2016

The Contribution of the Cerebello-thalamo-cortical Circuit to the Pathology of Non-dopaminergic Responsive Parkinson's Disease Symptoms

Shannon C. Lefaivre
Wilfrid Laurier University, lefa3540@mylaurier.ca

THE CONTRIBUTION OF THE CEREBELLO-THALAMO-CORTICAL CIRCUIT TO THE PATHOLOGY OF NON-DOPAMINERGIC RESPONSIVE PARKINSON'S DIESASE SYMPTOMS

Shannon C. Lefaivre

Honours Bachelor of Science in Kinesiology, University of Waterloo, 2012

THESIS

Submitted to Kinesiology and Physical Education, Faculty of Science
in partial fulfillment for the requirements for

Master of Science

Wilfrid Laurier University

ABSTRACT

It has been well established that motor symptoms in Parkinson's disease (PD) are primarily associated with dopaminergic degeneration in the basal ganglia. However, symptoms which respond poorly to dopaminergic replacement, such as tremor, gait, and balance deficits, point to an alternative pathology to dysfunction of the basal ganglia. Over-activity of the cerebellum has been demonstrated in PD, however it is not entirely clear how the cerebellum might be affecting motor symptoms. A lack of consensus exists regarding how cerebellar over-activity might be influencing PD tremor, and whether resting and postural tremor are differentially influenced by cerebellar dysfunction. It is also unclear how cerebellar over-activity might be affecting gait and balance deficits in PD, even though the cerebellum is an important subcortical structure for the control of gait and balance. Thus, the aim of the current thesis was to assess how cerebellar over-activity may be influencing symptoms which respond poorly to dopamine replacement in PD by inhibiting cerebellar activity using repetitive transcranial magnetic stimulation (rTMS). Additionally, a direct comparison was made between the effects of stimulation targeted to the medial versus lateral cerebellum with the aim to localize the effect of cerebellar over-activity. Fifty PD participants were randomly assigned to receive stimulation over either the medial cerebellum (n=20), lateral cerebellum (n=20) or sham stimulation (n=10). 900 pulses at 1Hz were delivered at an intensity of 120% resting motor threshold determined from the first dorsal interosseous muscle representation in the primary motor cortex. Tremor was assessed quantitatively using a wireless finger accelerometer to record tremor. Balance was measured with objective, computerized protocols: modified clinical test of sensory integration and balance (m-CTSIB) and postural stability testing (PST). Spatiotemporal gait parameters were

measured quantitatively during self-paced walking. All assessments were performed before and after either real or sham stimulation. Resting tremor frequency was reduced in tremor-dominant individuals, regardless of whether stimulation was applied over the medial ($p=0.024$) or lateral ($p=0.033$) cerebellum, but not in the sham group. Additionally, inhibition of the cerebellum did not result in modulation of gait and balance outcome measures. Hence, dysfunction of the cerebellum may be a contributing factor to resting tremor, but not gait and balance deficits in PD. Importantly, the improvements in resting tremor occurred without detriment to gait or balance, demonstrating the therapeutic potential of this stimulation protocol. Low frequency rTMS over the medial or lateral cerebellum provides promise of an alternative treatment for resting tremor in PD, a symptom that is poorly responsive to dopaminergic replacement.

ACKNOWLEDGEMENTS

There are a number of individuals who must be wholeheartedly thanked for their contributions to the success of this project.

Dr. Quincy Almeida for his mentorship and guidance through the confusing world that is research. I admire your utter devotion to improving the lives of individuals living with Parkinson's disease; I can only hope to carry forth your passion in my own way to help others.

Matt Brown, for teaching this young grasshopper everything there is to know about TMS. I cannot express how much your support throughout this entire project was appreciated; it would not have been possible without you. I will be forever thankful for your patience and time spent teaching. Dr. Richard Staines must also be thanked for his generosity; allowing me to use the equipment in his TMS lab throughout the learning process was vital to the success of this project.

The Movement Disorders Research and Rehabilitation Centre (MDRC) student research team including Kaylena Ehoetz Martens, Iko Pieruccini-Faria, Carolina Silveira, Eric Beck, Brittany Intzandt and Matt Lasswell. We truly are a MDRC family, and without your support and motivation throughout this project I would not have succeeded.

Thank you to all of the participants involved in this project, without whom this thesis would not have been possible. Interacting with each and every one of you was always the highlight of my day. Your positive outlook on life is truly inspiring, and your lessons taught me more than just about Parkinson's disease.

The devotion of student volunteers did not go unnoticed, particularily Moumita Paul and Yi Wang. We made a great team, and without your time this project could not have run as smoothly. Your perseverance through long days of data collection is much appreciated.

A loving thank you must be reserved for my parents, who have always been a constant source of encouragement throughout all academic endeavours. Thank you for being there through the struggles and successes of graduate school, and pushing me to move forward when I was feeling defeated. Finally, my dog Finley; who is always happy to see me, regardless of whether or not my results are significant.

Contents

List of Tables and Figures:

Chapter 2:

Chapter 3:

Chapter 4: Grand Discussion

Chapter 1: Prologue

Parkinson's disease

Degeneration of dopaminergic neurons in the basal ganglia is responsible for the motor symptoms of Parkinson's disease (PD). This neurodegenerative disease is progressive in nature, and typically presents four cardinal movement symptoms: tremor, rigidity, bradykinesia and postural instability. Motor symptoms do not become present until the amount of dopaminergic loss is substantial, and beyond repair. Typically, motor symptoms appear once 50-60% of dopaminergic neurons in the substantia nigra are lost, and striatal dopamine is diminished by 80-85% [1]. Without a cure for PD, treatments are focused on symptom management, allowing individuals to live more comfortably with the disease for a longer duration.

The gold standard for treatment of PD symptoms is dopaminergic replacement therapy, which acts to supplement dopamine that has been lost in the basal ganglia [2–4]. Although this is an effective method of treatment, the benefits of the medication vary over time[4]. Prolonged treatment in this manner increases the susceptibility of the individual to a realm of negative side effects. One side effect is the wearing off phenomenon, which is associated with motor fluctuations and characterized by the appearance or worsening of motor symptoms occurring before a scheduled dose of dopaminergic medication[3,4]. As the required dose for management of motor symptoms becomes higher, so does the risk of developing dyskinetic movements in the head, limbs, trunk and respiratory muscles. Dyskinetic movements are characterized by involuntary, purposeless movements which occur during both rest and voluntary movements [2,4,5]. Therefore, dopaminergic replacement is an effective shorter-term therapy for improving symptoms of rigidity and bradykinesia, however, the medication is less effective at improving

tremor, and has no effect on postural stability or balance control. This makes evident the need to look for alternatives to pharmaceutical therapy for the treatment and management of PD motor symptoms. Alternatives such as techniques to stimulate brain activity have the potential to enhance our understanding of brain functions and thus, the development of more effective treatments for PD symptoms.

Cortical Excitability and Transcranial Magnetic Stimulation

Transcranial magnetic stimulation (TMS) has been investigated as a non-invasive treatment option for PD symptoms [6–11]. TMS induces electric currents in the brain by producing magnetic pulses which are able to pass through the skull with little impedance. These magnetic fields are produced from a rapid electric current which circulates within the stimulation coil resting on the scalp[12]. The electric currents induced in the cortex travel perpendicular to the magnetic field in the coil, meaning that cortical stimulation travels in a direction toward the centre of the cortex [13]. The induced electrical current has the ability to depolarize neurons, and generate action potentials within specific cortical areas. For depolarization of a neuron to occur, the induced electric current must be strong enough to pass through the neuronal membrane[12,14].

