Abstract

Introduction: Parkinson Disease (PD) is characterized by bradykinesia along with altered oscillations in the basal ganglia (BG) and cortex resulting from loss of dopaminergic neurons in the substantia nigra. In addition, PD is linked to decreased activation of the Supplementary Motor Area (SMA) attributed to altered cortico-basal ganglia pathways resulting in poverty of movement.

Purpose: The main goal of the study was to determine whether 5 Hz rTMS over SMA improved motor control - indexed by a serial targeting task (STT) and a handwriting task in individuals with PD. Secondary aims were to 1) record change in cortical oscillations using electroencephalogram (EEG) during STT performance, and 2) to consider the role of the SMA in motor imagery in individuals with PD as indexed by a break test.

Methods: In a cross-over design, individuals with PD were randomized to receive either 5 Hz or control rTMS over the SMA. Three experimental tasks were administered prior to and immediately after the rTMS intervention with right hand; the STT, a handwriting task, and a break test. EEG was collected during STT and cortical excitability was indexed by assessing resting motor threshold (RMT).

Results: Participants showed an improved motor performance in STT post 5 Hz rTMS. There was concurrent reduction in θ and α oscillations during the reaction time phase of the task in frontocentral and central EEG channels along with lowered RMT in M1 post 5 Hz rTMS. In the handwriting task, participants showed improvement in stroke size, axial pressure after 5 Hz rTMS. Both groups’ (5 Hz and control) performance on the break test was similar.
**Conclusion:** The findings of this thesis suggest that 5 Hz rTMS is beneficial at least in the short-term for individuals with PD. 5 Hz rTMS improved motor performance, shifted cortical oscillations and cortical excitability. However, these effects were noted for only a single session. The results of this thesis may contribute to future research related to development of rTMS as a therapeutic option for individuals with PD.
Preface

Chapters of this thesis will be published as multi-authored manuscripts in peer-reviewed journals. Details of authors’ contributions are provided.

Submitted Manuscripts

Chapter 2: Randhawa BK, Meehan SK, Dao E, McKeown MJ, Boyd LA.
Repetitive Transcranial Magnetic Stimulation over Supplementary Motor Area benefits Motor Task Performance and Modulates Cortical Oscillations in Individuals with Parkinson Disease.

Contribution: 80% - contributed to study design, performed the research, conducted data analyses, and manuscript preparation. Dao E helped in collection of data and manuscript preparation. Drs. Meehan, McKeown, Boyd provided feedback during data analysis and final manuscript.

Chapter 3: Randhawa BK, Farley B.G., McKeown MJ, Boyd LA.
Repetitive Transcranial Magnetic Stimulation over Supplementary Motor Area Improves Handwriting in Individuals with Parkinson Disease.

Contribution: 90% - contributed to study design, performed the research, conducted data analyses, and manuscript preparation. Dr. Farley contributed in study design, data analysis and manuscript preparation. Dr. McKeown contributed to recruitment of participants and data analysis. Dr. Boyd provided feedback during data analysis and contributed in final manuscript.

Chapter 4: Randhawa BK, Deutsch JE, Boyd LA.
Effect of 5 Hz Repetitive Transcranial Magnetic Stimulation over Supplementary Motor Area on Imagery Ability in Individuals with Parkinson Disease.

Contribution: 90% - contributed to study design, performed the research, conducted data analyses, and manuscript preparation. Dr. Deutsch contributed in study design, data analysis and manuscript preparation. Dr. Boyd provided feedback during data collection, data analysis and contributed in final manuscript.

Ethical review and approval for this thesis was performed by the UBC Clinical Research Ethics Board (H09-00673) and Vancouver Coastal Health Research Institute (V09-0346).
# Table of Contents

Abstract ............................................................................................................................. ii

Preface .............................................................................................................................. iv

Table of Contents ............................................................................................................. v

List of Tables ..................................................................................................................... ix

List of Figures ................................................................................................................... x

List of Abbreviations ..................................................................................................... xi

Acknowledgements ......................................................................................................... xiii

Dedication ......................................................................................................................... xiv

Chapter 1  Introduction ................................................................................................. 1

1.1 Parkinson Disease ................................................................................................. 1

1.2 A Model of Basal Ganglia Circuitry ................................................................. 1

1.5 Motor symptoms in PD ....................................................................................... 7

1.5.1 Bradykinesia .................................................................................................. 7

1.5.2 Decreased Stroke Size in Handwriting ....................................................... 8

1.5.3 Motor Imagery Ability .................................................................................. 9

1.6 Therapeutics ......................................................................................................... 10

1.6.1 Pharmacological ......................................................................................... 10

1.6.2 Surgical or Invasive Brain Stimulation ....................................................... 10

1.6.3.1 Repetitive Transcranial Magnetic Stimulation .................................... 11

1.7 Transcranial Magnetic Stimulation - Theoretical Framework ...................... 12

1.7.1 TMS Safety ............................................................................................... 14

1.8 Objectives and Hypothesis of the Thesis ......................................................... 14
1.8.1 Specific Aim 1: To study the impact of 5 Hz rTMS over SMA on hand motor control in individuals with PD.

1.8.2 Specific Aim 2: To study the impact of 5 Hz rTMS on cortical oscillatory activity in α and β bands (8-35 Hz) and corticomotor excitability in M1 in individuals with PD.

1.8.3 Secondary Aim: To explore the impact of 5 Hz rTMS over SMA on the ability to imagine movements in individuals with Parkinson Disease.

1.9 Significance of the Study

1.10 Experimental Design of the Study

Chapter 2 Repetitive Transcranial Magnetic Stimulation over Supplementary Motor Area benefits Motor Task Performance and Modulates Cortical Oscillations in Individuals with Parkinson Disease

2.1 Preamble

2.3.1 Participants

2.3.2 TMS Protocol

2.3.4 EEG Data Collection and Processing

2.4 Statistical Analysis

2.4.1 STT

2.4.2 STT-EEG

2.4.3 RMT

2.5 Results

2.5.1 S

2.5.2 STT-EEG

2.5.3 RMT

2.6 Discussion
Chapter 3  Repetitive Transcranial Magnetic Stimulation over Supplementary Motor Area Improves Handwriting in Individuals with Parkinson Disease .................................49
  3.1  Preamble..........................................................................................................................49
  3.2  Introduction .........................................................................................................................49
  3.3  Methods ...............................................................................................................................52
      3.3.1  rTMS Protocol and Participant Characteristics ..........................................................52
      3.3.2  Handwriting .................................................................................................................52
  3.4  Analysis ..................................................................................................................................53
  3.5  Statistical Analysis ................................................................................................................54
  3.6  Results ...................................................................................................................................54
      3.6.1  Complete Loops .............................................................................................................54
      3.6.2  Upstroke and Downstroke ............................................................................................55
  3.7  Discussion ............................................................................................................................56

Chapter 4  Effect of 5 Hz Repetitive Transcranial Magnetic Stimulation over Supplementary Motor Area on Imagery Ability in Individuals with Parkinson Disease...71
  4.1  Preamble..................................................................................................................................71
  4.2  Introduction ............................................................................................................................71
  4.3  Methods ..................................................................................................................................74
      4.3.1  rTMS Protocol and Participant Characteristics ..........................................................74
      4.3.2  Assessment of MI ...........................................................................................................74
  4.4  Data Analysis ..........................................................................................................................75
  4.5  Results ....................................................................................................................................76
  4.6  Discussion ..............................................................................................................................77
  4.7  Conclusion .............................................................................................................................78

Chapter 5  Conclusion and Future Directions.............................................................................84
5.1 Overview .................................................................................................................................................. 84
5.2 Summary of Results ................................................................................................................................. 84
  5.2.1 rTMS over SMA benefits Motor Task Performance and Modulate Cortical Oscillations in Individuals with PD .............................................................................................................. 84
  5.2.2 rTMS over SMA improves Handwriting in Individuals with PD ......................................................... 85
  5.2.3 Effect of rTMS over SMA on MI Ability in Individuals with PD ....................................................... 86
5.3 Limitations of the Thesis .......................................................................................................................... 86
5.4 Conclusion and Future Directions ......................................................................................................... 89
BIBLIOGRAPHY ........................................................................................................................................... 91
APPENDIX 1: Movement Profiles: STT ....................................................................................................... 110
APPENDIX 2: EEG PSD Graphs .................................................................................................................. 112
APPENDIX 3: Handwriting Raw Data ......................................................................................................... 120
List of Tables

Table 1-1: Experimental design of the study.................................................................18
Table 2-1: Participant characteristics.................................................................41
Table 2-2: Mean (SD) and P-value for motor performance in STT.............................42
Table 2-3: P-Values for cortical oscillations and channel locations (EEG)............46
Table 2-4: Average means (SD) and P-value for RMT............................................48
Table 3-1: Average means (SD) and P-value for all segments, up-strokes and down-
strokes..........................................................................................................................62
Table 4-1: Imagery scores of all participants..........................................................80
List of Figures

Figure 1-1: Direct and indirect loops of basal ganglia ..................................................2
Figure 1-2: Pathways affected in PD .............................................................................2
Figure 1-3: Supplementary motor area (SMA) connections in the Macaque Monkey. ...4
Figure 1-4: Anatomical connections within the BG-thalamocortical circuitry ...............5
Figure 2-1: Talairach coordinates of SMA .................................................................26
Figure 2-2: Sterotaxic system for coil placement .........................................................26
Figure 2-3 (A-D): Serial Targeting Task (STT) ............................................................29
Figure 2-4: Headplot of electrode regions ...................................................................31
Figure 2-5 (A-F): Behavioural data from motor performance during STT .......45
Figure 2-6 (A-C): Data from cortical oscillations during motor performance (STT) ...47
Figure 2-7: Data from RMT over primary motor cortex ..............................................48
Figure 3-1: Handwriting task ......................................................................................53
Figure 3-2 (A-X): Behavioural data from handwriting task with SEM bars ..........70
Figure 4-1: Break Test: Continuous thumb finger opposition and metronome .......75
Figure 4-2 (A and B): Break test results for all participants in the study .................82
Figure 4-3: Spearman Correlation Coefficient for all participants in the study .......83
Figure 5-1: Limitations in recruitment of participants ..................................................89
Figure A-1-1(A-F): Pre-Post 5 Hz rTMS - Movement profiles ................................110
Figure A-1-2 (A-F): Pre-Post control rTMS - Movement profiles ..............................111
Figure A-2 (A-X): PSD graphs for all participants (Task-Baseline) .........................119
Figure A-3-1: Pre-Post 5 Hz rTMS: Handwriting ......................................................120
Figure A-3-2: Pre-Post control rTMS: Handwriting ..................................................120
List of Abbreviations

ANJ - Average Normalized Jerk
ANOVA - Analysis of Variance
BG - Basal Ganglia
C-BG – Cortico-Basal Ganglia
Cen - Central
DBS - Deep Brain Stimulation
DLPFC - Dorsolateral Pre-Frontal Cortex
ECT - Electroconvulsive Therapy
EEG - Electroencephalogram
EMG - Electromyography
FC - Frontro-central
FCR - Flexor Carpi Radialis
fMRI - functional Magnetic Resonance Imaging
GPe - Globus Pallidus externus
GPi - Globus Pallidus internus
H&Y - Hoehn and Yahr’s score
KVIQ - Kinesthetic and Visual Imagery Questionnaire
L_SM - Left Sensorimotor
LFP - Local Field Potential
LTD - Long-Term Depression
LTP - Long-Term Potentiation
M1 – Primary Motor Cortex
MEG - Magneto-encephalography
MEP - Motor Evoked Potentials
MI - Motor Imagery
MT - Movement Time
nPAP - Number of Peak Acceleration Points
Occ - Occipital
PD - Parkinson Disease
PET - Positron Emission Tomography
PMC - Premotor Cortex
PSD - Power Spectral Density
R_SM - Right Sensorimotor
RMT - Resting Motor Threshold
RT - Reaction Time
rTMS - Repetitive Transcranial Magnetic Stimulation
SMA - Supplementary Motor Area
SNr - Substantia Nigra reticulata
STN - Subthalamic Nucleus
STT - Serial Targeting Task
UPDRS-III - Unified Parkinson Disease Rating Scale – III (motor)
VA/VL - Ventero-Anterior/Ventero-Lateral
α - Alph
β - Beta
γ - Gamma
θ - Theta
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Dedication

To My Mom Dad
Chapter 1 Introduction

1.1 Parkinson Disease

Parkinson Disease (PD) is the second most common neurodegenerative disease\(^1\) and affects 100,000 Canadians and their families\(^2\) PD is characterized by bradykinesia, tremor, rigidity and postural instability resulting directly and indirectly from death of dopaminergic neurons in the substantia nigra and subsequent reduction of dopamine in the striatum which alters excitability within cortico-basal ganglia (C-BG) pathways. Generally, current therapeutic approaches (e.g., medication, neurosurgery, brain stimulation, etc.) attempt to improve motor symptoms by modulating excitability within these C-BG pathways.

1.2 A Model of Basal Ganglia Circuitry

The classic neural network model of BG involves two pathways: direct and indirect as shown in Figure 1-1; however, the precise manner by which these pathways interact both normally and in PD is not universally agreed upon.\(^3\) In the direct pathway, striatal neurons project to the globus pallidus internus (GPI) and substantia nigra reticulata (SNr) to reduce inhibition to VA/VL (ventero-anterior/ventero-lateral) complex of thalamus (disinhibition). Theoretically, the net effect of the direct pathway is to increase cortical excitability and facilitate movement. In the indirect pathway, striatal neurons project to the globus pallidus externus (GPe - inhibitory), from GPe to the subthalamic nucleus (STN - inhibitory) and from STN to the GPI/SNr (excitatory). This increased SPI/SNR output, raises inhibition in the VA/VL. Together, the action of the indirect pathway is considered to reduce cortical excitability and generally inhibit movement.
Figure 1-1: Direct and indirect loops of basal ganglia: Direct loop facilitates movement and indirect pathway inhibits movement. GPe: globus pallidus externus, GPi: globus pallidus internus, STN: subthalamic nucleus, SNc: substantia nigra.

Figure 1-2: Pathways affected in PD: The balance between direct and indirect pathways is altered, leading to a diminished ability of the BG to control the thalamic output to the cortex. This imbalance results increased inhibition of the thalamus due to failure of direct motor loop. GPe: globus pallidus externus, GPi: globus pallidus internus, STN: subthalamic nucleus, SNc: substantia nigra.
In PD, reduced dopamine, results in an imbalance between the direct and indirect pathways (Figure 1-2). Normally, dopamine release has the general effect of exciting the direct pathway (D1 receptors) and inhibiting the indirect pathway (D2 receptors). But in PD, degeneration of dopaminergic neurons results in reduced input from SNr, which alters striatal control of VA/VL output to the cortex and hence decreases the overall level of excitation in the motor cortex.

