodopa [140, 149]. How does one reconcile the lack of changes in the hand tapping speed despite significant reduction in plasma levodopa level? A tantalizing suggestion is that the long-term LCIG therapy with continuous dopaminergic stimulation somehow resulted in pharmacodynamic changes of levodopa and augmented the LDR [129], thereby improving the motor fluctuations and indeed it has been demonstrated that LCIG therapy improves motor function and quality of life in patients with advanced PD [150]. Another possibility is that the hand tapping speed was influenced by dyskinesia. Dyskinesia scores improved from being mild-moderate at the beginning of the study with the LCIG pump ON to being minimal-mild after the pump was switched OFF and hence it is conceivable that the hand tapping speed was lower than expected for a given levodopa level at the beginning of the study and the expected improvement in hand tapping speed with the

improvement in dyskinesia was offset by wearing off of levodopa. Yet another but less likely possibility is that the hand tapping speed may not be a sensitive measure of bradykinesia, but it has been previously demonstrated that it can reliably detect changes in motor function [123, 129] and indeed the experiment on effects of levodopa (Chapter5) demonstrated clear differences in hand tapping speed based on the medication state.

Side-to-side comparisons

In the present study, there was no significant side-to-side difference in cortical excitability but this was likely due to the small number of subjects in this study and does not invalidate the previous finding of significant relative reduction in SICI on the more affected side (Chapter 4).

In terms of the reduced RMT in our subjects, TMS studies in PD patients who have had deep brain stimulation (DBS) surgery have shown that RMT in OFF-DBS condition is not significantly altered by switching to ON-DBS state or administration of the dopamine agonist apomorphine, despite clear improvements in motor function [39], suggesting that RMT is not related to motor performance in PD unlike in normal controls where MT correlates with fine finger movements [27], and hence that clinical manifestations of PD are not primarily the result of any deficit in the excitability of individual motor neurons and their local density. Therefore, it was unlikely for the reduced RMT to have significantly influenced the results.

The fact that SICI correlated with plasma levodopa level rather than hand tapping speed raises questions about its role in the PD pathophysiology. However, interpretation of this result is not straight forward due to the fact that most of the

patients had at least mild-moderate dyskinesia in the present study and as yet the effects of dyskinesia on cortical excitability remains unclarified.

6.6.1 Limitations of the study

The main limitation of this study is the small number of patients and hence the findings cannot be generalised and has to be replicated in a larger group of patients. However the fact that consistent and significant findings were detected even with the small number of patients support the validity of the findings from this study.

6.7 CONCLUSION

SICI is reduced in patients with PD but the degree to which this is reduced appears to depend on disease severity, being more likely to be observed in those with advanced disease. Further, SICI correlates with plasma levels of levodopa. Taken together, the findings suggest that SICI is influenced by both the disease severity as well as plasma levodopa levels.

6.7.1 Clinical implications

The findings from the present study raise the possibility that long term LCIG therapy can improve the pharmacodynamics of levodopa. Specifically, the fact that hand tapping speed was maintained despite low plasma levels of levodopa suggests that the LDR response may have been re-established with LCIG therapy.

Conclusions

This thesis has provided significant insights into the significance of cortical excitability changes in both SMS and PD.

In SMS, SICI was markedly reduced despite the patients still being on high doses of benzodiazepines but there was dissociation between measures of cortical hyperexcitability, spinal hyperexcitability, serum anti-GAD antibodies and clinical symptoms in stiff man syndrome. The results suggest that spinal hyperexcitability and muscle stiffness and spasms are unlikely to be simply due to cortical disinhibition of spinal circuits. This study highlights the current incomplete understanding of the pathophysiology of SMS. Further studies of the relationship between cortical excitability and other features of SMS such as associated psychiatric symptoms, as well as studies of the relationship between cortical excitability, brainstem excitability and features of SMS may give further insights into the pathophysiology and treatment of SMS.

In PD, the most significant finding was that relatively advanced PD patients treated with LCIG tended to have greater reduction in SICI than those with milder disease. Additionally, there was a strong positive correlation between plasma levodopa level and SICI such that there was progressive reduction in SICI together with plasma levodopa level in the OFF-LCIG phase, suggesting that SICI is influenced by the severity of disease and drugs used to treat the disease. Results from studies in Chapters 4 and 5 suggest that SICI may be a compensatory phenomenon and notably the fact that there was positive correlation between decrement in hand tapping speed and SICI suggest that SICI tends to be reduced on those with less decrement perhaps

as a compensatory mechanism but normal in those with greater decrement suggesting failure of compensatory mechanism.

The studies and results contained herein confirm there is much value in further careful research of cortical excitability changes in Parkinson's disease and other related movement disorders.

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