TMS is an avenue for inducing electric currents within the cortex, where the effects essentially act through modulation of cortical excitability [12,15–17]. Stimulation at high frequencies (5Hz or above) induces facilitatory changes in cortical excitability, through what is thought to be long term potentiation (LTP) mechanisms[14,15]. Meanwhile, stimulation at low frequencies (1Hz or below) induces inhibitory changes in cortical excitability, through what is thought to be long term depression (LTD) mechanisms[14,15].

Long term potentiation is a use-dependent strengthening of a synapse, which can increase the excitatory post-synaptic potentials following stimulation for a duration of time beyond the period of activation [18,19]. A common form of LTP requires activation of NMDA receptors in the synapse during post-synaptic depolarization. This activation during the early phase of long term potentiation causes a rise of calcium ion concentration in the dendritic spine by allowing calcium ions to pass through the NMDA receptor channel. The increase in calcium ion concentration may be enough to trigger LTP alone, however other mediating factors, such a proteins may also contribute. An increase in the number of AMPA receptors at the synapse has also been demonstrated as an important component of LTP expression. The late phases of LTP require protein synthesis if the synaptic changes are to persist beyond 30-60 minutes. Synapses at which LTP occurs undergo structural changes to permit late phase (long lasting) changes in excitability. Synaptic changes include the growth of dendritic spines, enlargement of existing spines, as well as either growth of post-synaptic densities or splitting to form two functional synapses[19]. Long term potentiation mechanisms differ at mossy fibre synapses, as well as cerebellar parallel fibre synapses, in that activation of NMDA receptors is not required. It is instead a rise in presynaptic calcium ions and the activation of presynaptic receptors by glutamate which plays a strong facilitatory role in mossy fibre LTP. However the need for new protein synthesis for late phase LTP is a common factor [19], and becomes important when lasting changes in cortical excitability are desired.

In contrast, long term depression refers to a decrease in synaptic strength which is thought to either prevent a saturation of LTP, by placing a synapse in a temporary state of heightened responsiveness, or to serve as a replacement for information previously associated with LTP. LTD has also been suggested to function as an amplification mechanism, allowing

signals from surrounding potentiated synapses to become of greater strength [19,20]. NMDA receptor activation and a rise in post-synaptic calcium ions also play a key role in a common form of LTD, however it is the stimulation intensity which dictates the excitability changes. It has been suggested that the sub-unit composition of the NMDA receptors is regulated by activity at the synapses, and thus governs the expression of either LTD or LTP. While an increase in AMPA receptors contributes to LTP, it is a decrease in AMPA receptors that accompanies LTD expression [19]. Given that cerebellar synapses lack NMDA receptor expression[20,21], there exists three post-synaptic events which must occur for LTD induction in the cerebellum. First, the climbing fibres initiate a large calcium ion influx by causing post synaptic depolarization that is sufficient to activate voltage-gated calcium ion channels in the dendrites. Second, parallel fibres activate metabotropic receptors and non-NMDA excitatory amino acid receptors. Finally, an influx of sodium ions occurs via AMPA receptors (activated by parallel fibres) and voltage-gated sodium ion channels. Importantly, activation of both parallel fibres and climbing fibres (or directly Purkinje neurons) are necessary for the induction of LTD in the cerebellum[20,21]. LTD has been suggested to be related to motor performance errors, such that a synapse is signalled to be weakened following an error. According to existing models, it is the climbing fibres which dictate changes in synaptic strength[20].

Modulation of synaptic strength, and thus, cortical excitability, requires repeated activation or inhibition of the associated cortical area[6], particularly if the changes are intended to be long-lasting. The induced effects of stimulation on a cortical area is dependent on many parameters: the frequency or rate at which the pulses are delivered, the intensity of the stimulus, the duration of stimulation period (total number of pulses) and the cortical location of

stimulation[22]. Therefore, the stimulation effects found will be reflective of the parameters chosen.

Repetitive transcranial magnetic stimulation (rTMS)

Repetitive transcranial magnetic stimulation (rTMS) involves repetitive pulses being delivered, and allows for cortical changes in excitability to outlast the period of stimulation. With repeated sessions of stimulation, it is possible that the cumulative effects of rTMS may interact directly with the mechanisms of cortical plasticity, and prolong the period for which the cortical changes due to LTD and LTP exist beyond the period of stimulation. Studies have suggested that the threshold for inducing inhibitory effects in the cortex is lower than that of facilitatory effects, and the inhibitory effects were able to accumulate quicker[23]. In a review article by Fregni et al. (2005), it was found that the studies showing significant and long lasting effects were the protocols that demonstrated immediate benefits following an acute period of stimulation. This might suggest that immediate motor benefits are predictive of long lasting benefits[16], and could suggest the potential efficacy of a stimulation protocol for use as a therapy.

What is known about rTMS as a treatment in PD?

Several studies have explored the effectiveness of rTMS for PD. For example, Lomarev et al. (2006) found that two rTMS sessions per week for a total of eight weeks was able to significantly improve gait speed during a ten metre walking task, and upper limb bradykinesia for individuals with PD[6]. These motor improvements persisted for at least one month following the end of the stimulation sessions. During each stimulation session, 300 magnetic pulses at 100% motor threshold and 25Hz was delivered to each of four cortical targets: left and right

abductor pollicis projection in the motor cortex, as well as left and right dorsolateral prefrontal cortex[6]. In another study by Hamada et al, 1000 magnetic pulses were delivered in trains of 50 stimuli at 5Hz and 110% active motor threshold of the right tibialis anterior muscle[7]. Sessions were performed once each week for a total of eight weeks, with the pulses being applied over the supplementary motor area. This group found modest improvements in Unified Parkinson's Disease Rating Scale motor sub-section (UPDRS-III) scores following the intervention, with changes lasting up to 4 weeks beyond culmination of treatment[7]. Other protocols, such as stimulation Monday to Friday, for two weeks (ten total sessions) over the vertex demonstrated no therapeutic potential[8]. Arias et al administered 100 pulses in trains of 50 stimuli at 1Hz and an intensity of 90% resting motor threshold. This group determined the protocol to provide benefits not greater than placebo on the performance of a walking task, grooved pegboard and total UPDRS scores[8]. Though the examples provided are not comprehensive of all rTMS therapeutic protocols, it is clear that a stimulation target alternative to the motor cortex may provide some additional benefits.

Neural Circuitry Related to rTMS Application in PD

The Basal Ganglia in Parkinson's disease

Arguably the most important property of TMS is the stimulation target, which requires a great understanding of cortical circuitry. The primary brain structure implicated in the pathophysiology of PD is the basal ganglia, more specifically the degeneration of dopaminergic producing neurons in the substantia nigra pars compacta. Dopamine is a vital neurotransmitter that is implicated in the basal ganglia pathways responsible for movement production; the lack of

neurotransmitter in the disease state results in altered connectivity within these pathways, ultimately affecting movement output.