### 1.3 Supplementary Motor Area – Basal Ganglia Connections

The supplementary motor area (SMA) is located on the medial aspect of the brain (dorso-medial frontal cortex), anterior to the leg representation of the primary motor cortex.\(^4, 5\) Functionally, the SMA plays a key role in movement preparation and generation on the basis of memory, internal cues and external cues.

The SMA is an important target of basal ganglia and cerebellar output. The SMA receives relatively more input from the basal ganglia (as much as 3-4 times more projections) than the cerebellum.\(^6\) These connections are reciprocal with the SMA projecting efferently back to the basal ganglia (GPI).\(^7-9\) Thus, an anatomical loop exists between the SMA and basal ganglia (Figure 1-3). The SMA also has connections to STN,\(^10\) which may modulate ongoing activity in GPI and GPe circuits.\(^11\) However, due to reduced input from the basal ganglia, individuals with PD suffer from dysfunction in the SMA (decreased activation),\(^12-19\) that results from decreased function in SMA-basal ganglia loop. Therefore, the aim of modern therapeutic techniques such as transcranial magnetic stimulation over SMA or other cortical areas (primary motor cortex, dorsolateral prefrontal cortex) is to enhance activity in the direct pathway or STN, decrease the activity in indirect pathway, or stimulate dopaminergic neurons.
1.4 Pathological Oscillations and Abnormal Cortical Excitability

Oscillations are a widespread feature of normal brain activity that result from regular periodic oscillatory firing of single neurons with unique intrinsic biophysiological properties or from network architectures with interneurons and/or feedback connections.\textsuperscript{20, 21} Although oscillations exist in variety of different frequencies in different neuronal systems, primate and deep brain stimulation (DBS) studies suggest that altered oscillations in BG and associated regions of thalamus and cortex have a role in the pathophysiology of PD.\textsuperscript{22, 23}

Several circuits in the cortico-subcortical network have been proposed as possessing intrinsic oscillatory activity in the basal ganglia as shown in Figure 1-4:\textsuperscript{21} 1) GPe-STN, 2) cortical-STN, 3) STN-GPi, 4) Striatum-Thalamic connections, 5) STN-Thalamic connections and 6) cortical-striatum connections. The STN plays a central role in this network. The in-phase high-frequency synchronization observed in the STN
of individuals with PD is most likely caused by synchronous activity occurring in other areas – especially GPe and cerebral cortex, both of which send input to the STN.\textsuperscript{24, 25} These two areas are integral to the corticostriatal–GPe–STN–GPi (“indirect” pathway) and cortico–STN–GPi–thalamic circuitries, respectively.\textsuperscript{26} The oscillatory activity input from cortex to STN is through the motor cortex (M1) and SMA. The SMA sends efferents to striatum,\textsuperscript{7, 8} projecting to GPi both directly and indirectly.\textsuperscript{9} In addition, the SMA has connections to STN,\textsuperscript{10} to control ongoing activity in the cortical-basal ganglia circuit.\textsuperscript{11} The SMA also has dense projections to M1.\textsuperscript{27-33} Thus, in each of these circuits, the SMA may modify the neuronal activity in the basal ganglia, STN and M1, to change ongoing oscillatory activity. However, individuals with PD suffer from dysfunction in the SMA (decreased activation),\textsuperscript{12-19} therefore its role in the modulation of oscillations in the basal ganglia may be limited in individuals with PD.

![Anatomical connections within the BG-thalamocortical circuitry.](image)

Figure 1-4: Anatomical connections within the BG-thalamocortical circuitry. Red arrows denote excitatory connections, black arrows identify inhibitory connections. Green numbers and arrows refer to internuclear mechanisms to generate or maintain oscillations. PPN: Pedunculopontine nucleus; CM: Centromedian nucleus of the thalamus. Figure modified from Gatev et al.\textsuperscript{21}

Oscillatory activity in the basal ganglia has been subdivided into roughly four bands, $\theta$ (<8 Hz), $\alpha$ (8-12 Hz), $\beta$ (13-35 Hz), and $\gamma$ (>35 Hz).\textsuperscript{22, 34} Deep brain stimulation
studies have revealed the presence of exaggerated activity in β band (13-35 Hz) in people with PD, which has been purported as one source of the movement impairment associated with this disease. In neurologically intact healthy people, low-frequency oscillations relate to long-range communication between different brain regions, whereas high frequency (high γ) oscillations reflect local cortical processing. The α band has been related to motor activity and is disrupted by both sensory and motor-related processing, local α activity has classically been assumed to indicate inactivity (i.e., ‘idling’) of the underlying cortex. In contrast, it has been assumed that the β band plays role in controlling motor activity. In healthy individuals, oscillations in the β band are suppressed before and during motor tasks and increase to inhibit movement. However, in people with PD, exaggerated oscillatory 13-35 Hz synchrony has been observed, and interpreted as playing a role in movement impairment and limb tremor. Levy et al demonstrated that the synchronization of neuron pairs in the GP is limited to oscillatory activity occurring in the tremor frequency range of 15–30 Hz. Lower frequencies (2-10 Hz) have also been recorded in STN and GPi of non-dyskinetic individuals with PD; this effect is exaggerated in medicated individuals. In addition, synchronous high frequency oscillations (> 60 Hz) have also been recorded in STN and GPi of treated individuals with PD. A more in-depth explanation of oscillatory changes in individuals with PD is provided in Chapter 2.

Individuals with PD also suffer from impaired cortico-motoneuronal output at rest. Some reports suggest that impaired cortical excitability is reflected in higher amplitude of motor evoked potentials (MEP) in individuals with PD as compared to healthy individuals. However, this finding is controversial as altered MEP amplitude
and/or higher motor thresholds are not universally reported in people with PD. MEP size reflects global excitability of the corticospinal pathway. Enhanced MEP size at rest may be related to an excessive tonic activity in the whole cortico-motoneuron system (including the spinal level), and seems to be associated with rigidity and bradykinesia in PD.\textsuperscript{50} This impaired motor inhibitory control may be attributed to altered inhibitory output to the cortex via the basal ganglia and SMA.

1.5 Motor symptoms in PD

Individuals with PD suffer from dysfunction in the basal ganglia and cortical motor areas (i.e., net decreased activation).\textsuperscript{12-19} The net result is under-activation of cortico-subcortical loops, which in turn leads to a series of functional changes in basal ganglia circuits that mediate the cardinal motor features of PD.

1.5.1 Bradykinesia

Bradykinesia is defined as slowness of a performed/executed movement. It is sometimes erroneously used interchangeably with term the hypokinesia, which actually refers to decrease in movement amplitude.\textsuperscript{52} For consistency in this thesis, I will use term 'bradykinesia' to describe slowed movement in individuals with PD. The degree of bradykinesia is generally correlated with the rate and progression of PD.\textsuperscript{53, 54} It is the clinical sign associated with PD that is most closely affiliated with difficulties in performing activities of daily living.\textsuperscript{55, 56}

It is known that PD causes bradykinesia as well as impaired scaling of movement speed with respect to distance.\textsuperscript{57-60} Bradykinesia becomes more evident when individuals with PD are externally directed to perform aimed movements. Because scaling of speed and accuracy of movement are linked in motor tasks, I employed a
movement task in this study, which enabled consideration of these elements (speed and accuracy) separately (as described in Chapter 2).

Further, neuroimaging studies provide evidence that the SMA, putamen and cerebellum are associated with planning and execution in motor tasks.\textsuperscript{61,62} However, in individuals with PD, bradykinesia reduces the capability to control and regulate the magnitude of velocity/acceleration in voluntary movements.\textsuperscript{63-65} I explored this further in chapter 2 using a novel, externally directed motor task that requires modulation of both components of motor control, that is, speed and accuracy.

1.5.2 Decreased Stroke Size in Handwriting

Handwriting in individuals with PD is characterized by under-scaled movements resulting in diminution of letter size, reduced speed and slow acceleration.\textsuperscript{66-70} Deficits in handwriting often begin with hypometric movements and then may progress to micrographia as the disease advances. Severe micrographia is present in 15% of individuals with PD.\textsuperscript{71}

Past work demonstrates that the basal ganglia along with the SMA, plays an important role in movement and velocity scaling, specifically in planning for movement amplitude.\textsuperscript{72} Normally, basal ganglia activity is adjusted in response to a required movement velocity and amplitude.\textsuperscript{73,74} But in individuals with PD, basal ganglia (i.e., substantia nigra \textit{pars compacta}) degeneration impairs the control of movement amplitude and duration, and the rate of force production;\textsuperscript{67,71,75,76} the net result is hypometric and bradykinetic movements. The functional consequences of hypometric movements in handwriting will be discussed further in Chapter 3.
1.5.3 Motor Imagery Ability

Motor imagery (MI) is the mental representation of movement without any actual body movement.\textsuperscript{77, 78} It is a complex cognitive process, which involves the use of sensory and perceptual memories as they relate to motor actions. There are various types of MI; the two most commonly used in rehabilitation are external (visual) and internal (kinaesthetic) imagery. External imagery refers to watching oneself as an external observer, e.g., watching oneself in a mirror, and is also known as visual imagery. In internal or kinaesthetic imagery, a person imagines movement from an inner perspective, performing a movement mentally and experiencing the actual situation and sensations.\textsuperscript{79} MI practice has been shown in other populations (e.g., stroke, spinal cord injury) to be a safe, effective method to facilitate motor learning and movement initiation, as such MI may be a novel and effective method to facilitate motor function in individuals with PD.

Motor imagery is a self-generated or internally guided activity; the SMA is predominantly involved in self-generated actions along with the basal ganglia.\textsuperscript{27, 80} Imaging studies report activation of SMA during the imagining of movements both in healthy people\textsuperscript{81-85} and in individuals with PD.\textsuperscript{85} However, PD is linked to dysfunction of the SMA (i.e., decreased activation)\textsuperscript{12-19} attributed to reduced input from the basal ganglia. Past work has not convincingly demonstrated that individuals with PD can generate or visualize movements mentally.\textsuperscript{86, 87} These studies are discussed further in chapter 4.

Brain imaging work exploring the neural correlates of MI in healthy people has demonstrated the importance of the basal ganglia and SMA during mentally imagined
movements.\textsuperscript{15-17} However, the network activated during MI has been shown to be shifted by PD\textsuperscript{15} to be more reliant on the SMA to the exclusion of the basal ganglia. Because PD disrupts basal ganglia function and causes motor deficits, it is tempting to conclude that this disease will also impact MI. This hypothesis is explored further in chapter 4.

1.6 Therapeutics

1.6.1 Pharmacological

The ‘gold standard’ of pharmacological treatment of PD is administration of levodopa, the precursor of dopamine. Unlike dopamine, levodopa can cross blood brain barrier where it can then be metabolized into dopamine by the enzyme dopa-decarboxylase.\textsuperscript{88, 89} Because this enzyme is found throughout the body, very large doses of levodopa are required to be effective which can result in severe nausea. To mitigate this problem, dopa-decarboxylase inhibitors such as carbidopa or benserazide (which do not cross the blood brain barrier) are often given with the levodopa dose. There are two drawbacks of levodopa administration: 1) Standard levodopa administration creates imbalance in corticostriatal circuitry, producing abnormal pattern of neuronal discharges, resulting “off” and “on” states with occasional dyskinesia;\textsuperscript{90, 91} and (2) The effect of levodopa is short-lasting and may make the basal ganglia unstable, where pharmacological and compensatory mechanisms act in opposing directions.

1.6.2 Surgical or Invasive Brain Stimulation

The most commonly used surgical intervention for people with PD is deep brain stimulation (DBS) of the STN or GPi.\textsuperscript{92} Although DBS is effective, allowing reduction in medication doses and lessening the side effects of medication, its application is limited
to a small, well-defined PD population. It carries risk of serious surgical side effects along with other negative possible outcomes including psychosis, compulsive behaviours and depression.\textsuperscript{93, 94}

The second type of surgical intervention in individuals with PD is pallidotomy and/or thalamotomy, which are designed to eradicate tonic inhibition of motor output. Pallidotomy induces drastic reduction in GPi efferent activity to motor thalamus and influences dyskinesia. Thalamotomy, on the other hand, reduces thalamocortical drive and enhances bradykinesia. Therefore, both of these surgeries have the potential to actually worsen these motor features.\textsuperscript{91}

1.6.3 Non-Invasive Brain Stimulation

1.6.3.1 Repetitive Transcranial Magnetic Stimulation

Repetitive transcranial magnetic stimulation (rTMS) is a potential treatment for individuals with PD, as it has been shown to impact some motor functions in individuals with PD.\textsuperscript{95} A recent meta-analysis by Elahi et al\textsuperscript{96} and Fregni et al\textsuperscript{97} concluded that high frequency rTMS (applied across different stimulation sites and parameters) has a beneficial effect on the motor signs associated with PD as indexed by motor section of Unified Parkinson Disease Rating Scale (UPDRS). At this time, no randomized control trials have verified the clinical efficacy of rTMS as a therapeutic intervention for people with PD. However, based on promising results from previous pre-clinical studies, for this thesis I aimed to assess the impact of 5 Hz rTMS over SMA on motor symptoms, motor function and control, oscillatory patterns in the brain, and imagery ability in individuals with PD. The theoretical framework is further discussed below.
1.7 Transcranial Magnetic Stimulation - Theoretical Framework

Transcranial magnetic stimulation (TMS) is a non-invasive method of stimulating neurons in the human cerebral cortex. It modifies neuronal activity locally and at distant sites when delivered in series or trains of pulses. When delivered repetitively at frequencies ≥ 5 Hz, rTMS transiently enhances motor excitability; at frequencies ≤ 1 Hz, rTMS transiently depresses excitability. The mechanisms of these changes centers on stimulation of long-term potentiation (LTP) or long-term depression (LTD) of individual synapses in the central nervous system.

Individuals with PD suffer from dysfunction in the SMA (i.e., decreased activation) due to reduced input from the basal ganglia. Past work has demonstrated that high frequency rTMS (10 Hz) can facilitate activation in SMA, dorsolateral pre-frontal cortex (DLPFC) and M1 in individuals with PD. Based on past work showing that rTMS over DLPFC may induce dopamine release in the basal ganglia and increase cortical excitability, I extended past work to determine whether 5 Hz rTMS over SMA would impact motor control and function in individuals with PD.

Secondly, the SMA has dense projections to M1 and spinal cord. SMA particrates in movement preparation, planning and coding of movements ahead of execution (e.g., motor memory) and then transmits this preparatory activity to M1. However, in people with PD activation of the SMA is diminished leading to decreased motor initiation, preparation and execution. Basal ganglia degeneration further impairs the control of movement amplitude and duration, and the rate of force production; the net result is bradykinetic movements. Thus, in the present
study, we considered the potential of 5 Hz rTMS to alter SMA-basal ganglia-M1 circuitry to investigate immediate effects on improved motor control (speed and accuracy) and stroke size (handwriting) in individuals with PD.

In addition, Aizawa et al\textsuperscript{111} reported that M1 neurons receive inputs from SMA, rather than from the thalamus or the parietal cortex during motor imagery (which requires internally cuing of movements). Thus, activating SMA using excitatory rTMS may facilitate movement in two ways. First, enhanced excitability in SMA may reinforce the formation of memories for movement. Second, it may restore the SMA’s participation in movement preparation.

The third rationale for using 5 Hz rTMS over SMA is to alter cortical (M1) activity. Imaging and neurophysiologic studies have demonstrated that PD causes a pathological pattern of neuronal firing in different brain areas. This pathological pattern is associated with altered resting state activity of primary motor and premotor cortices as well as STN and globus pallidus in the advanced stages of the PD,\textsuperscript{21, 112} which may lead to pathological synchronization within β band (13-30 Hz) oscillations, generated by GPe-STN circuit. This synchronization is correlated with bradykinesia and rigidity in PD.\textsuperscript{21, 113, 114}

Cortical over-activity noted in people with PD also results in lower resting motor threshold (RMT) and higher amplitude of MEPs as compared to healthy individuals.\textsuperscript{49-51} Altered cortical activity in people with PD results in shifts in resting motor threshold as compared to healthy individuals.\textsuperscript{49-51} This is attributed to impaired cortico-motoneuronal output to the cortex. Thus, by stimulating SMA in individuals with PD, we may shift
cortical excitability closer to normal through SMA connections with M1 and the basal ganglia.

In this study, I chose to stimulate SMA and not M1 for two reasons: 1) Past work has shown that SMA is underactive in individuals with PD, and 2) the stimulation intensity was 110% of RMT, which would result in uncomfortable muscle twitches if stimulated over M1. Further, SMA may be a more promising therapeutic target given its known role in motor planning and movement control. As this was a preliminary study, I chose stimulation frequency of 5 Hz to determine its effect on motor and functional tasks.

1.7.1 TMS Safety

TMS is a safe, non-invasive, and relatively painless method for stimulating the cortex. All the stimulation parameters used in the present study were within published safety standards. The risk of seizure or other adverse events for our study participants was real but minimal. To our knowledge there has never been an incident of seizure associated with rTMS or single-pulse TMS in an individual with PD and no adverse events occurred within the population examined for this study. The Clinical Ethics Board at the University of British Columbia ethically approved all study procedures (H09-00673).

1.8 Objectives and Hypothesis of the Thesis

The main goal of this study was to determine whether 5 Hz rTMS over SMA improved bradykinesia indexed by a serial targeting task – STT and a handwriting task in individuals with PD. I proposed that 5 Hz rTMS over SMA would improve motor symptoms (bradykinesia) in people with PD. The second aim of the study was to record
cortical activity (oscillations) using electroencephalogram (EEG) during a motor task (the STT) to index the effects of rTMS on cortical oscillatory activity, especially in α and β bands (8-35 Hz). I proposed that along with improvement in motor performance, there would be decrease in α and β oscillations during performance of the motor task. The third goal of the study was to determine the role of SMA in imagining movements (motor imagery) in individuals with PD. I proposed that 5 Hz rTMS over SMA would facilitate motor imagery ability in individuals with PD.

1.8.1 Specific Aim 1: To study the impact of 5 Hz rTMS over SMA on hand motor control in individuals with PD.

SMA has connections with primary motor cortex, which function both to transmit preparatory activity and generate actual movements. I hypothesized that 5 Hz rTMS over SMA would facilitate targeting speed and accuracy as compared to the delivery of control 5 Hz rTMS over SMA (Hypothesis 1). I expect that stimulating SMA using 5Hz rTMS would facilitate improved motor control. I also hypothesized that 5 Hz rTMS over SMA would improve stroke size and speed in individuals with PD, as indexed by improvements in handwriting (Hypothesis 2).

1.8.2 Specific Aim 2: To study the impact of 5 Hz rTMS on cortical oscillatory activity in α and β bands (8-35 Hz) and corticomotor excitability in M1 in individuals with PD.

Oscillations within the β band (13-35 Hz) generated in the basal ganglia – thalamocortical circuits are prevalent in PD. The exaggerated oscillatory activity in the basal ganglia in people with PD suggests a link with impaired movement. As an exploratory investigation, I hypothesized that excitatory rTMS over SMA would reduce oscillatory activity in α and β bands during a motor control task (Hypothesis 3), as recorded through EEG.
SMA has efferent outputs to the striatum\textsuperscript{7,8} and projects to GPi both directly and indirectly\textsuperscript{9} to form an important cortico-subcortical loop. Studies suggest that disrupted function of the cortico-subcortical loop in people with PD results in altered influence on M1; the net result is higher RMT in individuals with PD.\textsuperscript{49} I proposed that 5 Hz rTMS over SMA, would influence M1 excitability. Based on this information, I hypothesized that 5 Hz rTMS over SMA would decrease RMT in M1 as compared to participants with PD who received control rTMS over SMA (Hypothesis 4).

1.8.3 **Secondary Aim: To explore the impact of 5 Hz rTMS over SMA on the ability to imagine movements in individuals with Parkinson Disease.**

The SMA functions in part to support movement preparation and generation on the basis of memory; however, individuals with PD suffer from a poor ability to activate this brain region. Because PD disrupts basal ganglia function and causes motor deficits, it is tempting to conclude that this disease will also impact MI. However, no work has directly assessed the question of whether or not people with PD can imagine movement. One impediment to work considering MI ability in individuals with PD is the need for a reliable and valid MI assessment scale. We recently established reliability and validity of the kinesthetic and visual imagery questionnaire (KVIQ) in individuals with PD.\textsuperscript{118} Because the KVIQ is a long questionnaire and given the short timeframe that rTMS aftereffects persist following brain stimulation, I was unable to administer it pre-post rTMS. Therefore, I recorded baseline imagery ability using the KVIQ and assessed pre-post rTMS imagery ability via a break test.\textsuperscript{119} Past work has demonstrated that 5Hz rTMS can alter activation of the SMA but none has considered the effect of brain stimulation on MI. Based on this knowledge, I explored the hypothesis
that 5 Hz rTMS over SMA will enhance the ability to imagine movements as compared to control rTMS over SMA as indexed by a break Test\textsuperscript{119} (Hypothesis 5).

1.9 Significance of the Study

The proposed study is the first study to link changes in motor control, function, oscillatory patterns, cortical excitability and MI before and after the application of 5 Hz rTMS. As such it is an essential first step in determining what functions may be altered by 5 Hz rTMS in individuals with PD. In turn, it may illustrate a potential new intervention for individuals with PD.

1.10 Experimental Design of the Study

The study involved three experimental tasks; each was designed to consider a unique function of the motor system. The serial targeting task was employed to assess changes in upper extremity motor control, the handwriting task to test motor function and the break test to consider the ability to mentally imagine movement. Each test was completed prior to, and immediately after, the rTMS intervention (Table 1-1). To control for the possibility that PD affected participants so severely that they were unable to imagine movement baseline imagery ability was assessed prior to the delivery of rTMS using the KVIQ in the first testing session. As the KVIQ is long, (taking over 20 minutes to administer), it was not repeated. To consider the impact of rTMS on cortical oscillations, EEG was collected during the serial targeting task. In addition, cortical excitability changes were indexed by assessing resting motor threshold prior to, and after rTMS intervention. To limit the impact of variability in the motor ability and symptoms of my participants, I employed a cross-over design where each individual in the study received both active 5 Hz rTMS and control, sham stimulation in separate
sessions. The order of stimulation type was randomly assigned. To allow for effects of stimulation to dissipate, at least 1 week separated each rTMS session.

| Time |
|-----------------|-----------------|-----------------|
| **PRE-Intervention** | **INTERVENTION** | **POST-Intervention** |
| KVIQ | RMT<sub>pre</sub> | rTMS | RMT<sub>post</sub> | Break Test |
| Break Test | | (5 Hz or Control) | | Handwriting |
| Handwriting | | | | |
| STT (Motor Task) + EEG | | | STT + EEG |
| Table 1-1: Experimental design of the study. Participants were randomly allocated to receive either 5 Hz or Control rTMS first. Following a 1-week washout period they were crossed-over to the opposite form of stimulation. All procedures and testing were identical pre-post rTMS, and in the two arms of the cross-over. |
Chapter 2 Repetitive Transcranial Magnetic Stimulation over Supplementary Motor Area benefits Motor Task Performance and Modulates Cortical Oscillations in Individuals with Parkinson Disease

2.1 Preamble

Chapter 2 explores the impact of 5 Hz rTMS over SMA on motor tasks, cortical oscillations and motor excitability in individuals with PD. In this chapter, I first present the effect of 5Hz rTMS over SMA on motor performance in individuals with PD while performing a novel motor task (serial targeting task); EEG data were collected simultaneously from the same group. EEG data were used to characterize the impact of 5 Hz rTMS on cortical oscillations during controlled movement. Finally, I present results showing altered cortico-motor excitability in M1 following with 5Hz rTMS over SMA.

2.2 Introduction

PD is a neurodegenerative disease\(^1\) caused by loss of dopamine in the striatum. Reduced dopamine alters functioning of nigrostriatal pathways\(^52\) as well as cortico-basal ganglia loops\(^65\) leading to bradykinesia, impaired movements, abnormal oscillatory activity and altered cortical excitability.

PD leads to a number of movement deficits in addition to bradykinesia, including impaired scaling of movement speed with respect to distance\(^57,60\) and poor accuracy which may be characterized by variability of endpoints for discrete movements.\(^58\) Neuroimaging studies provide evidence that SMA, putamen and cerebellum are important components of a network that is active to plan and execute movements that are fast and accurate.\(^61,62\) However in individuals with PD, basal ganglia impairment
reduces the capability to control and modify velocity/acceleration associated with aimed movements.\textsuperscript{63-65}

There is growing evidence that abnormal oscillations in the basal ganglia and associated regions of the thalamus and cortex may play role in pathophysiology of PD.\textsuperscript{22, 23} Oscillatory activity in the basal ganglia may be subdivided into roughly four bands, $\theta$ (<8 Hz), $\alpha$ (8-12 Hz), $\beta$ (13-35 Hz), and $\gamma$ (>35 Hz).\textsuperscript{22, 24} In healthy individuals, circuits in the cortico-subcortical network are known to possess intrinsic oscillatory activity. The STN plays central role in this network. Low-frequency oscillations are related to long-range communication between different brain regions,\textsuperscript{36} whereas high frequency ($\gamma$) activity has been related to local cortical processing.\textsuperscript{37-39} Activity within the $\alpha$ band appears to be shifted by both sensory and motor-related processing,\textsuperscript{40} local $\alpha$ band activity has classically been assumed to indicate inactivity (‘idling’) of the underlying cortex. Normally, the decrease of oscillatory activity in the $\alpha$ band is considered prerequisite for active information processing.\textsuperscript{38} Praamstra et al.\textsuperscript{120} suggested that reduction in activity in the $\alpha$ band in healthy individuals is related to termination of a state of heightened sensitivity to expected stimulation. In contrast, the $\beta$ band appears to play role in controlling motor activity and, suppression of $\beta$ oscillations has been reported before and during motor tasks.\textsuperscript{41, 42} However in PD, excessive $\beta$ band synchronization (~ 20 Hz) is reported and has been linked to movement impairment. Recently, Kuhn et al.\textsuperscript{42} reported that suppression of $\beta$-power local field potential (LFP) prior to movement, with an onset latency that strongly correlated with mean reaction time in individuals with PD performing visually guided choice reaction task. Similarly another experiment by Williams et al.,\textsuperscript{121} pointed to the role of $\beta$-power
suppression in movement preparation in a choice reaction paradigm, where warning cues were presented prior to the go cue. Furthermore,\textsuperscript{122} reported lower β band suppression and α band augmentation in individuals with PD-off medication as compared to healthy during movement phase of the visually guided choice reaction task. These studies suggest a role for the α and β bands in the generation of voluntary movement.

Recent studies suggest that oscillatory activity in the basal ganglia is modulated by input from the cortex to the STN, especially via M1 and SMA. SMA sends efferents to the striatum,\textsuperscript{7, 8} projecting to GPi both directly and indirectly.\textsuperscript{9} In addition, the SMA has connections with the STN,\textsuperscript{10} to control ongoing activity in the cortical-basal ganglia circuit.\textsuperscript{11} The SMA has also dense projections to M1.\textsuperscript{27-29, 31, 33} Given its central location in each of these circuits, the SMA may be able to modify neuronal activity in the basal ganglia, STN and M1, and possibly shift ongoing oscillatory activity.

To date, only a few studies have focused on the possibility that rTMS may influence oscillations in the brain\textsuperscript{123} and/or basal ganglia function in healthy people or individuals with PD. Recently, Gaynor et al\textsuperscript{124} showed decreased LFP in the STN in the 8-35 Hz band following single-pulse TMS over SMA or M1 in individuals with PD. Matsunaga et al\textsuperscript{101} noted that 5 Hz rTMS over SMA can induce short-lasting facilitation of excitability of M1, in healthy individuals. In addition, by combining rTMS and positron emission tomography (PET) the impact of cortical stimulation on endogenous dopamine release has been considered. 10 Hz rTMS over DLPFC releases dopamine in the ipsilateral caudate nucleus,\textsuperscript{102} while 10 Hz rTMS over M1 stimulates dopamine release in ipsilateral putamen.\textsuperscript{104, 105} Recently, Hamada et al\textsuperscript{95} showed improved Unified
Parkinson Disease Rating Scale – III (UPDRS-III) scores immediately following 5Hz rTMS over SMA. However, as the UPDRS is a global measure of motor state these data do not reveal which aspects of motor performance changed in response to rTMS. To date, no work has employed a controlled motor task in conjunction with rTMS to determine the effect of altered SMA excitability on motor control and pathological oscillations in individuals with PD.