There are two main pathways within the basal ganglia that modulate movement: the direct pathway, associated with promoting movement production, and the indirect pathway, associated with the inhibition of movement production[24–26]. Both pathways originate from populations of neurons in the striatum which express opposing types of dopaminergic receptors. Activation of D1 receptors in the striatum produces an overall excitatory effect on the neurons associated with the direct path, while activation of D2 receptors produces an overall inhibitory effect on neurons of the indirect path[25]. In the direct path, cortical information from the striatum is transmitted directly to the globus pallius internus and the substantia nigra pars reticulata, the output nuclei of the basal ganglia, via an inhibitory GABA-ergic projection. In contrast, the indirect path transmits cortical signals from the striatum indirectly through an inhibitory GABA-ergic projection from the globus pallidus externus to the subthalamic nucleus, and an excitatory projection from the subthalamic nucleus to the output nuclei[24,25]. Signals from both pathways relay in the thalamus before being transmitted back to the cerebral cortex and brainstem to exert effects on movement production.

Dopaminergic loss in the substantia nigra pars compacta has a major effect on the connectivity of these pathways, and thus, movement production. The lack of the neurotransmitter dopamine results in decreased activity within the striatal neurons of the direct pathway. Meanwhile, a lack of dopamine promotes increased activity in the striatal neurons of the indirect pathway. The increased activity results in increased inhibition of the globus pallidus externus, coupled with disinhibition of the subthalamic nucleus which potentiates to increased excitation of the basal ganglia output nuclei [25]. Taken together, decreased activity from the direct

(movement producing) pathway, in conjunction with increased activity in the indirect (movement inhibiting) pathway, results in a net inhibitory output from the basal ganglia nuclei. Thus, dopaminergic loss is associated with an overall reduction in the production of movement [24,25].

This makes the basal ganglia a logical target for the application of magnetic stimulation due to the role of the structure in the pathophysiology of the disease, however, there are several issues with using the basal ganglia as a target. First, due to the depth of the basal ganglia within the brain, the stimulation intensity required to reach an adequate depth would be above the range of tolerable stimulation, or the capabilities of TMS devices. Second, if such an intensity was attained to reach the depth of the basal ganglia, the electrical current induced in the cortex would be very diffuse, not allowing for focal stimulation. Finally, areas superior to the basal ganglia within the cortex would be stimulated as the electrical current travelled toward the structure, not allowing for the basal ganglia to be targeted with TMS in isolation. It has now been made evident that a more superficial structure, alternative to the basal ganglia, must be chosen as a stimulation target.

The Cerebellum in Parkinson's disease

As previously mentioned, the onset of motor symptoms in PD does not occur immediately upon the initiation of degeneration of dopaminergic neurons, but after a substantial loss. This might suggest the existence of a compensatory mechanism which functions to preserve normal basal ganglia functioning and delay the onset of symptoms[27]. There is increasing evidence of multi-synaptic anatomical connections that exist between the cerebellum and the basal ganglia [28–30].

The dentate nucleus projects to both the striatum and globus pallidus externus of the basal ganglia. A disynaptic projection to the striatum, by way of the thalamus, originates in both motor and non-motor areas of the dentate nucleus[28–30]. This suggests that the cerebellum might influence this pathway, which is implicated in information processing in the basal ganglia. The subthalamic nucleus of the basal ganglia also has a topographically organized disynaptic projection to the cerebellar cortex through the pontine nuclei. This connection enables the involvement of integration of the basal ganglia-cerebellar functions on both a motor and non-motor level[28–30]. The subthalamic neurons project to both the crus II posterior (area of the cerebellum which receives input from regions of the prefrontal cortex and frontal eye fields), as well as the sensorimotor area of lobule VIIB (an area which receives input from the primary and premotor cortices)[28]. These identified reciprocal connections between the basal ganglia and the cerebellum create an anatomical basis to question the involvement of the cerebellum in the pathology of PD and the associated motor symptoms.

The presence of dopamine D1-3 receptors in the cerebellum allows this structure to receive dopaminergic innervation from the ventral tegmental area/ substantia nigra pars compacta[31]. Knowing that the substantia nigra pars compacta is the primary site of dopaminergic loss in PD, it is no surprise that expression levels of D1-3 dopamine receptors are consequently altered in the cerebellum. Dopamine receptors, along with the mRNA for tyrosine hydrolase and dopamine active transporter, are present in the cerebellar vermis (lobules 9 and 10). Since mRNA is only associated with neural cell bodies and dendrites, the presence of dopamine producing mRNA in the cerebellum suggests that these gene products must exist intrinsically within the cerebellum[31]. Thus, the presence of dopamine within the cerebellum may help explain the connectivity and signalling between the basal ganglia and cerebellum.

Post-mortem analysis of the cerebellum in PD patients showed decreased levels of D1 and D3 dopamine receptor mRNA in lobule 9, along with decreased levels of tyrosine hydrolase and dopamine receptor mRNA in lobule 10 [31]. Similarly, lesions of the paravermal cerebellum increases receptor D1 levels in the contralateral striatum, suggesting that cerebellar nuclear and cortical projections modulate D1 receptor expression of the striatal direct pathway[31]. Within the striatum, a co-expression of dopamine D1-D3 receptors exists within the same cells, where the D1 and D3 receptors exert opposing effects on the D2 receptors. The specific reduction of D1 and D3 receptors in the cerebellum, without modification in D2 receptor expression, suggests that the dopamine receptors of the cerebellum behave similarly to those in the striatum[31]. Degeneration of striatal dopaminergic neurons would therefore be related to dysfunction not only in the basal ganglia, but also in the basal ganglia-thalamic and cerebellum-thalamic connections. This might suggest that this pathological damage in the cerebellum, that is unique to PD, might contribute to motor symptom generation.

There are characteristic changes that occur in the cortical connectivity patterns in PD: hyper-metabolism in the striatum, thalamus, pons and cerebellum, together with hypo-metabolism in the supplementary motor area, premotor and parieto-occipital association areas[28]. Increased activation of the cerebellum in PD compared to controls is consistently present not only throughout motor task execution and motor learning tasks, but also at rest[28,30,32]. Functional magnetic resonance imaging shows that in predominantly akinetic/rigid PD patients, the amount of spontaneous cerebellar activation at rest is heightened. In these individuals, there were weakened connections between the striatum-cortex and striatum-cerebellum, paired with increased connectivity between the cortex and cerebellum during self-initiated movements[28]. It has been suggested that the hyperactivity in the cerebellum might be

a compensatory strategy to help overcome the dysfunction in the basal ganglia[28,32]. Understanding the connection between the cerebellum and basal ganglia may lead to potential treatment options for those symptoms associated with PD that are not responsive to dopaminergic treatment.

In order for the hyper-activation and connectivity changes in the cerebellum to be considered a compensatory mechanism, the responses of the cerebellum should exhibit physiological adaptations that act to counterbalance the abnormal functioning of the basal ganglia. For example, the supplementary motor area is a main input region in the basal ganglia motor circuit, where decreased activity in PD is thought to be the result of inadequate striato-thamalo-cortical facilitation[28,32]. Since the supplementary motor area is heavily implicated in initiating internally generated movements, pairing this underactivity in the striato-thalamo-cortical loop with dopamine depletion could provide a mechanism to explain akinesia in PD. Interestingly, where hyper-activity was found in the cerebellum, individuals with PD were able to complete motor tasks in a manner comparable to healthy participants[28]. This might suggest that the increased activity in the cerebello-thalamo-cortical loop compensated for the lack of activity in the striato-thalamo-cortical loop in such a way that motor function was able to remain near normal[32]. To further support this notion of compensation, activity or connectivity of the cerebello-thalamo-cortical loop demonstrated a positive correlation with UPDRS scores (a clinical assessment scale), while that of the striato-thalamo-cortical loop demonstrated a negative correlation[28]. Recruitment of the cerebello-thalamo-cortical loop increases with PD progression, suggesting that as the impairment in the striato-thamalo-cortical loop becomes greater, activity in the latter becomes more important for compensation.

On the opposing side, increased cerebellar activity could be seen as negatively contributing to the pathophysiology of PD symptoms. While the cerebello-thalamo-cortical loop may serve to compensate for akinetic/rigid symptoms, it may actively contribute to other symptoms, such as tremor, poor balance and gait impairments. Given that the connection between the basal ganglia and the cerebellum is reciprocal, hyperactivity in the cerebellum could stem from the inability of the basal ganglia to suppress activity of inappropriate or abnormal output of circuits.[30]. The subthalamic nucleus, or the "driving force" of the basal ganglia[26], has significantly heightened activity in PD. The subthalamic nucleus output is excitatory (glutamatergic), and the activity can be described in PD as abnormal bursting and oscillatory in nature. Propagation of the chronically increased excitatory drive from the subthalamic nucleus to the cerebellum via the pontine nuclei could account for the overactivity of the cerebellum. This has been confirmed in rat brains, whereby activation of neurons in the deep cerebellar nuclei occurs following high frequency stimulation of the subthalamic nucleus[30]. Therefore, altered cerebello-thalamo-cortical circuit activity might actually contribute to PD symptoms, and help to explain why not all symptoms are responsive to dopaminergic replacement therapy, or the result of striatal dopaminergic degeneration.

The Contribution of the Cerebellum to Motor Symptoms in PD

Cerebellar Contributions to Tremor Generation

Parkinsonian tremor exists in the most distal extremities, and can occur both during rest or movement. Resting tremor persists at 4-5 Hz, while postural tremor is characterized by a higher frequency of 8-12 Hz[27]. Dysfunction of the basal ganglia and striatal dopamine degeneration are more strongly linked with the bradykinesia and rigidity associated with PD, and

seem to be less implicated in the generation of tremor[27]. Given that peripheral deafferentation fails to modulate resting tremor, it can be concluded that the underlying mechanism must be related to a more central abnormality[28]. There is increasing evidence that the cerebello-thalamo-cortical pathway contributes to the pathophysiology of tremor. There is a characteristic tremor-related pattern of cortical activation in PD, consisting of concurrent increases in activation of the cerebellum, motor cortex and putamen. This pathway demonstrates a correlation with clinical tremor ratings, but not that of akinesia or rigidity. Similarly, activity in this cortical pattern has been found to be elevated in tremor-dominant PD, while not in akinetic-rigid PD, suggesting specific involvement in the generation of tremor. Further, it has been shown that deep brain stimulation (DBS) of the ventrolateral nucleus reduces the activity of this pattern, while DBS of the subthalamic nucleus reduces both tremor and akinetic/rigid activity simultaneously[28]. This further supports the notion suggesting that tremor may be generated by a metabolic network which is distinct from that of akinesia/rigidity. It has long been thought that tremor may be generated by neural mechanisms which are actively working to compensate for akinesia and rigidity[28]. This could help to explain the differential symptomology between individuals with PD.

Individuals with PD who experience tremor symptoms demonstrate a strengthened functional connectivity between the basal ganglia and the cerebello-thalamo-cortical pathway, suggesting this pathological interaction may result in tremor generation. Tremor reset is a tool used to confirm the contribution of a specific stimulated brain area to the generation or transmission of tremor[33]. The cerebello-thalamo-cortical pathway allows stimulation effects from TMS on the cerebellum to be transmitted to the thalamus, basal ganglia and primary motor cortex, which interrupts the ongoing tremor drive, resulting in a tremor reset. Activity in the

basal ganglia is the likely trigger to tremor generation in the cerebello-thalamo-cortical circuit, specifically dysfunction in the vermis/paravermis region. Reduced excitability in the cerebello-thalamo-cortical pathway might allow for abnormal neuronal activities to occur at the thalamus and primary motor cortex, which is the site of integration of cerebellar and basal ganglia loops[34].

It is debated whether the cerebellum contributes to both rest and postural tremor, or whether the cereblello-thalamo-cortical loop may have a more significant effect on one specific form of tremor. A study using paired-pulse TMS over the cerebellum and primary motor cortex found that the amount of cerebellar inhibition in the primary motor cortex was proportional to the tremor reset index[33]. While this protocol did not alter the frequency of tremor, it did result in the reset of postural, but not resting, tremor. It has been suggested that pathways for generation of resting and postural tremor originate from differing cortical areas. This notion can be supported based on the key differences in the frequency between resting and postural tremor (rest tremor has a lower frequency) and the stimulation targets which produced the highest tremor reset index[33]. While stimulation of the primary motor cortex reset both varieties of tremor, the reset index was higher for postural tremor. In contrast, cerebellar stimulation only reset postural tremor, having no effect on resting tremor. Ni et al suggested the isolated involvement of the cerebellum in postural tremor, with the effects likely mediated by the basal ganglia and not the primary motor cortex following stimulation.

Pallidal dopamine depletion levels are a strong indicator of tremor severity, and does not seem to be related to striatal dopamine depletion[34]. While the basal ganglia does have a role in the generation of tremor, it is the cerebello-thalamo-cortical circuit that drives the tremor pattern on a cycle-by-cycle basis. This property of tremor generation may help to explain why tremor-

dominant PD have reportedly better symptom prognosis, characterized by lower risk of dyskinesia and motor fluctuations in response to dopaminergic replacement therapy. In comparison to akinetic/rigid PD, it is possible that tremor-dominant PD is less due to basal ganglia pathologies, and is more associated with abnormalities associated with the cerebello-thalamo-cortical circuit[27]. Whether this is true for both postural and rest tremor or specifically, postural tremor is still debated in research.

Cerebellar Contributions to Gait Disturbances

The cerebello-thalamo-cortical pathway has also been suggested to play a role in the pathophysiology of gait disturbances in PD. While gait parameters such as velocity, stride length and stride time have demonstrated improvements related to dopaminergic therapy, other parameters such as step frequency (cadence) and double limb support time appear to be non-responsive to dopamine replacement. Dopamine may benefit gait indirectly by reducing the effects of rigidity and bradykinesia , without effectively improving all aspects of gait[35]. This suggests the involvement of separate pathways underlying the pathology of gait disturbances in PD, where spatial aspects of gait seem more related to dopamine, and thus the involvement of the basal ganglia, than do temporal aspects of gait[36–38].

Double limb support time is thought to reflect postural stability during gait. This gait parameter is increased in PD, indicative of impaired dynamic balance control mechanisms [36–38]. Decreased acetylcholinesterase activity in the midbrain and cerebellum were found to be correlated with the severity of balance and gait impairments in PD. During gait, it has been suggested that hyper-activation in the vermis may act to compensate for a lack of lateral gravity shift[28]. This property, likely caused by hypo-activation in the left cerebellar hemisphere, is thought to be responsible for the small, shuffling steps characteristic of individuals with PD[28].

Thus, it may be possible to find benefits in gait through modifying cortical activity in the cerebellum.