Therefore, the current study aimed to investigate the effect of 5 Hz rTMS over SMA on motor performance indexed via a novel serial targeting task (STT). In addition, we considered whether 5Hz rTMS over SMA impacts cortical oscillations during STT performance in individuals with PD.

The first aim of the study was to investigate the immediate effects of a single session of 5 Hz rTMS over SMA on motor control indexed via STT performance in individuals with PD. We hypothesized that 5 Hz rTMS over SMA would facilitate targeting speed and end-point accuracy as compared to the delivery of sham, control rTMS over SMA.

The second aim of the study was to determine the effect of 5Hz rTMS on cortical oscillations in individuals with PD. Our main emphasis was on change in α and β band. As a secondary analysis the θ and γ frequencies were considered to facilitate understanding of the overall effect of rTMS on all frequencies ranging from 1 – 50 Hz. We hypothesized that stimulation over SMA with 5 Hz rTMS would decrease α and β oscillatory activity during motor task performance.

The third aim of this study was to determine if 5 Hz rTMS over SMA would alter M1 cortical excitability. Some imaging and neurophysiologic studies have demonstrated
that PD shifts neuronal activity resulting in impairment of motor cortex activation.\textsuperscript{49-51} This is attributed to impaired cortico-motoneuronal inhibitory output to cortex. Given the robust connections between SMA and M1,\textsuperscript{9, 109} we anticipated that 5 Hz rTMS over SMA in individuals with PD would facilitate M1 excitability as shown by decreased resting motor thresholds following stimulation.

2.3 Methods

2.3.1 Participants

Ten individuals with PD (Mean age: 70.5 years; 9 male, 1 female) participated (Table 2-1). All had a neurologist-confirmed diagnosis of PD and were on-medication (dopamine supplements). The medication list for all participants is reported in Table 2-1. All were right hand dominant except one participant. PD predominantly affected the right side for 6 of the 10 participants. To characterize disease status UPDRS-III and Hoehn and Yahr’s (H&Y) scores were determined by a physiotherapist prior to testing (Table 2-1). Due to time constraints, the UPDRS-III was not repeated post rTMS. Exclusion criteria included: 1) age above 80; 2) cognitive dysfunction (i.e., Montreal Cognitive Assessment <24); 3) any psychiatric disturbances; 4) any neuromuscular, skeletal, cardiovascular conditions that might interfere in participating in the study; 5) any past history of seizures/epilepsy, substance abuse or head trauma, stroke, tumour or 6) severe PD (H&Y stage >3), making it difficult to maintain sitting balance, or unable to move in response to a command to perform movement. Additional exclusion criteria for functional magnetic resonance imaging (fMRI) anatomical scanning and TMS mapping included pacemaker, pregnancy, metallic objects in body or claustrophobia.
This study employed a cross-over design, with all participants receiving active 5 Hz rTMS and as a control, inactive sham 5 Hz rTMS over SMA in separate sessions; a minimum of one week separated each testing day. The order of rTMS type was randomly assigned. Participants were tested while on their regular medication schedule; interviews confirmed that medication status did not change during the period of study participation. To control for medication-induced fluctuations in function, all participants were tested at the same time of day for each of the two sessions, two hours before their next medication dosage. That is, testing occurred during the second phase of medication cycle, in an effort to establish the maximum additional benefit that might be conferred by rTMS. All participants gave informed, written consent and all procedures were institutionally and ethically approved. To minimize variability, all participants used their right hand for behavioural testing, while repetitive stimulation was delivered over left SMA.

2.3.2 TMS Protocol

For all TMS stimulation, a 70-mm figure-of-eight air-cooled coil was used, with a Magstim SuperRapid stimulator (Magstim Company, Ltd.). For TMS participants were seated in a semi-reclined dental chair with their arms bent and supported by armrests. During stimulation of both M1 for thresholding and SMA for rTMS, the TMS coil was oriented tangentially to the scalp with the handle pointing back and away from midline at 45 degrees. The magnetic stimulus had a biphasic waveform with a pulse width of 400 us. On a separate day, prior to the start of the experiment, each participant had an anatomical MRI scan at the UBC 3T MRI Centre (T1 images TE = 5 ms, TR = 24 ms, 40° flip angle, NEX = 1, thickness = 1.2 mm, FOV = 256 mm). These images were imported into Brainsight™ TMS neuronavigation software (Rogue Research Inc.) to
allow for stereotaxic registration of the participant's brain with the TMS coil for online control of the trajectory of stimulation and to ensure consistency of stimulation location across experimental days. Each participant's brain was transformed into standard Talairach space using Brainsight software. This enabled standardization of rTMS delivery over known coordinates for SMA: -5,-3,52.4,126 as shown in Figure 2-1.

Motor evoked potentials (MEPs) were used to determine the coil position that evoked the maximal response ("hot spot") in the right flexor carpi radialis (FCR). MEP amplitude was monitored by surface electromyography (EMG) over the participants right FCR using the evoked potential unit of the Super Rapid² control unit (Magstim Super Rapid², Magstim Company, Ltd). Once the location and trajectory of the coil was determined for this "hot-spot" it was marked using Brainsight™ to minimize variability of coil location over experimental sessions. The motor cortical hotspot was verified during each experimental session, as well as pre- and post-rTMS. Following determination of the motor cortical "hot spot", RMT was defined as the percentage of stimulator output intensity that elicits a MEP >50µV in 5 out of 10 trials. Figure 2-2 shows the site of stimulation as recorder in Brainsight™ for left M1 and left SMA.
Figure 2-1: Talairach coordinates of SMA. The coordinates are in millimetres from the origin of the standard space of Talairach and Tournoux (1988), where $x$ indicates the distance from the midline in the coronal plane, $y$ indicates the distance from the anterior commissure (Vca line) in the sagittal plane, and $z$ indicates the vertical distance from a plane crossing both the anterior and posterior commissures. Figure modified from Picard et al.4

Figure 2-2: Sterotaxic system for coil placement. Brainsight™ is used to locate Left SMA (as per Talairach coordinates) and Left M1; markers were placed to ensure accuracy of coil placement.
Individuals were randomly assigned to first participate in 5 Hz or control rTMS group. All participants were naïve to TMS and were blinded to group assignment. Using an identical custom sham coil that looks and sounds exactly like an active rTMS coil control (sham) stimulation was delivered over SMA. rTMS stimuli were delivered at 110% of RMT at a frequency of 5 Hz over the SMA, for 1200 pulses (approximately 6 minutes). One participant reported discomfort at 110% intensity and thus was stimulated at 100% RMT. All stimulation parameters were in accordance with published safety standards.

2.3.3 Serial Targeting Task (STT- Motor Behaviour Task)

The STT consisted of a sequence of circular (2.6 cm in diameter) serial targets that were presented sequentially at various equidistant (12.12 cm) spatial locations (Figure 2-3). Target locations were randomly generated but counterbalanced so that an equal number appears on the left and right sides of the computer screen. Participants were seated in a chair in front of a computer screen (37.7 x 30 cm) and used their right hand to control an adapted mouse to move a cursor (red dot, ~ 2 cm diameter) between the sequential targets. The mouse cursor was located on a desk in front of the computer screen. The chair was adjusted for height to allow the right arm to be comfortably placed on the table with the elbow below shoulder height. Participants were instructed to move as fast and accurately as possible between the targets by taking the most direct route between the start point and the end point target. Participants were required to hold the cursor inside the target for 500 msec to trigger the presentation of the next target. On each day participants moved between 60 targets both before and after rTMS (Figure 2-3). Each participant performed the same randomly generated sequence of target locations.
Target presentation and mouse position were controlled using custom software (Labview 8.1, National Instruments Co., Austin, TX). The mouse (windows-based) was physically centered on the pad. The computer program then centered the mouse on the screen before the first target. Sensitivity settings were identical for all participants. Mouse position was sampled at 200 Hz according to the Cartesian pixel coordinates of the screen's resolution (1280 x 1050) and stored for offline analysis.

Custom software (Labview v.8.1; National Instruments Co.) was used to extract behavioral and kinematic measures. The raw Cartesian pixel coordinates for each sampling period were converted into distance by calculating the tangent between each the x,y pixel coordinates of each sampling period. This tangent was converted to mm according to the scale of the screen (3.3 pixels per mm) and low-pass filtered at 5 Hz. Reaction Time (RT) was defined as the time (in seconds) from target presentation to the time to initiate movement toward that target that exceed 1.2 times the diameter of the target. Movement Time (MT) was defined as the time from target appearance to the presentation of the next target, corrected for the duration of the RT and the 500 ms inside the target. End point accuracy was defined as the tangent (in mm) between the center of the target and the participant's end point. End point accuracy was indexed as absolute error and recorded as overall deviation from center of the target without considering the direction.

Kinematic variables were extracted using the same custom software used to extract the behavioral variables. The first derivative of the magnitude by time waveform was used for velocity while the second derivative was used for measures of acceleration. Peak velocity (mm/sec) was defined as the peak amplitude during the first
50% of the distance between the start and end target positions. Peak acceleration (mm/sec\(^2\)) was defined as the peak amplitude of the second derivative during the first 50% of the distance between the start and end target locations. Peak deceleration (mm/sec\(^2\)) was defined as the minimum during this period.

**Figure 2-3 (A-D): Serial Targeting Task (STT).**
Adapted mouse to track targets; (B) Sample condition of STT showing target as white circle and cursor as red dot; the distance to the target (amplitude) and width of the target was the same for all trials; (C) Sample sequence of targets for STT. Sixty total targets appeared one at a time in a random sequence. Participants began the trial in the center of the screen, then were instructed to move as fast and accurately as possible to the target when it appeared. Once the target was hit, participants had to stay in...
it for 500 ms before the next target was displayed; (D) STT phases: Rest - corresponded to 500 msec prior to the presentation of the target; Reaction Time - the time from target appearance to the initiation of movement; Movement Time - the time between the start and the end of the movement.

### 2.3.4 EEG Data Collection and Processing

To consider the impact of rTMS over SMA on oscillatory brain activity EEG data were recorded during STT performance both before and after 5Hz rTMS and control rTMS using a 32 Ag/AgCl channel electrode cap (EasyCap, Herrsching-Breitbrunn, Germany) referenced to linked mastoids. The data were recorded over 20 active channels (as shown in Figure 2-4) using 10-20 placement system. Eye artifacts were recorded by surface electrodes placed above and to the side of right eye. Channel impedance was less than 5 kΩ. EEG data was recorded at 250 Hz, amplified 40,000X and filtered (1-200Hz, 6dB octave roll-off, Grass Neurodata Acquisition System 12B), digitized (250 Hz, using custom software, Labview 8.6, PCI-6078E Series A/D board with a SCB-99 pin-out), and stored for further analysis.

EEG data were analyzed using the EEGLab toolbox (Institute for Neural Computation, University of California – San Diego, San Diego, CA) for MATLAB v2009 (The Mathworks, Natick, MA). All EEG channels were inspected for noise artifacts. As a result of this analysis EEG data from the occipital channels (Oz, O1 and O2) were excluded from further analysis due to excessive noise in the majority of participants. The remaining channels were then re-referenced offline to the global average of the remaining EEG channels and corrected for movement and ocular artifacts. The data were bandpass filtered at 1-100 Hz and notch filtered at 60 Hz. Each channel was then converted to unit variance by normalizing to the mean amplitude of the channel. Next the data were averaged over five electrode regions: Fronto-central (FC: Fz, Fcz, F3, F4,
FC3, FC4), Central (Cen: Cz, Pz and Cpz), Left Sensorimotor (L_SM: Cp3, C3 and P3) and Right Sensorimotor (R_SM: Cp4, C4 and P4).

![Headplot of electrode regions.](image)

The average EEG signal for each electrode group was then epoched according to the timing of the STT. The epoched data consisted of three phases: the ‘rest phase’ corresponded to 500 msec prior to the presentation of the target, the ‘reaction phase’ was defined as the time from target appearance to the initiation of movement and ‘movement’ was defined as time between the end of reaction and the end of the movement. Power spectral density (PSD - with hanning window of 1024 samples and overlap of 512 samples) was then calculated for each epoch and electrode combination. For group analyses these epochs were averaged for each electrode region and participant. The mean change in power for each electrode group and frequency was calculated.

To account for potential changes in baseline EEG activity before and after rTMS, EEG was collected for 60 seconds while participants were at rest, fixating on a central point. A baseline epoch for each electrode group was then extracted using only the
middle thirty seconds of the 60-second block. The PSD was calculated for each electrode combination as described above. Finally, the baseline PSD was subtracted from the STT EEG data to account for non-specific rTMS shifts (Figure A-1).

PSD was quantified by calculating the area under the curve for the various frequency bands (θ: 0-8 Hz, α: 8-12 Hz, β: 13-35 Hz and γ: 35-50 Hz). Area under the curve was calculated by taking the integral for each range of frequencies (Appendix-1, Figure A-1). EEG was collected for all individuals in this study except participant 3 who was excluded from EEG analysis due to data artifacts.

As this was a single session (cross-over) study, participants first performed the STT while EEG was recorded (pre-TMS), then underwent rTMS and then performed STT again while EEG data were recorded a second time (post-TMS). As the effect of rTMS has been shown to last induce approximately 25-minute the performance of the STT and EEG data collection were completed in this time following stimulation delivery.

2.4 Statistical Analysis

2.4.1 STT

Separate 2 (Session: 5 Hz, control rTMS) by 2 (Time: pre-, post stimulation) repeated measures Analysis of Variance (ANOVA) was performed for each dependent variable. All statistical analyses were performed using SPSS software (v.14) and the threshold for significance was set to P ≤ 0.05.

2.4.2 STT- EEG

Separate 2 (Session: 5 Hz, control) X 3 (Task phase: rest, reaction, movement) repeated measures ANOVA on the area under the curve for each frequency range (θ, α,
β and γ) were performed using SPSS software (v.14). Significant interactions (Session by Task) were decomposed using T-tests.

### 2.4.3 RMT

A 2 (Session: 5 Hz, control rTMS) by 2 (Time: pre-, post stimulation) repeated measures ANOVA was performed for RMT.

### 2.5 Results

#### 2.5.1 STT

The means for all variables in STT are shown in Table 2.2 and Figure 2.5. Example data from one participant for movement, velocity and acceleration profiles, are shown in Appendix – 1.

**Reaction Time (RT):** The two-way ANOVAs considering reaction time revealed a main effect of Time ($F(1,9) = 6.87, P = 0.03$). The main effect can be attributed to a decrease in the time to initiate movement following appearance of the target in both the 5 Hz and control rTMS groups. There was not an interaction effect for RT ($P=0.64$).

**Movement Time (MT):** The two-way ANOVAs upon movement time failed to reveal any significant effects of Session ($P = 0.96$) or Time ($P=.67$). There was not an interaction effect for MT ($P=0.98$).

**Peak Velocity:** A trend for an interaction between Session and Time was noted ($F(1,9) = 4.51, P = 0.06$) largely as the result of higher peak velocity after control rTMS (32.56 mm/sec), and reduced peak velocity after 5 Hz rTMS (-10.32 mm/sec).

**Peak Acceleration and Peak Deceleration:** Significant interactions between Session and Time were noted both for peak acceleration ($F(1,9) = 6.63, P = 0.03$) and
peak deceleration ($F(1,9) = 7.49, P = 0.02$). Participants were faster to accelerate and decelerate after control rTMS (by 513.1 mm/sec$^2$, and -518 mm/sec$^2$ respectively) as compared to after 5 Hz rTMS (-170.78 mm/sec$^2$ and 56 mm/sec$^2$ respectively; Figure 2-5 D and E).

**End Point Accuracy (EP):** The two way ANOVA upon end-point accuracy revealed a Session by Time interaction ($F(1,9) = 6.96, P = 0.03$). This interaction can be attributed to a decrease in the deviation from the center of the target following 5 Hz rTMS (Figure 2-5 F).

### 2.5.2 STT - EEG

The mean change in power for each electrode group and frequency is shown in Table 2.3.

**θ-band:** There was a trend for a main effect of Task phase on θ oscillations measured from FC channel electrodes ($F(1,8) = 3.73, P = 0.06$), suggesting different portions of the task (rest, reaction and movement) affected θ oscillations differently in both 5 Hz and control groups.

**α-band:** A significant interaction between Session and Task for α oscillations was measured from Cen channel group ($F (1, 8) = 4.18, P = 0.04$; Figure 2-6 B). Follow up paired t-tests revealed that after 5 Hz rTMS α oscillations were significantly lower from control rTMS during reaction phase of the task ($P = 0.01$); this was not the case during rest or movement.

**β- and γ-band:** Contrary to our hypothesis, there were no effects of Session or Time for the β- or γ-bands.
2.5.3 RMT

A Session by Time interaction was noted for RMT (*F*(1,9) = 5.25, *P* = 0.05). This was the result of lower RMT after 5 Hz (*P* = 0.02). This was not the case following control rTMS (Table 2-4, Figure 2-7).

2.6 Discussion

In individuals with PD 5 Hz rTMS over SMA resulted in more accurate but slower movements during performance of a novel motor task. In addition, 5 Hz rTMS over SMA influenced cortical oscillations in the α range in the central channel grouping, and increased M1 cortical excitability as reflected by lower resting motor thresholds. It is possible that the changes noted in this study were the result of 5 Hz rTMS altering activity in the SMA, which may have facilitated movements by modifying subcortical circuitry (SMA-STN-basal ganglia circuitry). We speculate that 5 Hz rTMS over SMA may have influenced subcortical circuitry and facilitated movements in two ways. First, 5 Hz rTMS over SMA may have modified activity in the basal ganglia and motor cortex through the GP direct route and subsequently changed motor performance via modulating oscillations in the α band. Secondly, past work\textsuperscript{102, 104, 105} suggests that 5 Hz rTMS induces the release of dopamine in the basal ganglia. Though we did not directly assess the release of dopamine, it is also possible that rTMS induced a dopamine release in the basal ganglia, which influenced voluntary movement and changed oscillatory behaviour. In addition, our data also suggest that stimulation over SMA facilitated M1 excitability. This effect was also a likely source of the changed motor performance and shifted cortical oscillations that were observed.
The behavioural results of the current study are in line with previous studies that report beneficial effects of rTMS in individuals with PD\textsuperscript{98, 130, 131} In the present study, we presumably induced a change in the functional interactions between SMA, M1, STN and basal ganglia via the cortico-subcortical loops. Animal studies suggest that the SMA is richly linked with both M1 and the basal ganglia. The SMA acts with basal ganglia to both prepare and execute movement.\textsuperscript{132-135} In the present study, we propose that 5Hz rTMS over SMA may have facilitated motor performance as shown by more controlled (slower) but more accurate movements following stimulation. Further, after 5 Hz rTMS α oscillations were significantly lower during the reaction time phase, which may indicate enhanced preparation for the upcoming movement. Evidence from the literature suggests that α activity correlates with working memory\textsuperscript{136-138} and attention. The decrease in α activity is considered beneficial for visual detection and discrimination performance.\textsuperscript{139} The decrease in α oscillations in the current study suggests that participants may have been able to process information related to the target more effectively during reaction phase. In turn enhanced preparation may have led to more accurate STT performance as shown by less end point deviation about the target.

From our findings we also speculate that 5 Hz rTMS may have modulated the impact that SMA had on the STN and basal ganglia. The SMA send efferents to the STN and this an important route for modulation of ongoing cortico-basal ganglia activity. Further, the STN plays a significant role in the execution of motor tasks through projections to output nuclei (GPe and SN). Past work proposed that the STN plays a key role in the speed-accuracy trade off.\textsuperscript{11, 140} It may be that 5Hz rTMS over SMA altered activity in the STN, and hence helped individuals with PD to generate more
accurate movements. The net result was slower but more accurate movements after 5Hz rTMS as compared to control rTMS.

In addition, we observed modulation of θ oscillations during different task phases in both groups under the FC channels. Others have suggested that θ amplitude has a characteristic FC distribution and may be related to recurrent co-activation between frontal cortex and the anterior cingulate and/or the hippocampus. Previous EEG/ magneto-encephalography (MEG) research found that θ amplitude increases over FC regions during cognitively demanding tasks (e.g., working memory) that scales with cognitive load and correlated with selective attention. However, the significance of differential modulation observed in θ in the current study after both rTMS is unclear. The decrease in θ oscillations during reaction phase and increase in other phases of the task in this study in both rTMS groups suggest that θ band was more sensitive to task phases than to rTMS. Future studies are warranted to tease out the role of θ in individuals with PD.

Contrary to our hypothesis, we did not notice any significant change in β oscillation in either group. This finding may be due to three reasons: First, the literature suggests a role for dopamine in modulating coherent β oscillatory activity in the basal ganglia and STN. It has been reported that dopamine can reduce overall abnormal oscillations in individuals with PD in an attempt to normalize the brain connectivity. Kuhn et al. reported suppression of STN β oscillations following ‘Levodopa’ medication in individuals with PD. In the present study, all participants were tested on medication; possibly medication effects masked antikinetic β oscillations. Second, β oscillations have been related by some authors to tremor. None of the participants
the present study had tremor. Third, the STT task requires relatively continuous movements. However, much of the literature reporting change in β oscillations used discrete Go-NoGo task tasks\(^{42, 122}\) which reveal increases in β when individuals are cued to stop the ongoing task. Finally, modulation of β oscillations associated with movement have largely been shown by LFP recordings from DBS, with only one revealing similar results using scalp EEG. In the current study we employed scalp EEG but with a continuous task; both may have reduced our ability to demonstrate modulation of the β oscillations.

Finally, we discovered that 5Hz rTMS over SMA lowered RMT in M1. This finding suggests that stimulation over SMA altered the excitability of interconnected brain regions. In the current study, we recorded RMT over M1 which is an indicator of membrane-related aspects of pyramidal cell excitability\(^{148}\) reflecting corticospinal excitability. Chen et al\(^{48}\) reported enhanced corticospinal motor output excitability in individuals with PD during rest, pre-movement and post movement periods, however, others have reported no change in RMT in individuals with PD.\(^{149}\) Valls-Sole et al\(^{150}\) suggested that the excessive tonic corticospinal activity may be coupled with relative failure of volitional recruitment. Therefore, individuals with PD may have to recruit more neurons to generate motor output. In this study we report lowering of RMT post 5 Hz rTMS, suggesting that rTMS over SMA shifted M1 neuronal activity; the net effect may be more effective recruitment of neurons.

Taken together with past work\(^{102, 105}\) it appears possible that the improved endpoint accuracy during motor task performance and shifts in cortical oscillations following 5 Hz rTMS over SMA may be related to an increase in dopamine in the BG. Earlier
studies of people with PD have reported release of dopamine in caudate and putamen after rTMS over M1 and DLPFC, respectively. \textsuperscript{102, 105} Electrophysiological and animal studies suggest that cortico-striatal neuronal connections influence striatal dopamine release\textsuperscript{151-154} through modulation of dopamine neuron firing or direct effects on dopamine nerve terminals. \textsuperscript{102} All participants in this study were on Levodopa medication. The literature\textsuperscript{155} suggests that the maximum impact of Levodopa on movement times (RT and MT) occurs 60 minutes after administration, this effect starts deteriorating after 2-2.5 hours and reaches pre-medication level at 4 hours. We tested participants at this second (declining) phase of the medication cycle, to understand the effect of rTMS in a relatively lower dopamine supplement state. In the present study, we did not measure the release of dopamine directly, but given our effects on endpoint accuracy and α oscillations we speculate that one possible mechanism for these changes may be that endogenous dopamine was released by our stimulation over SMA. Studies that employ PET scanning in conjunction with rTMS over SMA will have to be run to test this hypothesis.

Several limitations impact the conclusions that may be drawn from this work. First, our participants were stimulated on-medication. Thus, it is possible that we would have noted larger differences following rTMS if we tested individuals who were off-medication. We did control for any medication cycle effects by testing individuals in the same relative phase of their medication cycle, two hours before their next medication dosage. That is, testing was performed during second phase of medication cycle, to record the maximum add-on effect of rTMS. However, we did note significant effects of rTMS on motor control despite individuals in this study being on medication. Second, we did not measure MEP amplitude/ MEP recruitment curves to record cortical
excitability over a range of stimulation intensities. However, we recorded RMT changes pre-post rTMS and observed change in M1 excitability. Further, our work provides justification for future studies that do employ recruitment curves. Other methodological limitations include the small sample size, heterogeneity of participants in terms of age, disease duration, medication use, and gender. In addition given our small sample size we are prone to family wise error rates. Given that this is the first study to consider the impact of 5 Hz rTMS over SMA in individuals with PD our data may be used for to power future work to detect significant effects associated with stimulation over SMA.

To our knowledge this is the first study to show the impact of 5Hz rTMS over in individuals with PD during the performance of serial targeting movements. We noted that individuals with PD were slower but more accurate after 5Hz rTMS over SMA. In addition, there was concurrent reduction in α oscillations during the reaction time phase of the task in the central channel grouping. These effects were tested only in the short term. Future work will have to consider whether multiple sessions of rTMS over SMA confer a cumulative benefit on motor performance.
<table>
<thead>
<tr>
<th>P</th>
<th>Sex</th>
<th>Age</th>
<th>MOCA</th>
<th>UPDRS-III</th>
<th>H&amp;Y</th>
<th>DH</th>
<th>More Affected Side</th>
<th>Disease Duration (Years)</th>
<th>Medication (Daily Dose - mg)</th>
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<td>R</td>
<td>10</td>
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<td>1.5</td>
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<td>M</td>
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<td>7</td>
<td>1</td>
<td>R</td>
<td>R</td>
<td>8</td>
<td>Sinemet CR 200-50 TID Azilect 1mg /day QD</td>
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<tr>
<td>6</td>
<td>M</td>
<td>77</td>
<td>29</td>
<td>9</td>
<td>1.5</td>
<td>R</td>
<td>L (more axial)</td>
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<td>M</td>
<td>72</td>
<td>26</td>
<td>7</td>
<td>1.5</td>
<td>R</td>
<td>R (more axial)</td>
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<td>5</td>
<td>1.5</td>
<td>R</td>
<td>R (more axial)</td>
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<td>Sinemet Cr 100/25 q5H Teva-Pramipexole 1 TID Azilect 0.5 QD</td>
</tr>
<tr>
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<td>6</td>
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<td>R</td>
<td>L</td>
<td>4</td>
<td>Sinemet 100/25 QID Pramipexole 0.5 QID</td>
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<td>R</td>
<td>R</td>
<td>3</td>
<td>Sinemet Cr 200/50 QID</td>
</tr>
</tbody>
</table>

P: Participant,  Age: years, F: Female, M: Male, R: Right; L: Left, MoCA: Montreal Cognitive Assessment, UPDRS-III: Unified Parkinson’s Disease Rating Scale-motor section, H & Y: Hoehn and Yahr’s stages, DH: Dominant Hand, QD: One/day, BID: Two times/day, TID: Three/day, QID: Four/day, q5H: Five/day.

Table 2-1: Participant characteristics.
<table>
<thead>
<tr>
<th>Kinematic Variable</th>
<th>TMS</th>
<th>P value</th>
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</thead>
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<td></td>
<td>5 Hz</td>
<td>Control</td>
</tr>
<tr>
<td><strong>Reaction Time</strong></td>
<td>Pre</td>
<td>0.46 (0.08)</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>0.43 (0.07)</td>
</tr>
<tr>
<td><strong>Movement Time</strong></td>
<td>Pre</td>
<td>0.90 (0.21)</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>0.88 (0.20)</td>
</tr>
<tr>
<td><strong>Peak Velocity</strong></td>
<td>Pre</td>
<td>1.58 (0.35)</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>1.53 (0.43)</td>
</tr>
<tr>
<td><strong>Peak Acceleration</strong></td>
<td>Pre</td>
<td>17.56 (4.45)</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>15.45 (4.0)</td>
</tr>
<tr>
<td><strong>Peak Deceleration</strong></td>
<td>Pre</td>
<td>-15.36(3.89)</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>-15.08 (4.88)</td>
</tr>
<tr>
<td><strong>End Point Accuracy</strong></td>
<td>Pre</td>
<td>6.29(1.21)</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>5.86 (0.76)</td>
</tr>
</tbody>
</table>

NS: not significant; *1: significant main effect of TMS; *2: significant main effect of TIME; *3: significant interaction

Table 2-2: Mean (SD) and P-value for motor performance in STT.
Figure 2-5 (A-F): Behavioural data from motor performance during STT with standard error of mean (SEM) bars.
<table>
<thead>
<tr>
<th>Oscillations</th>
<th>Channel Location</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\theta$ (&lt;8 Hz)</td>
<td>FrontoCentral</td>
<td>0.06$^{(NS,2)}$</td>
</tr>
<tr>
<td>$\alpha$ (8-12 Hz)</td>
<td>Central</td>
<td>0.04$^{*3}$</td>
</tr>
<tr>
<td></td>
<td>FrontoCentral</td>
<td>0.07$^{(NS,1)}$</td>
</tr>
</tbody>
</table>

NS: not significant; $^1$: significant effect of TMS; $^{*2}$: significant effect of Task; $^{*3}$: significant change in interaction (TMS*Task)

Table 2-2: P-Values for cortical oscillations and Channel locations (EEG).
Figure 2-6 (A-C): Data from cortical oscillations during motor performance (STT) with SEM bars.
<table>
<thead>
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<th>Variable</th>
<th>TMS</th>
<th>P value</th>
</tr>
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<td></td>
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<td>Sham</td>
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<tr>
<td><strong>RMT</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>63.1 (10.55)</td>
<td>62.3 (8.08)</td>
</tr>
<tr>
<td>Post</td>
<td>60.2 (9.14)</td>
<td>61.2 (8.59)</td>
</tr>
</tbody>
</table>

NS: not significant; *1: significant effect of TMS; *2: significant TIME; *3: significant interaction

Table 2-3: Average means (SD) and P-value for RMT.