The pedunculopontine nucleus demonstrated connectivity with the cerebellum in healthy subjects and individuals with PD who lack severe gait disturbances [28]. In PD who had severe gait disturbances, this connection did not exist. It was found that when DBS was applied to the pedunculopontine nucleus, a consequent increase in blood flow within the cerebello-thalamo-cortical pathway resulted[28]. This might suggest that the cerebello-thalamo-cortical pathway may be an effective stimulation target to improve gait disturbances in PD.

Cerebellar Contributions to Balance Control and Postural Instability

An important intermediary to understanding how the cerebellum influences gait disturbances is to first understand its contributions to balance. There is increasing evidence that postural instability in PD cannot be attributed to only the loss of striatal dopaminergic neurons. The vermis and fastigial nuclei are important structures in the cerebellum which contribute to balance control. These regions are implicated in exerting control over extensor muscle tone in order to maintain upright balance and stance. Also under the control of these regions is the vestibular and reticular nuclei, which act to modulate the rhythmic activation of both flexor and extensor muscle groups. The cerebellum also utilizes sensory feedback from the limbs to exert postural changes for balance control.

Impairments in sensory integration have been suggested to have a negative impact on balance in PD[39–43], such that deficits in proprioceptive processing require individuals to rely mainly on visual feedback for balance control. It has been suggested that vision dependence in PD could be a cerebellar mechanism which allows utilization of relatively intact cerebellar

pathways in order to avoid the dysfunctional basal ganglia. Individuals with PD therefore rely on an intact visually guided network, that is the cerebellum[27]. This heightened reliance on vision puts individuals with PD at a greater risk of loss of balance when vision is compromised or not available, such as in dark environments[43,44]. It is possible that altering the activity within the cerebellum-basal ganglia connections may improve sensory integration, and thus postural stability, by decreasing the reliance on vision.

There seems to exist a PD-specific effect related to thalamic cholinergic denervation such that the ability to integrate sensory information for balance control is negatively affected[45]. The pedunculopontine complex sends cholinergic inputs to the thalamus, cerebellum, brainstem nuclei, basal ganglia and spinal cord[45]. Neurons in the thalamus which respond to proprioceptive stimuli, are functionally related with magnocellular neurons receiving cerebellar projections in the ventrolateral posterior nucleus. This might suggest that some level of cerebellar modulation of proprioceptive information might occur in the thalamus neurons before they are sent to motor cortical areas[46]. When comparing individuals with PD who experience falls against those that do not, there is not a significant difference in the level of striatal dopaminergic degeneration. Interestingly, PD who experience falls have significantly decreased pedunculopontine (thalamic) cholinergic innervation [43]. The level of cholinergic denervation was found to be associated both with poor balance during assessments, as well as increased UPDRS scores. Treatment with acetylcholinesterase significantly reduces the number of falls, suggesting that degeneration of the pedunculopontine cholinergic system might be associated with contributing to the pathology of postural instability[45]. Thus, modifying activity within the cerebello-thalamo-cortical loop has the potential to improve symptoms of postural instability in PD.

Modulation of the Cerebello-Thalamo-Cortical Pathway

Thesis Objective

The need to investigate the role of the cerebellum in the generation of tremor, as well as gait and balance deficits has been made evident. The current thesis will aim to understand how the cerebellum might be influencing these symptoms which respond poorly to dopaminergic replacement, by modulating cerebellar over-activity using a repetitive transcranial magnetic stimulation (rTMS) protocol. Direct comparisons are made between the effects on motor symptoms of an inhibitory rTMS protocol targeted at the medial versus lateral cerebellum, controlled by a sham stimulation group. Chapter two aims to investigate how the cerebellum might be differentially affecting resting and postural tremor in PD, with the use of a wireless finger accelerometer to objectively measure tremor before and after rTMS. Chapter three goes further to investigate whether cerebellar over-activity might also be influencing gait and balance deficits in PD, by using objective and computerized assessments of balance and spatiotemporal gait parameters before and after rTMS. Finally, chapter four provides a general discussion, offering a summary of the findings and implications for guiding future research toward further understanding the cerebellum in Parkinson's disease.

References

[1] Wirdefeldt K, Adami H-O, Cole P, Trichopoulos D, Mandel J. Epidemiology and etiology of Parkinson's disease: a review of the evidence. Eur J Epidemiol 2011;26:S1–58.

[2] Thanvi B, Lo N, Robinson T. Levodopa-induced dyskinesia in Parkinson's disease: clinical features, pathogenesis, prevention and treatment. Postgrad Med J 2007;83:384–8.

[3] Pahwa R, Lyons KE. Levodopa-related wearing-off in Parkinson's disease: identification and management. Curr Med Res Opin 2009;25:841–9.

[4] Almeida QJ, Hyson HC. The Evolution of Pharmacological Treatment for Parkinson ' s Disease. Recent Pat CNS Drug Discov 2008;3:50–4.

[5] Rothwell J. Transcranial magnetic stimulation as a method for investigating the plasticity of the brain in Parkinson ' s Disease and dystonia. Parkinsonism Relat Disord 2007;13:417–20.

[6] Lomarev MP, Kanchana S, Bara-Jimenez W, Iyer M, Wassermann EM, Hallett M. Placebo-controlled study of rTMS for the treatment of Parkinson's disease. Mov Disord 2006;21:325–31.

[7] Hamada M, Ugawa Y, Tsuji S. High-frequency rTMS over the supplementary motor area for treatment of Parkinson's disease. Mov Disord 2008;23:1524–31.

[8] Arias P, Vivas J, Grieve KL, Cudeiro J. Controlled trial on the effect of 10 days low-frequency repetitive transcranial magnetic stimulation (rTMS) on motor signs in Parkinson's disease. Mov Disord 2010;25:1830–8.

[9] Khedr EM, Farweez HM, Islam H. Therapeutic effect of repetitive transcranial magnetic stimulation on motor function in Parkinson's disease patients. EurJNeurol 2003;10:567–72.

[10] Lefaucheur J-P, Drouot X, Von Raison F, Ménard-Lefaucheur I, Cesaro P, Nguyen J-P. Improvement of motor performance and modulation of cortical excitability by repetitive transcranial magnetic stimulation of the motor cortex in Parkinson's disease. Clin Neurophysiol 2004;115:2530–41.

[11] Benninger, D.H., Berman, B.D., Houdayer, E., Pal, N., Luckenbaugh, D.A., Schneider, L., Miranda, S. & Hallett M. Intermittent theta-burst transcranial magnetic stimulation for treatment of Parkinson disease. Neurology 2011;76:601–9.

[12] Arias-Carrión O. Basic mechanisms of rTMS: Implications in Parkinson's disease. Int Arch Med 2008;1:2.

[13] Hallett M. Transcranial magnetic stimulation: a primer. Neuron 2007;55:187–99.

[14] Kobayashi M, Pascual-leone A. Transcranial magnetic stimulation in neurology. Lancet Neurol 2003;2:145–56.

[15] Cantello R, Tarletti R, Civardi C. Transcranial magnetic stimulation and Parkinson ' s disease. Brain Res Rev 2002;38:309–27.

[16] Fregni F, Simon DK, Wu a, Pascual-Leone a. Non-invasive brain stimulation for Parkinson's disease: a systematic review and meta-analysis of the literature. J Neurol Neurosurg Psychiatry 2005;76:1614–23.

[17] Rossini PM, Rossi S. Transcranial magnetic stimulation: diagnostic, therapeutic, and research potential. Neurology 2007;68:484–8.