Figure 2-7: Data from RMT over primary motor cortex with SEM bars.
Chapter 3  Repetitive Transcranial Magnetic Stimulation over Supplementary Motor Area Improves Handwriting in Individuals with Parkinson Disease

3.1 Preamble

In Chapter 2, I explored one aspect of motor control (targeting speed and accuracy) during an externally cued motor task.

Here in Chapter 3, I have analyzed the impact effect of 5 Hz rTMS over SMA on a second aspect of motor control, handwriting (stroke size and speed; internally cued) in individuals with PD. This task was chosen for two reasons. First, it represents a functional skill that is often disrupted by PD. Second, in contrast to the STT, performance of the handwriting task was internally driven (i.e., not cued by any external triggers). I present here the effect of 5 Hz rTMS over SMA on stroke size and speed while writing.

3.2 Introduction

Individuals with PD have impaired control of movement amplitude and duration.\textsuperscript{70} Normally, the basal ganglia play a role in movement and velocity scaling,\textsuperscript{72} but in individuals with PD, degeneration in the basal ganglia related to deficits in dopamine leads to decreased activity in the SMA and less efferent feedback from the basal ganglia-thalamocortical motor loop. Consequently, individuals with PD show altered activation patterns in the SMA\textsuperscript{12-19} and less cortico-cortical excitability. Taken together, these changes in brain function may be one source of the hypometric and bradykinetic movements that are associated with PD.
The SMA is located on medial aspect of the forebrain (dorso-medial frontal cortex), highly influenced by the basal ganglia and has strong projections to motor cortex. It receives efferent projections from the basal ganglia, processes information and transmits preparatory activity\textsuperscript{117} to M1 to generate actual movements (in the absence of external cues) that are appropriately scaled kinematically to meet task requirements. The SMA also plays a key role in motor selection for sequentially structured tasks like handwriting. Data from healthy controls suggest that the cortical control of handwriting requires activity in the SMA, motor cortex and basal ganglia in order to produce the finely-graded precision grip that is required.\textsuperscript{72} However, due to poor basal ganglia and SMA function, individuals with PD present both with movements that are too slow and hypometric to meet the context requirements of the task (i.e., bradykinesia), and impairments in the initiation and sequencing of complex or simultaneous movements (i.e., akinesia).

Hypometric movements resulting in diminution of letter size, reduced speed and slow acceleration typically characterizes handwriting in individuals with PD.\textsuperscript{66-70} Specifically, hypometric movements may be related to the impaired capability of individuals with PD to maintain adequate muscle forces and reduced ability to process concurrent and forthcoming movement information while writing. Therefore, handwriting is one model by which to characterize a functional movement in individuals with PD.

TMS offers a non-invasive technique to enhance or alter activity in cortico-cortical loop. TMS may modify neuronal activity locally or at distant sites when delivered in series or trains of pulses. rTMS, at frequencies $\geq 5$ Hz, transiently enhances motor excitability,\textsuperscript{156} whereas rTMS, $\leq 1$ Hz, transiently depresses excitability.\textsuperscript{99} Past work
suggested that rTMS can induce a release of dopamine in the basal ganglia and increase cortical excitability in people with PD. Using positron emission tomography (PET), Strafella et al showed that in healthy individuals 10 Hz rTMS over the dorsolateral prefrontal cortex induced dopamine release in the caudate. Similarly, stimulating over motor cortex has been shown to evoke the release of dopamine in the putamen. However, past work has not characterized the impact of rTMS on a functional motor skill. For this thesis, I chose to stimulate over SMA based on its known role in handwriting and previous reports of altered function in this area in individuals with PD. As indexing dopamine release associated with rTMS using PET imaging is both difficult and costly, for this thesis I choose to study only the impact of brain stimulation over SMA on motor function; my work may serve as the basis for future studies of dopamine release in the basal ganglia associated with rTMS over SMA. Thus, in the present study, we considered the potential of 5 Hz rTMS over SMA to alter functional ability, indexed as handwriting, in individuals with PD.

Handwriting is an ideal task for the study of motor function in PD. Cursive writing requires coordinated flexion-extension movements in hand to produce up and down strokes and progression left to right. In this study, we designed an experiment with simple writing pattern – cursive “i”s or loops, as each loop has two curved strokes – an upstroke and a downstroke - with an element of coordination progressing left-to-right.

Given that enhancing activity in the SMA with 5 Hz rTMS may alter activity in the basal ganglia, we predicted that 5 Hz rTMS over the SMA would improve handwriting size and speed along with fluency in individuals with PD. Consequently, following 5 Hz rTMS over SMA individuals with PD should be able to scale speed and amplitude to
produce the desired stroke pattern. Tuelings et al\textsuperscript{157} reported that individuals with PD show greater dysfluency in writing tasks involving wrist flexion than in the tasks involving wrist extension. Therefore, we hypothesize that individuals with PD will demonstrate more improvement in the downstroke than upstroke following 5 Hz rTMS. To our knowledge, the present study is the first study to assess the effect of rTMS over SMA on handwriting in individuals with PD.

3.3 Methods

3.3.1 rTMS Protocol and Participant Characteristics

A detailed explanation of our rTMS methods is provided in Chapter 2; to avoid repetition it is not presented here. The same group of individuals with PD completed this portion of the study following the same group allocation and experimental outline as has already been provided (Table 1-1 and 2-1).

3.3.2 Handwriting

All participants were tested on a handwriting task while sitting at a table adjusted for height to allow the right arm to be comfortably placed with the elbow below shoulder height. Participants were asked to write repetitive cursive loops or “l”s in their everyday style and preferred speed using a special ink pen on an 8 ½ by 11-inch paper placed on top of a digitizing tablet (WACOM Intuos3 tablet-9X12). The orientation of the paper on the tablet was determined by the participant. The paper contained rectangular boxes of 2 cm height and 8 inches in length and participants were instructed to match the height of their loops to the size of the box (Figure 3-1). Before starting the experiment, all participants were shown a sample of cursive loops or “l”s and allowed to practice a trial to familiarize with the digitizer and the writing task. Two trials of 15 second each were
recorded. For each condition (active vs. control), data was collected twice – prior to rTMS (pre) and after rTMS (post) on the same day.

Figure 3-1: Handwriting task showing rectangular box of 2 cm height and 8 inches in length; participants were instructed to match the height of their loops to the size of the box.

3.4 Analysis

Kinematic variables of handwriting were quantified using ScriptAlyzer software (NeuroScript, LLC; Tempe, AZ, USA). ScriptAlyzer was used to record position data (X-Y coordinates) and then calculate the kinematic parameters of interest. Position data (x-y displacement) were sampled at a frequency of 200 Hz with a spatial resolution of 0.002 cm. For each loop, the software used the zero velocity crossing to identify two segments, an upstroke and a downstroke. For all participants, the first loop (upward and downward stroke) was eliminated by the software from each trial. For any trials where freezing or hand repositioning events occurred, the software would also automatically eliminate the segment immediately before and after the event. After software elimination, stroke segments for each trial were visually inspected in the raw data and final data to verify that the software correctly identified and eliminated only those segments where freezing or hand repositioning occurred in the trial. On average, 20 segments were analyzed per subject.

Finally, for each trial, ScriptAlyzer used the averaged segment data to calculate the following kinematic parameters of interest: (1) size, (to study effect of rTMS on writing size) which included vertical size (displacement in y-axis), absolute size
(resultant of x and y axes displacement) and roadlength (actual path length of the stroke); (2) speed, which included peak vertical velocity, peak vertical acceleration, average absolute velocity and relative time to peak vertical velocity; (3) handwriting smoothness or dysfluency, which was quantified by average normalized jerk (ANJ) per trial in the vertical direction, and the number of peak acceleration points (nPAP).\textsuperscript{157} ANJ is unit-less and normalized for stroke duration and length.\textsuperscript{158} Higher ANJ scores and increased nPAP in handwriting are reflective of dysfluent writing or dyskinesia.\textsuperscript{157, 159} (4) Average pen pressure (z coordinate). I first analyzed average parameters of the complete loop for both trials. Then I separated the upstrokes and downstrokes to determine if rTMS impacted the two segments of the loop differently.

3.5 Statistical Analysis

For each set of dependent variables, we considered handwriting data first for whole, completed loops; upstrokes and downstrokes were analyzed separately. \textsuperscript{2} (Session: 5 Hz, control TMS) by \textsuperscript{2} (Time: pre-, post stimulation) ANOVAs with repeated measures corrections were performed on the mean of each dependent variable using SPSS software (v.14). Significant interactions (Session X Time) were decomposed with follow up T-tests. The threshold for significance was set to $P \leq 0.05$.

3.6 Results

3.6.1 Complete Loops

a) Size: As shown in Table 3-1 and Figure 3-2, there was a significant interaction between Session and Time ($F (1,9) = 5.59$, $P = 0.04$) due to increased global vertical size for the 5 Hz group following stimulation
at the post-test as compared to control group. There was no significant effect of Session or Time on the absolute size or roadlength.

b) **Speed**: There were no interaction effects for speed variables. However, a significant main effects of Time were noted for peak vertical velocity \((F(1,9) = 10.67, P = 0.01)\) and average absolute velocity \((F(1,9) = 10.24, P = 0.01)\). This suggests that both groups wrote faster at the post-test, regardless of stimulation type. There was no significant main effect of Session or Time on time to reach peak velocity for all segments.

c) **Smoothness**: Similar to speed, there were no interaction effects for smoothness parameters. However, there was significant main effect of Time for both ANJ and nPAP irrespective of rTMS group \((F(1,9) = 13.50, P = 0.004; F(1,9) = 8.82, P = 0.02\) respectively).

d) **Average Pen Pressure**: There was no interaction effect for pen pressure. However, average pen pressure was decreased by 5 Hz rTMS (-94.295) and increased by control rTMS (3.64); however these effects did not reach statistical significance.

### 3.6.2 Upstroke and Downstroke

a) **Size**: There was significant interaction between Session and Time in vertical size of upstroke \((F(1,9) = 9.62, P = 0.01)\). No interaction was observed in downstrokes. Unlike the complete loop, there was a significant main effect of Session on absolute size in upstroke \((F(1,9) = 5.95, P = 0.04)\). There was no main effect of Session on downstroke. However, there was significant main effect of Session on roadlength in both upstroke and
downstrokes ($F_{(1,9)} = 11.34, P = 0.008; F_{(1,9)} = 8.18, P = 0.02$ respectively).

**b) Speed:** There was no significant interaction between Session and Time in peak vertical velocity or average absolute velocity of upstrokes. However, there was significant main effect of Time on downstrokes stemming from increased peak vertical velocity ($F_{(1,9)} = 8.69, P = 0.02$) and average absolute velocity in both groups ($F_{(1,9)} = 9.23, P = 0.01$). Finally, both groups (5Hz and control) took longer to reach peak vertical velocity, during downstrokes at the post-test. This was illustrated by a main effect of Time for downstrokes ($F_{(1,9)} = 5.63, P = 0.04$).

c) **Smoothness:** Similar to speed, there was main effect of Time on smoothness parameters. Both groups (5 Hz and control) had improved smoothness in writing at the post-test. This was evident by main effects of Time in upstrokes ($F_{(1,9)} = 8.66, P = 0.02$), downstrokes ($F_{(1,9)} = 6.48, P = 0.03$), ANJ ($F_{(1,9)} = 8.71, P = 0.02$) and nPAP ($F_{(1,9)} = 8.834, P = 0.02$).

d) **Average Pen Pressure:** There was significant interaction between Session and Time in upstroke ($F(1,9) = 4.93, P = 0.05$). This was due to the decreased pen pressure following 5 Hz rTMS (Figure 3-1). No interaction was observed in downstroke.

**3.7 Discussion**

This is the first study to report that single session of 5 Hz rTMS over SMA improves size of handwriting, a functional fine motor task, in individuals with PD. Specifically, 5 Hz rTMS over SMA increased the global size of “l”s during cursive
handwriting in individuals with PD. In addition, it was also noted that following 5 Hz rTMS less pen pressure was applied by people with PD. During handwriting, the pen tip proceeds in x and y directions and it is the offset in the x and y directions that dictates the production of curved reversal as in writing “l”s.\textsuperscript{160, 161} Van Gemmert et al\textsuperscript{162} reported that individuals with PD undershoot the required size during handwriting when they produce strokes of 2 cm or larger. In the present study, we provided individuals with a visual target box that was 2 cm in height which may have provided a visual cue for movements. Despite the visual information provided by our box, following 5 Hz rTMS individuals in our study improved the global size of their “l”s. I speculate that 5 Hz rTMS helped participants to better modulate and coordinate joints in both the x and y directions, however, I did not measure hand joints force modulation or coordination directly during the task. Future studies may record electromyography (EMG) and/or run motion analysis to better understand force modulation and coordination between different joints of upper extremity during writing.

The data from this thesis data suggest that 5 Hz rTMS over SMA might have altered cortico-striatal connectivity perhaps by activating an otherwise hypoactive, SMA and its projections to the basal ganglia, M1 and other motor areas. The basal ganglia plays an important role in motor behavior. According to a hypothetical model of basal ganglia, the putamen, the major input nucleus, controls globus pallidus-internus (GPI), the major output nuclei, both directly and indirectly. In PD, this balance between putamen and GPI/GPe is altered due to loss of neurotransmitter dopamine. SMA acts with the basal ganglia to prepare movements.\textsuperscript{132-135} SMA receives input from the basal ganglia\textsuperscript{132, 135, 163, 164} and then via efferents reciprocally connects back to the striatum\textsuperscript{7, 8, 165}
projecting to GPi both directly and indirectly. Thus, a crucial cortico-subcortical loop is formed (Figure 1-4). Our data suggest that 5Hz rTMS may have influenced basal ganglia output, thus enabling participants to better control movement amplitude and force to generate a vertical size closer to 2 cm post 5 Hz rTMS.

Improvement in handwriting in individuals with PD after 5 Hz rTMS suggests that stimulation over this brain region may be a potential therapeutic target. Although the present study considered only one session of rTMS Hamada et al reported improvement in the motor scores of an outcome measure scale – UPDRS after 8 sessions of 5 Hz rTMS over SMA. Taken together these two studies support the notion that SMA could be a potential stimulation site capable of influencing both general motor function and fine motor control.

We found that participants improved significantly in writing size for upstrokes as compared to downstrokes after 5 Hz rTMS over SMA. This may be attributed to the fact that individuals with PD have more tonic activation of flexor muscles and reduced control of wrist flexion. Writing curved loops involves finger and wrist extension in writing upstrokes and finger/wrist flexion while writing downstrokes. Therefore, rTMS may have facilitated the easier movement of wrist extension that is required by upstrokes, which are less affected by PD. This finding is contrary to our hypothesis. One explanation for the finding that 5 Hz rTMS over SMA aided upstrokes may be the dosage of pulses delivered and/or stimulation intensity used in this work. I delivered 1200 rTMS pulses at 110% of resting motor threshold. Future studies may wish to consider whether more pulses and/or higher stimulation intensities modulate the size of downstrokes. In addition, the use of electromyography over the wrist flexors and
extensors would be required to directly assess the impact of 5 Hz rTMS over SMA on the muscle activity. However, our handwriting data do indicate that participants were able to generate larger letters at their preferred speed after 5 Hz rTMS over SMA than after control rTMS, perhaps this was attributable to better control of movement following stimulation.

In addition, there was reduction in average pen pressure during upstrokes after 5 Hz rTMS over SMA. Past literature suggests that basal ganglia and SMA dysfunction in individuals with PD leads to an inefficient recruitment of muscle force, deficits in amplitude scaling and/or velocity scaling and rigidity in muscle groups, resulting in jerky movements. After 5 Hz rTMS over SMA individuals in the present study applied less pressure (in the z-direction) while writing upstrokes. It is possible that 5 Hz rTMS over SMA altered the function of the SMA-basal ganglia-M1 loop, so that M1 was better able to modulate the force of movements. Indeed we noted an increase in M1 cortical excitability following 5 Hz rTMS (see chapter 2). However, we neither measured the release of dopamine nor recorded EMG during writing to assess changes at the neuromuscular level. To better understand the mechanism of improvement, future studies should attempt to quantify dopamine release in different areas of the brain, especially in the basal ganglia, after 5 Hz rTMS on SMA in individuals with PD. In addition EMG may be collected to assess change in firing pattern in different muscles during writing in individuals with PD.

This study also revealed main effects of time during the writing of complete loops and down strokes. Regardless of stimulation type, a higher peak vertical velocity and average absolute velocity were noted. Since both groups improved, the possibility of a
placebo effect cannot be excluded. Importantly, it is also possible that these effects were attributable to motor learning. This possibility provides support for future motor learning and rehabilitation trials for handwriting function in people with PD.

Taken together, my results suggest that 5 Hz rTMS over the SMA can influence several key aspects of handwriting including vertical size and axial pressure. Although this thesis cannot elucidate the exact mechanism by which 5 Hz rTMS stimulated these effects it is possible that brain stimulation over SMA alters the net function of the SMA-basal ganglia-M1 loop which leads to improved handwriting function. Our data are an essential first step in determining the utility of rTMS over SMA in individuals with PD. Future work will first have to categorize more precisely the mechanism of the changes in function reported here.
<table>
<thead>
<tr>
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<th>All segments</th>
<th>Up-stroke</th>
<th>Down-stroke</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 Hz</td>
<td>Control</td>
<td>P value</td>
</tr>
<tr>
<td>Vertical Size (cm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>1.87 (0.09)</td>
<td>1.85 (0.12)</td>
<td>0.04*</td>
</tr>
<tr>
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<tr>
<td>Pre</td>
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<td>2.05 (0.22)</td>
<td>NS</td>
</tr>
<tr>
<td>Post</td>
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<td>2.06 (0.26)</td>
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</tr>
<tr>
<td>Roadlength (cm)</td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>2.29 (0.23)</td>
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</tr>
<tr>
<td>Post</td>
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<td>Peak Vertical Velocity (cm/sec)</td>
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<td></td>
</tr>
<tr>
<td>Pre</td>
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<td>7.54 (1.08)</td>
<td>0.01**</td>
</tr>
<tr>
<td>Post</td>
<td>7.99 (1.90)</td>
<td>7.98 (1.62)</td>
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</tr>
<tr>
<td>Average Absolute Velocity (cm/sec)</td>
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<td></td>
</tr>
<tr>
<td>Pre</td>
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<td>4.61 (1.17)</td>
<td>0.01**</td>
</tr>
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</tr>
<tr>
<td>----------------------------------------------</td>
<td>--------------</td>
<td>----------------------</td>
<td>----------------------</td>
</tr>
<tr>
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<td>Post</td>
<td>Pre</td>
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<td>Control</td>
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</tr>
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<tr>
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<td>Post</td>
<td>72.31 (63.43)</td>
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</tr>
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<tr>
<td></td>
<td>Post</td>
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<td>Pre</td>
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<td>546.43 (204.47)</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>564.64 (154.47)</td>
<td>550.07 (254.76)</td>
</tr>
<tr>
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<td></td>
<td></td>
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<td></td>
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</tbody>
</table>

**NS**: not significant; **1**: significant main effect of TMS; **2**: significant main effect of TIME; **3**: significant interaction

Table 3-1: Average means (SD) and P-value for all segments, up-strokes and down-strokes.
D) **Absolute Size - All Segments**

E) **Absolute Size - Upstroke**

F) **Absolute Size - Downstroke**
Relative Time to Peak Velocity - All Segments

Relative Time to Peak Velocity - Upstroke

Relative Time to Peak Velocity - Downstroke
Figure 3-2 (A-X): Behavioural data from handwriting task with SEM bars.
Chapter 4   Effect of 5 Hz Repetitive Transcranial Magnetic Stimulation over Supplementary Motor Area on Imagery Ability in Individuals with Parkinson Disease

4.1 Preamble

Thus far I have explored the impact of rTMS on motor control and oscillatory patterns in the brain (Chapter 2), and a functional movement, handwriting (Chapter 3) in individuals with PD. I present here the results of effect of 5 Hz rTMS over supplementary motor area on motor imagery ability in individuals with PD.

4.2 Introduction

Motor imagery (MI) is the mental representation of movement without any actual body movement.\textsuperscript{77, 78} It is a complex cognitive process, which involves the use of sensory and perceptual memories as they relate to motor actions. MI can be employed to enhance motor performance and learn new motor tasks in healthy people.\textsuperscript{79, 166} There are various types of MI; the two most commonly used in rehabilitation are external (visual) and internal (kinaesthetic) imagery. External imagery refers to watching oneself as an external observer, e.g., watching oneself in a mirror, and is also known as visual imagery. In internal or kinaesthetic imagery, a person imagines movement from an inner perspective, performing a movement mentally and experiencing the actual situation and sensations.\textsuperscript{79} MI practice has been shown in some populations to be a safe, effective method to facilitate motor learning and for initiation of movements. However, to date little work has considered whether MI might facilitate motor function in individuals with PD. Motor learning is traditionally defined as the acquisition of skilled movements as the result of practice.\textsuperscript{86} Several studies have reported improvement in motor learning or performance following mental practice of simple motor tasks.\textsuperscript{167-170, 171}
These studies also support the notion that motor imagery and actual motor performance involve very similar neural circuitry. Lotze et al\textsuperscript{172} found that SMA, PMC, and M1 are equally activated during both actual and imagined movements. Further, motor imagery is a self-generated or internally guided activity; the SMA is predominantly involved in self-generated actions.\textsuperscript{80, 173} Imaging studies report activation of SMA during MI of movements both in healthy people\textsuperscript{81-85} and in individuals with PD.\textsuperscript{85} However, PD is linked to decreased activation of the SMA\textsuperscript{12-17} attributed to reduced input from basal ganglia. Thus, it is possible that enhancing activity in SMA using 5 Hz rTMS will facilitate imagined movements and/or functional arm use.

Research into MI has been performed in individuals with stroke,\textsuperscript{167, 174-176} spinal cord injury,\textsuperscript{177, 178} and PD.\textsuperscript{87, 179} There is evidence in the literature showing that MI based practice may be an effective adjunct rehabilitation tool, with the majority of studies involving individuals with stroke.\textsuperscript{180, 181} Yet, past work has not convincingly demonstrated that individuals with PD can generate or visualize movements mentally. To date, several studies have considered whether MI can positively alter behaviour (e.g., motor learning)\textsuperscript{86} or function (e.g., daily activities of life)\textsuperscript{87} in individuals with PD. However, this work has not directly tested MI ability. Past work considering the impact of MI on individuals with PD did not use a questionnaire to assess baseline imagery ability or a standardized test of MI. Instead, they \textit{inferred} the effects of MI on motor behaviour using pre-post designs that assessed changes in behaviour associated with MI. For example, in a single session, Yaguez et al\textsuperscript{86} trained individuals with PD using a combination of physical motor and MI practice; neither improved movement. A more positive result was demonstrated by Tamir et al\textsuperscript{87} who noted that MI practice reduced losses of daily function attributable to bradykinesia. Though this past work is important,
neither of these studies directly indexed MI ability in people with PD. Until recently another possible explanation for the lack of evidence for MI in individuals with PD centers on the lack of a reliable, valid scale to index MI. We recently established the reliability and validity of the KVIQ. Though the KVIQ is a reliable questionnaire for individuals with PD it does suffer from ceiling effects and it is timely to administer. Owing to the need for a faster assessment and a direct test of imagery in PD, in the present study the break test was used to assess differences in a MI ability following 5Hz rTMS over SMA. We chose break test as it is widely used in stroke and healthy populations to assess the MI ability. The break test relies on the evaluation of the congruence between the time to physically perform a movement and the time to mentally imagine the same motion. Theoretically, these two times are identical; as the difficulty of the task increases both physical and MI ability should “break” at the same point. However, in individuals with poor MI ability the break points for physical and mental imagery will differ. In this study, we hypothesized that 5 Hz rTMS would enhance activity in SMA in individuals with PD, which may in turn increase activity in BG and the cortex through the globus pallidus direct route. As the literature suggests that MI and physical performance largely share the same neural circuitry, we anticipated that rTMS over SMA would improve the congruence between the break point for imagined and executed movement. Thus, we predicted that as compared to pre-rTMS, a post assessment immediately following 5 Hz rTMS over SMA, would improve the congruence between MI and physical break points in individuals with PD. To our knowledge, the present study is the first study to assess the effect of 5 Hz rTMS over SMA on MI in individuals with PD. Because this is the first study to consider the impact
of rTMS on M1 a main aim was to generate effect sizes for use to appropriately power future work.

4.3 Methods

4.3.1 rTMS Protocol and Participant Characteristics

A detailed explanation of our rTMS methods is provided in Chapter 2; to avoid repetition it is not presented here. The same group of individuals with PD completed this portion of the study following the same group allocation and experimental outline as has already been provided (Table 1-1 and 2.1).

4.3.2 Assessment of MI

To consider general capability for MI, and ensure that all participants could engage in MI, each individual in this study completed the KVIQ prior to the administration of rTMS. However, because of the length of time to complete (~ 25 minutes), this test was not repeated following rTMS. Instead KVIQ data were used to categorize baseline MI ability. For the KVIQ, participants were tested in a quiet room. The imagery ability of all participants was assessed with the KVIQ on the day before the first rTMS session, regardless of whether or not they were randomized to begin the study with 5Hz or control rTMS. Completion of the KVIQ followed the procedures outlined by Randhawa et al.¹¹⁸ and Malouin et al.¹⁸⁴ Subsequently, all participants performed a break test¹¹⁹ involving physical and mental simulation of a continuous thumb-fingers opposition sequence to the sound of metronome (Figure 4-1). Participants imagined touching each finger with their thumb starting with the little finger, on right hand only. All participants practiced the task once physically and once mentally to understand it before a break point was determined. The speed of a metronome, initially set at 40 beats per minute, was increased every 5 seconds by 4 beats/minute,
until the participant reported that the imagined hand could no longer keep up with the imposed speed. The movement sequence was physically executed first (so that participants could understand the movement pattern and examiner could visually see movement being performed as instructed) and mentally performed second to determine the break point (time at which participants could no longer keep up with the metronome) for each. Break points were compared for maximum physically executed and imagined movement speeds by calculating a difference score between the two. We compared performance using the right hand pre and post rTMS. The same protocol was followed for both rTMS (5 Hz and control) intervention days.

Figure 4-1: Break Test: Continuous thumb finger opposition and metronome.

### 4.4 Data Analysis

To score the KVIQ, 10 items for each subscale (kinaesthetic and visual) were tallied. Then following the convention established by Malouin et al\(^{184, 185}\), the scores of the right upper limb (items # 3, #4, #5) were separated for each subscale.

For each session (pre/post, 5Hz/control), physical and mental break point data from break Test were used calculate a difference score. To account for the possibility that initial MI ability influenced break points, KVIQ scores were employed as a co-variant in ANCOVAs which compared pre to post intervention separately for the 5 Hz and control rTMS groups. Difference scores between mental and physical break points
were the dependent measures. Analyses employed SPSS software (v.14). The threshold for significance was set to \( P \leq 0.05 \). To understand if there was a relationship between the break test and the KVIQ, a spearman correlation coefficient was calculated between pre rTMS break test difference scores and the KVIQ right upper limb subscale. To generate data for future power calculations, we also computed effect sizes\(^1\) from post 5 Hz and control rTMS difference scores.

4.5 Results

All participants were able to imagine as indicated by their KVIQ scores except one participant. The lowest possible score on the KVIQ which would indicate poor imagery ability is a 6 (kinaesthetic and visual combined). In the present study, the scores for right upper limb imagery ranged from 11 to 27 as shown in Table 4-1, which suggest that all participants were able to imagine.