[18] Madison D V, Malenka RC, Nicoll R a. Mechanisms underlying long-term potentiation of synaptic transmission. Annu Rev Neurosci 1991;14:379–97.

[19] Malenka RC, Bear MF. LTP and LTD: an embarrassment of riches. Neuron 2004;44:5–21.

[20] Massey P V, Bashir ZI. Long-term depression: multiple forms and implications for brain function. Trends Neurosci 2007;30:176–84.

[21] Linden DJ. Long-term synaptic depression in the mammalian brain. Neuron 1994;12:457–72.

[22] Siebner HR, Rothwell J. Transcranial magnetic stimulation: new insights into representational cortical plasticity. Exp Brain Res 2003;148:1–16.

[23] Filipović SR, Rothwell JC, Bhatia K. Slow (1 Hz) repetitive transcranial magnetic stimulation (rTMS) induces a sustained change in cortical excitability in patients with Parkinson's disease. Clin Neurophysiol 2010;121:1129–37.

[24] DeLong MR, Wichmann T. Circuits and circuit disorders of the basal ganglia. Arch Neurol 2007;64:20–4.

[25] Smith Y, Bevan MD, Shink E, Bolam JP. Microcircuitry of the direct and indirect pathways of the basal ganglia. Neuroscience 1998;86:353–87.

[26] Strafella AP, Vanderwerf Y, Sadikot AF. Transcranial magnetic stimulation of the human motor cortex influences the neuronal activity of subthalamic nucleus. Eur J Neurosci 2004;20:2245–9.

[27] Lewis MM, Galley S, Johnson S, Stevenson J, Huang X, Mckeown MJ. The Role of the Cerebellum in the Pathophysiology of Parkinson ' s Disease. Can J Neurol Sci 2013;40:299–306.

[28] Wu T, Hallett M. The cerebellum in Parkinson's disease. Brain 2013;136:696–709.

[29] Carrillo F, Palomar FJ, Conde V, Diaz-corrales FJ, Porcacchia P, Fernández-del-olmo M, et al. Study of Cerebello-Thalamocortical Pathway by Transcranial Magnetic Stimulation in Parkinson ' s Disease. Brain Stimul 2013;6:582–9.

[30] Kishore A, Meunier S, Popa T. Cerebellar influence on motor cortex plasticity : behavioral implications for Parkinson ' s disease. Front Neurol 2014;5:1–8.

[31] Hurley MJ, Mash DC, Jenner P. Markers for dopaminergic neurotransmission in the cerebellum in normal individuals and patients with Parkinson's disease examined by RT-PCR. Eur J Neurosci 2003;18:2668–72.

[32] Yu H, Sternad D, Corcos DM, Vaillancourt DE. Role of hyperactive cerebellum and motor cortex in Parkinson ' s disease. Neuroimage 2007;35:222–33.

[33] Ni Z, Pinto AD, Lang AE, Chen R. Involvement of the Cerebellothalamocortical Pathway in Parkinson Disease. Ann Neurol 2010;68:816–24.

[34] Helmich RC, Janssen MJR, Oyen WJG, Bloem BR, Toni I. Pallidal Dysfunction Drives a Cerebellothalamic Circuit into Parkinson Tremor. Ann Neurol 2011;69:269–81.

[35] Foreman KB, Wisted C, Addison O, Marcus RL, Lastayo PC, Dibble LE. Improved Dynamic Postural Task Performance without Improvements in Postural Responses: The Blessing and the Curse of Dopamine Replacement. Parkinsons Dis 2012;2012:692150.

[36] Rochester L, Baker K, Nieuwboer A, Burn D. Targeting dopa-sensitive and dopa-resistant gait dysfunction in Parkinson's disease: selective responses to internal and external cues. Mov Disord 2011;26:430–5.

[37] Lord S, Baker K, Nieuwboer A, Burn D, Rochester L. Gait variability in Parkinson's disease: an indicator of non-dopaminergic contributors to gait dysfunction? J Neurol 2011;258:566–72.

[38] Almeida QJ, Frank JS, Roy E a, Patla AE, Jog MS. Dopaminergic modulation of timing control and variability in the gait of Parkinson's disease. Mov Disord 2007;22:1735–42.

[39] Tan T, Almeida QJ, Rahimi F. Proprioceptive deficits in Parkinson's disease patients with freezing of gait. Neuroscience 2011;192:746–52.

[40] Caudron S, Guerraz M, Eusebio A, Gros J, Azulay J, Vaugoyeau M. Evaluation of a visual biofeedback on the postural control in Parkinson ' s disease. Clin Neurophysiol 2014;44:77–86.

[41] Vaugoyeau M, Hakam H, Azulay J. Proprioceptive impairment and postural orientation control in Parkinson ' s disease. Hum Mov Sci 2011;30:405–14.

[42] Tagliabue M, Ferrigno G, Horak F. Effects of Parkinson's disease on proprioceptive control of posture and reaching while standing. Neuroscience 2009;158:1206–14.

[43] Horak FB. Postural orientation and equilibrium: what do we need to know about neural control of balance to prevent falls? Age Ageing 2006;35 Suppl 2:ii7–11.

[44] Jacobs J V, Horak FB. Abnormal proprioceptive-motor integration contributes to hypometric postural responses of subjects with Parkinson's disease. Neuroscience 2006;141:999–1009.

[45] Muller MLTM, Albin RL, Kotagal V, Koeppe RA, Scott PJH, Frey KA, et al. Thalamic cholinergic innervation and postural sensory integration function in Parkinson ' s disease. Brain 2013;136:3282–9.

[46] Popa T, Velayudhan B, Hubsch C, Pradeep S, Roze E, Vidailhet M, et al. Cerebellar Processing of Sensory Inputs Primes Motor Cortex Plasticity. Cereb Cortex 2013;23:305–14.

[47] Bologna M, Di Biasio F, Conte A, Iezzi E, Modugno N, Berardelli A. Effects of cerebellar continuous theta burst stimulation on resting tremor in Parkinson's disease. Parkinsonism Relat Disord 2015;21:1–6.

[48] Minks E, Mareček R, Pavlík T, Ovesná P, Bareš M. Is the cerebellum a potential target for stimulation in Parkinson's disease? Results of 1-Hz rTMS on upper limb motor tasks. Cerebellum 2011;10:804–11.

[49] Koch G, Brusa L, Carrillo F, Lo Gerfo E, Torriero S, Oliveri M, et al. Cerebellar magnetic stimulation decreases levodopa-induced dyskinesias in Parkinson disease. Neurology 2009;73:113–9.

[50] Popa T, Russo M, Meunier S. Long-lasting inhibition of cerebellar output. Brain Stimul 2010;3:161–9.

[51] Fierro B, Giglia G, Palermo A, Pecoraro C, Scalia S, Brighina F. Modulatory effects of 1 Hz rTMS over the cerebellum on motor cortex excitability. Exp Brain Res 2007:440–7.

[52] Langguth B, Eichhammer P, Zowe M, Landgrebe M, Binder H, Sand P, et al. Modulating cerebello-thalamocortical pathways by neuronavigated cerebellar repetitive transcranial stimulation (rTMS). Clin Neurophysiol 2008;38:289–95.

[53] Hardwick RM, Lesage E, Miall RC. Cerebellar transcranial magnetic stimulation: The role of coil geometry and tissue depth. Brain Stimul 2014;7:643–9.

[54] Ugawa Y, Uesaka Y, Terao Y, Hanajima R, Kanazawa I. Magnetic stimulation over the cerebellum in humans. Ann Neurol 1995;37:703–13.