Both groups’ (5 Hz and control) performance on the break test was similar (Table 4-1 and Figure 4-2). There was no statistical difference in the location of physical and mental break points following control rTMS \( (F(1,8) = 0.02, \ P = 0.90) \). However, following 5 Hz rTMS the difference between physical and mentally imagined break points was smaller than at the pre-test as shown in Table 4-1. But the large variability in responses this resulted in only a trend for improved estimation \( (F(1,8) = 4.82, \ P = 0.06) \).

There was moderate correlation between the break test and KVIQ scores \( (r = 0.46; \ \text{Figure 4-3}) \). There was also a moderate effect size for difference scores between 5 Hz and control group (Cohen’s \( d = 0.46 \)). Sample size calculations based on effect size demonstrate that at least 50 participants would be required to reach statistical significance of \( (P<0.05) \).\(^1\)
4.6 Discussion

Our results suggest that 5 Hz rTMS over SMA did not change MI ability in individuals with PD. Contrary to our hypothesis, we were unable to demonstrate a clear effect of 5Hz rTMS over control rTMS in individuals with PD. However, the data in this study represent an important first step and should allow for future work in this area to be adequately powered to overcome the inherent variability in responses of individuals with PD.

This is the first study to determine the effect of 5 Hz rTMS over SMA on MI ability in individuals with PD. Two reasons may contribute to the inconclusive results of this study. First, the break task may not be sensitive enough to record changes in imagery ability in individuals with PD. Secondly, the break test requires participants time sequential finger movements to speed of the metronome, both physically and mentally. Our low difference scores both before and after rTMS may result from impaired timing of movements\textsuperscript{188} and/or impaired time perception;\textsuperscript{189} each of these functions has been linked to BG function and are altered by PD.\textsuperscript{190, 191} However, from the data reported here it is unclear which of these factors impacted the ability of people with PD to perform break test.

Unfortunately, the reliability of the break test has not been established in individuals with PD. We propose that future work is needed to establish the reliability of the break test in PD population in both medicated and non-medicated states to better estimate MI ability and effects of rTMS. Consideration of the impact of rTMS on MI ability was one part of a larger study. Because the after effects associated with rTMS are fairly short (~30 minutes)\textsuperscript{130} we were only able to complete one repetition of the break test following 5 Hz and control stimulation. However, we speculate that more
repetitions of the break test pre-post rTMS would add power in the future studies. We did find moderate effect size (Cohen’s d = 0.46), which suggests that in future, at least 50 participants would be required to reach statistical significance of (P<0.05).\textsuperscript{187}

Although it is difficult to speculate on the role of the SMA in imagery ability in PD population, it is also possible that SMA is not the optimal stimulation site to influence imagery ability in individuals with PD. Future studies may attempt to stimulate other areas involved with MI, such as the parietal or frontal cortex, or to deliver more pulses to determine the impact of brain stimulation on MI ability in people with PD.

Last, the literature suggests that different types of imagery (visual and kinaesthetic) modulate corticomotor excitability differently. Stinear et al\textsuperscript{192} applied TMS over M1 in healthy individuals and showed that kinesthetic imagery modulates corticomotor excitability but that visual motor imagery does not. One of the drawbacks of the break test is that it allows undefined imagery types to be used for task completion. In the current study, participants were allowed to attempt the task visually or kinaesthetically. There is possibility that a few participants used kinaesthetic imagery and benefitted from 5 Hz rTMS, whereas others visualized the task and were unable to capitalize on the effects of stimulation. We did notice a trend towards better estimation of break point after 5 Hz rTMS, but data from a larger group of individuals is needed to verify these results.

### 4.7 Conclusion

We conclude that 5Hz rTMS over SMA did not influence performance of the break test in individuals with PD. However, the results of this study must be interpreted cautiously. First, the sensitivity of the break test needs to be compared in healthy individuals and individuals with PD to understand effect of rTMS on MI ability. Second,
the reliability of break test in PD needs be established for use in future studies. Finally, the type of imagery, visual or kinesthetic, should be pre-specified to determine effect of rTMS on modality specific imagery ability.
Table 4-1: Imagery scores of all participants: Right upper limb imagery score on KVIQ, imagery scores pre-post 5 Hz and control rTMS.

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<tr>
<th>P</th>
<th>5 Hzpre</th>
<th>5 Hz post</th>
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P = Participant, Phy = Physical, Men = Mental, DF = Difference Score, UE = Upper Extremity, AC = 5 Hz group, SH = control group.
A) P = Participant number, score on physical test, score on mental test during break test
Figure 4-2 (A and B): Break test results for all participants in the study.

P = Participant number, score on physical test, score on mental test during break test
Figure 4-3: Spearman Correlation Coefficient for all participants in the study.
Chapter 5  Conclusion and Future Directions

5.1 Overview

PD is a neurodegenerative disease\(^1\) characterized primarily by bradykinesia and hypometria resulting from loss of dopamine in the striatum, which alters functioning of the nigrostriatal pathways causing a poverty of movements. There is evidence that altered oscillations in the basal ganglia and associated regions of thalamus and cortex also play role in the pathophysiology of PD.\(^{22, 23}\) In addition, PD may also cause to dysfunction of the SMA (decreased activation)\(^{12-19}\) which may be attributed to reduced input from the basal ganglia. Modern therapeutic approaches attempt to improve motor symptoms and balance pathways within the basal ganglia. This thesis explored the impact of 5 Hz rTMS over SMA on motor performance and handwriting. Using a novel motor task (the STT), I attempted to explore motor control elements of an externally cued movement task. In the handwriting task, I explored stroke size and speed along with fluency of an internally driven functional motor task. The second goal of the study was to determine the effect of 5 Hz rTMS over SMA on cortical oscillations, especially on the α and β bands (8-35 Hz) through EEG recording during the STT. The third goal of this thesis was to index the impact of brain stimulation over SMA on motor imagery ability in individuals in PD.

This chapter will summarize and discuss the main findings of the thesis, address the limitations of the experiments and suggest directions for future research.

5.2 Summary of Results

5.2.1 rTMS over SMA benefits Motor Task Performance and Modulate Cortical Oscillations in Individuals with PD
Past work suggest that individuals with PD suffer from decreased activation in SMA that contributes to bradykinesia and abnormal synchronization of oscillations especially in β band along with altered cortical excitability in M1. In this study, I examined how 5 Hz rTMS over SMA impacted motor performance (indexed by the STT) and influenced cortical oscillations, especially in α and β band (collected/recorded via EEG), in individuals with PD. This is the first study to report that 5 Hz rTMS over SMA altered motor performance; following stimulation individuals with PD moved slower but with more accuracy during a serial targeting task. In addition, following 5 Hz rTMS there was a concurrent reduction in α oscillations during reaction phase of the task in the Cen channel grouping and a lower resting motor threshold in M1. These results suggest that 5Hz rTMS over SMA influenced movements possibly by modifying subcortical circuitry (SMA-STN-basal ganglia circuitry) through the GP direct route. As other studies of people with PD have reported release of dopamine in caudate and putamen after rTMS over M1 and DLPFC, it is possible that the altered motor performance noted after stimulation in this thesis was the result of dopamine released as well. Future work that employs PET scanning will have to verify this speculation. Overall, the results of the present study suggest that 5Hz rTMS over SMA may benefit individuals with PD and by changing motor performance and cortical α oscillations.

5.2.2 rTMS over SMA improves Handwriting in Individuals with PD

Previous research suggests that handwriting in individuals with PD is typically characterized by hypometric movements that lead to diminution of letter size and reduced speed and slowed acceleration of a pen tip. As handwriting is an important functional task in everyday living, in this study, I investigated the impact of 5 Hz rTMS on it in individuals with PD, via 5 Hz rTMS over SMA. It is encouraging to
report that that 5 Hz rTMS improved the vertical size of letters during handwriting, largely as a function of larger upstroke size during writing of cursive "l"s. The results of this study are in line with similar study by Hamada et al\textsuperscript{95} who reported improvement in motor scores of an outcome measure scale – UPDRS after 8 sessions of 5 Hz rTMS over SMA. Together with Hamada et al\textsuperscript{95} I support the notion that SMA could be a potential stimulation site capable of influencing motor function in people with PD.

5.2.3 Effect of rTMS over SMA on MI Ability in Individuals with PD

MI is a self-generated or internally guided activity; the SMA is predominantly involved in such self-generated actions.\textsuperscript{77,173} Imaging studies report activation of SMA during imagining movements both in healthy people\textsuperscript{81-85} and in individuals with PD.\textsuperscript{85} However, PD is linked to dysfunction of SMA (decreased activation) attributed to reduced input from basal ganglia. In this study, I attempted to alter activity in SMA using 5 Hz rTMS to influence MI ability, as indexed by break test, in individuals with PD. Contrary to my hypothesis, the results of this study suggest that 5Hz rTMS over SMA did not influence performance of break test in individuals with PD. However, the results of this study must be interpreted cautiously. Future studies, may first establish the reliability and sensitivity of the break test in individuals with PD, to understand effect of rTMS on MI ability.

5.3 Limitations of the Thesis

Several limitations impact the conclusions that may be drawn from this work. First, all participants were stimulated on-medication. It is possible that we would have noted larger differences in motor performance during motor task (STT) and handwriting, along with change in β oscillations following 5 Hz rTMS, if participants were tested off-medication. This thesis controlled for any medication cycle effects though, by testing
individuals in the same relative phase of their medication cycle, two hours before their next medication dosage. That is, testing was performed during second phase of medication cycle, to record the maximum add-on effect of rTMS.

Second, this thesis has a small sample size. Though 10 participants were included in all the experiments except EEG analysis (where one additional participant was excluded due to artifact in EEG recording), 14 participants were enrolled in this thesis. Figure 5-1 shows a flowchart of the difficulties encountered with data collection and analysis. Due to recruitment limitations, the sample of individuals who were examined were somewhat heterogeneous in terms of age, disease duration (3 to 10 years), hand dominance (right hand dominant: 9 out of 10), side affected (right side affected: 6 out of 10) and a gender (male: 9 out of 10). However, to control for variability in the sample participants were tested using their right hand. Participants were instructed to perform the tasks with dominant (right) hand because STT and handwriting tasks required dexterity, and would have created added difficulties in performance of the tasks if participants used their non-dominant (left) hand instead. In addition, PD is not strictly unilateral disorder, it affects both right and left hemispheres.

Third, there were few methodological limitations of the thesis, specifically in EEG collection/recording and imagery ability assessment. During the EEG experiment, participants were required to keep the EEG cap on during entire experiment. The cap was prepared with electrode gel in beginning of the experiment and placed according to 10-20 system, referenced to specific brain areas. As the duration of entire experiment (Table 1-1) was about 2 hours, it is possible that cap might have shifted and/or electrode gel might have dried to some extent. To control for these limitations, EEG signals were monitored throughout experiment to detect any changes in the
recording. However, in the future, EEG collection may be combined with fMRI to further our knowledge on neurobiological mechanisms associated with cortical changes. Finally, in the MI experiment in chapter 4, the break test was used to assess MI ability, as it is used widely in stroke populations. However, the reliability and validity of the break test in the PD population is still not established. To control for this limitation, all participants were first tested on baseline imagery ability on KVIQ. Though the KVIQ is a reliable questionnaire for individuals with PD it does suffer from ceiling effects and it timely to administer. Owing to the need for a fast assessment, in the present study the break test was used to assess differences in MI ability. However, in future, reliability of the break test in individuals with PD needs to be established.

Finally, the numbers of statistical tests in chapters 2 and 3 may leave open the possibility of a Type I error. However, as this is the first study of the effect of 5 Hz rTMS in individuals with PD it is an essential first step in understanding the impact of brain stimulation in this population. The data may need to be interpreted cautiously, but they do provide information that may be used both to justify and power future work in this field.
5.4 Conclusion and Future Directions

Taken together the findings from this thesis suggest that 5 Hz rTMS may be a therapeutic option for individuals with PD. As all the effects documented in this thesis were noted for only within a single session the next key step will be to validate these findings in a larger group of participants who are tested over multiple sessions of stimulation to determine if the benefits of rTMS over SMA are cumulative. In addition, this work may be extended to include higher frequencies (10 Hz\textsuperscript{104} or 25 Hz\textsuperscript{197}) and/or alternate stimulation sites (SMA, DLPFC, M1) to observe additive effect of rTMS. Further, future studies, may attempt to quantify dopamine release in different areas of brain, especially in the basal ganglia using PET, after high frequency rTMS over SMA in individuals with PD.
In the light of issues discussed in section 5.3, future studies should also attempt to consider the effect of 5 Hz rTMS over SMA in dopamine depleted (off-medicatin) individuals with PD. In addition, it may be desirable to have a more balanced sample of participants with PD in terms of gender, hand dominance and affected side. It might be of interest to assess participants with both right and left hand on motor tasks and compare results within/ between different groups of individuals affected by bradykinesia or tremor or dyskinesia. In the EEG experiment in chapter 2, no changes were observed in β oscillations, future studies may employ more discrete tasks such as a choice reaction task\textsuperscript{121} or a go-no go task\textsuperscript{42} that have been shown in other work to alter β power. Additionally, with respect to current results in chapter 4, in the future, MI ability may be indexed with tool that has known reliability in individuals with PD with specific type of imagery, e.g. visual or kinaesthetic.

Another investigative avenue to pursue would be to combine rTMS with skilled practice of movements and/or functional tasks, to understand the potential effect of rTMS on plasticity and improvement in motor symptoms of PD. Moreover, EEG may be recorded over extended number of electrodes to understand the overall cortical activity and connectivity, including occipital location to understand visuo-motor processing in individuals with PD. In addition, it will be interesting to run a similar study in healthy individuals with 1 Hz rTMS over SMA to create virtual SMA lesion and compare the temporary effects with symptoms seen in individuals with PD.
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APPENDIX 1: Movement Profiles: STT

Figure A-1-1(A-F): Pre-Post 5 Hz rTMS - Movement profiles
Figure A-1-2 (A-F): Pre-Post control rTMS - Movement profiles
APPENDIX 2: EEG PSD Graphs

A) Active-pre Frontal Central Real PSD Graph

B) Active-pre Frontal Central Reaction PSD Graph

C) Active-pre Frontal Central Movement PSD Graph
J) Sham-post Central Rest PSD Graph

K) Sham-post Central Reaction PSD Graph

L) Sham-post Central Movement PSD Graph
Figure A-2 (A-X): PSD graphs for all participants (Task-Baseline) before and after 5 Hz (active) and control (sham) rTMS.
APPENDIX 3: Handwriting Raw Data

Figure A-3-1: Pre-Post 5 Hz rTMS: Handwriting

Figure A-3-2: Pre-Post control rTMS: Handwriting