[55] Giuffrida JP, Riley DE, Maddux BN, Heldmann D a. Clinically deployable kinesia technology for automated tremor assessment. Mov Disord 2009;24:723–30.

[56] Heldman D a., Giuffrida JP, Chen R, Payne M, Mazzella F, Duker AP, et al. The modified bradykinesia rating scale for Parkinson's disease: Reliability and comparison with kinematic measures. Mov Disord 2011;26:1859–63.

[57] Heldman D a., Espay AJ, LeWitt P a., Giuffrida JP. Clinician versus machine: Reliability and responsiveness of motor endpoints in Parkinson's disease. Park Relat Disord 2014;20:590–5.

[58] Ilg W, Giese M a, Gizewski ER, Schoch B, Timmann D. The influence of focal cerebellar lesions on the control and adaptation of gait. Brain 2008;131:2913–27.

[59] Manto M, Bower JM, Conforto AB, Delgado-García JM, Da Guarda SNF, Gerwig M, et al. Consensus paper: Roles of the cerebellum in motor control-the diversity of ideas on cerebellar involvement in movement. Cerebellum 2012;11:457–87.

[60] Horak FB. Clinical assessment of balance disorders. Gait Posture 1997;6:76–84.

[61] Benninger DH, Iseki K, Kranick S, Luckenbaugh D a., Houdayer E, Hallett M. Controlled Study of 50-Hz Repetitive Transcranial Magnetic Stimulation for the Treatment of Parkinson Disease. Neurorehabil Neural Repair 2012;26:1096–105.

[62] Von Papen M, Fisse M, Sarfeld AS, Fink GR, Nowak D a. The effects of 1 Hz rTMS preconditioned by tDCS on gait kinematics in Parkinson's disease. J Neural Transm 2014;121:743–54.

[63] Bohnen NI, Müller ML, Dauer WT, Albin RL. Parkinson's disease: what role do pedunculopontine cholinergic neurons play? Future Neurol 2014;9:5–8.

[64] Tard C, Delval a., Devos D, Lopes R, Lenfant P, Dujardin K, et al. Brain metabolic abnormalities during gait with freezing in Parkinson's disease. Neuroscience 2015;307:281–301.

[65] Brugger F, Abela E, Hagele-Link S, Bohlhalter S, Galovic M, Kagi G. Do executive dysfunction and freezing of gait in Parkinson's disease share the same neuroanatomical correlates? J Neurol Sci 2015;356:184–7.

[66] Göttlich M, Münte TF, Heldmann M, Kasten M, Hagenah J, Krämer UM. Altered Resting State Brain Networks in Parkinson's Disease. PLoS One 2013;8.

[67] Helmich RC, Derikx LC, Bakker M, Bloem BR, Toni I. Spatial Remapping of Cortico-striatal Connectivity in Parkinson ' s Disease. Cereb Cortex 2010;20.

[68] Rubino A, Assogna F, Piras F, Di Battista ME, Imperiale F, Chiapponi C, et al. Does a volume reduction of the parietal lobe contribute to freezing of gait in Parkinson's disease? Park Relat Disord 2014;20:1101–3.

[69] Chan HF, Kukkle PL, Merello M, Lim SY, Poon YY, Moro E. Amantadine improves gait in PD patients with STN stimulation. Park Relat Disord 2013;19:316–9.

[70] Théoret H, Haque J, Pascual-Leone A. Increased variability of paced finger tapping accuracy following repetitive magnetic stimulation of the cerebellum in humans. Neurosci Lett 2001;306:29–32.

[71] Miall RC, Christensen LOD. The effect of rTMS over the cerebellum in normal human volunteers on peg-board movement performance. Neurosci Lett 2004;371:185–9.

Chapter 2: Cerebellar involvement in Parkinson's disease resting tremor
Submitted to: Cerebellum & Ataxias

Abstract

There exists a lack of consensus regarding how cerebellar over-activity might influence tremor in Parkinson's disease (PD). Specifically, it is unclear whether resting or postural tremor are differentially affected by cerebellar dysfunction. It is important to note that previous studies have only evaluated the influence of inhibitory stimulation on the lateral cerebellum, and have not considered the medial cerebellum. Thus, the aim of the current study was to compare the effects of a low-frequency rTMS protocol applied to the medial versus lateral cerebellum to localize the effects of cerebellar over-activity. Fifty PD participants were randomly assigned to receive stimulation over the medial cerebellum (n=20), lateral cerebellum (n=20) or sham stimulation (n=10). 900 pulses were delivered at 1Hz at 120% resting motor threshold of the first dorsal interosseous muscle. Tremor was assessed quantitatively (before and after stimulation) using the Kinesia Homeview system which utilizes a wireless finger accelerometer to record tremor. The main finding was that resting tremor frequency was reduced in tremor-dominant individuals, regardless of whether stimulation was applied over the medial (p=0.024) or lateral (p=0.033) cerebellum, but not in the sham group. Given that the cerebellum is overactive in PD, the improvements in resting tremor following an inhibitory stimulation protocol suggest that over-activity in cerebellar nuclei may be involved in the generation of resting tremor in PD. Low-frequency rTMS over the medial or lateral cerebellum provides promise of an alternative treatment for tremor in PD, a symptom that is poorly responsive to dopaminergic replacement.

Introduction

Parkinson's disease (PD) symptoms have generally been associated with dysfunction of the basal ganglia, and specifically, with the loss of dopaminergic producing neurons in the substantia nigra [1]. However, since increased activation levels of the cerebellum are also found in individuals with PD, it has been suggested that not all PD motor symptoms are due entirely to basal ganglia dysfunction [28,30,32]. Given the anatomical connections between the cerebellum and the basal ganglia, it has been suggested that increased cerebellar activity may also contribute to the pathophysiology of PD symptoms[28–30]. It is possible that increased excitatory output from the subthalamic nucleus in PD may be propagated to the cerebellum, via the pontine nuclei, and account for hyperactivity in cerebellar nuclei [30]. Understanding the effects of increased cerebellar nuclei activity in PD may be the key to gaining insight into some of the mechanisms underlying symptoms that are non-responsive or variably responsive to dopaminergic replacement.

Symptoms of bradykinesia and rigidity in PD demonstrate a stronger link with dysfunction of the basal ganglia and dopaminergic loss, while tremor seems to be less implicated with dopamine [27,28,34]. Given that tremor symptoms are generally less responsive to dopaminergic treatment, this might suggest that the pathophysiology of this symptom could be more related to over-activity in the cerebellum. Increased functional connectivity between the basal ganglia and cerebellum has been found in individuals with PD who experience tremor [33]; it has been suggested that while the basal ganglia may generate tremor, it is over-activity of the cerebellum that drives the tremor pattern [34]. In order to help understand the role of cerebellar over-activity in tremor, methods to suppress or reduce activity in the cerebellar nuclei provide a

means to assess how PD symptoms might change under a temporary state of cerebellar depression.

The assessment of changes in tremor following repetitive transcranial magnetic stimulation (rTMS) protocols designed to transiently inhibit activity in the cerebellar nuclei are methods utilized in research to help determine whether it might be over-activity in the cerebellum contributing to this symptom. Other forms of TMS protocols have also been used to understand the involvement of the cerebellum in PD tremor. For example, tremor reset is a measure used to confirm the contribution of a brain area to either the generation or transmission of tremor. If the cortical target of TMS is involved in the pathophysiology of tremor, the effect of the stimulation interrupts the ongoing tremor drive and causes a tremor reset. A paired-pulse TMS protocol by Ni et al. (2010) demonstrated the involvement of the cerebellum in PD tremor by showing a reset of postural tremor following single-pulse cerebellar and primary motor cortex (M1) stimulation. It is important to note, however, that despite tremor reset, there was no change in tremor frequency following stimulation, and the effects were found only for postural, but not resting tremor after cerebellar stimulation [33]. In contrast, a study by Bologna et al. (2015) demonstrated no changes in resting tremor severity following continuous theta burst stimulation (cTBS) over the lateral cerebellum. This group suggested no involvement of the cerebello-thalamo-cortical loop in the generation of PD tremor [47]. Another study by Minks et al. (2011), which utilized one single session of low frequency (1Hz) rTMS over the right lateral cerebellum found significant improvement in bradykinesia of gross motor skills of both hands following stimulation. Although there was a benefit to gross motor skills, fine motor skills worsened and was only seen on the hand ipsilateral to stimulation [48]. A trial with greater clinical benefits using rTMS was that of Koch et al. (2009) which employed a two-week treatment of bilateral

cerebellum using cTBS. This study resulted in reduced levodopa-induced dyskinesias (LIDs) for a period up to four weeks following the stimulation protocol [49]. These studies demonstrate a link between the modulation of cerebellar activity and some level of change in motor symptoms, suggesting the cerebellum may play a role in the pathophysiology of PD motor symptoms. The previous studies also demonstrate how inhibitory TMS protocols may be a useful tool for developing a better understanding of how the cerebellum might contribute to PD symptoms.

It is important to note that these previous studies applied stimulation targeted only to the lateral cerebellum, whereupon the cerebellar output nucleus (dentate) relays in the thalamus and basal ganglia. Additionally, these previous studies may not have had a pure sample of tremor dominant PD participants, which is important because not all individuals with PD present with tremor. Hence, this may have potentially lead to variable tremor results following cerebellar modulation. It may be beneficial to include a non-tremor dominant PD control group to ensure tremor results are definitive. Therefore, the aim of the current study was to use a low frequency rTMS protocol to transiently inhibit neural activity in the cerebellum of individuals with PD to further understand how over-activity in the cerebellum may contribute to tremor. Further, to localize the effects of over-activity in specific cerebellar nuclei, a direct comparison controlled by sham stimulation was done between the effects of stimuli applied over the medial versus lateral cerebellum. The assessment of changes in this less dopaminergic responsive PD motor symptom has the potential to uncover new knowledge about cerebellar pathophysiology in PD tremor. Further understanding of the involvement of cerebellar activity in PD has the potential to guide the development of treatments targeted to those symptoms which are less responsive to dopaminergic replacement therapy, such as tremor.

Methods

Participants

Fifty individuals diagnosed with idiopathic PD who met inclusion criterion were recruited for participation from the Movement Disorders Research & Rehabilitation Centre database (Wilfrid Laurier University, Waterloo, Ontario, Canada). For participant demographics, see Table 1. For safety, individuals who had received deep brain stimulation, implanted aneurysm clips or cochlear implants were excluded from participation. Previous history of seizures or the prescription of medications which lower the seizure threshold were also criterion for exclusion. Given the requirement to limit movements of the head and neck during stimulation, individuals with severe dyskinesia in the neck muscles were also excluded. Participants were blinded and randomized into three groups: Medial (n=20), Lateral (n=20) or Sham (n=10). This study was approved by the research ethics board at Wilfrid Laurier University, with all participants providing informed consent.

Stimulation Protocol

Stimulation was delivered using a Magstim Rapid2 with 70mm double air film coil (Magstim, UK) guided by TMS Manager navigation (Northern Digital Inc., Waterloo, Ontario). Stimulation intensity was based upon the resting motor threshold (RMT) of the right first dorsal interosseous (FDI) muscle. The measurement of resting electromyography (EMG) activity of the hand in individuals with PD is difficult due to the constant muscle activity from the generation of tremor, however, FDI has been shown in previous work to be capable of maintaining a level of muscle relaxation comparable to healthy individuals [33].

The motor hot spot for the FDI muscle was found in the primary motor cortex (M1) by systematically modifying coil placement and orientation until a consistently isolated FDI contraction was found. RMT was determined by decreasing stimulator output in 1% intervals and defined as the lowest stimulus intensity which produced a motor evoked potential of 50-100μV in amplitude in at least 5 out of 10 consecutive responses with the hand relaxed [15,29,50]. RMT served to standardize the stimulus intensity relative to individual motor thresholds and varying levels of cortical excitability across participants.

The stimulation protocol consisted of one single session, where 900 pulses at 1Hz were applied at 120% RMT. Previous studies have shown that 900 pulses is sufficient for cerebellar suppression [50,51]. In the absence of individual magnetic resonance images (MRI) to determine exact structure depth, the higher stimulation intensity was used to account for the increased distance between the coil and the cerebellum in comparison to the motor cortex [52,53]. The stimulation target was either the cerebellar vermis (medial group) or lateral cerebellum (lateral group). The vermis is located directly beneath the inion, and was located through surface palpation. The lateral cerebellum, or dentate nucleus, is located three centimetres lateral and one centimetre inferior to the vermis [54]. Lateral cerebellar stimulation was applied to the side of the participant that was most affected by PD symptoms. Side affected was determined by self-report and confirmed by clinician assessment. All stimulation (both real and sham) was applied while participants were seated with their face resting on a padded surface, creating both a comfortable position for the participants, while at the same time minimizing head and neck movement. Sham stimulation was employed by angling the coil at 90 degrees to the participants' scalp, following the same protocol as real stimulation. All stimulation (real and sham) was delivered while participants were in their ON medication state.

Outcome Measures

The Unified Parkinson's Disease Rating Scale motor sub-section (UPDRS-III) scores for each participant were assessed by a blinded movement disorders specialist before beginning the study. Participants were then assessed for baseline tremor severity before receiving either real or sham stimulation, and then assessed again immediately following the stimulation.

Quantitative measures of tremor were assessed for each hand using the Kinesia Homeview tablet, which is equipped with a wireless motion sensor that is placed on the index finger. The motion sensor consists of 3 accelerometers and 3 gyroscopes which are able to capture motion of the hands in the x,y and z planes at 128Hz. The output is then run through a previously validated algorithm by the Kinesia software and calculates a tremor severity score which ranges from 0 to 4 at a resolution of 0.1 [55–57]. This score, which has been demonstrated to be highly correlated with clinician ratings, is based upon kinematic measures including peak power, frequency of peak power and root mean square of angular velocity [55–57]. While in the seated position, tremor was recorded for 15 seconds separately for each hand. Participants were instructed to relax the hands between the legs to assess resting tremor, during which time the stroop task was presented to distract participants from tremor activity. Participants were instructed to raise both arms directly in front to shoulder height to assess postural tremor. .

Analysis

Given that the presence of motor symptoms is heavily dependent on PD subtypes, a two-way repeated measures ANOVA with two independent factors was run to account for either the presence or absence of tremor in participants. Participants who had high scores on the upper limb section of the UPDRS-III assessment compared to the lower limb section were classified and

tremor dominant (TD). Likewise, participants with higher scores in the lower limb compare to the upper limb section were classified as postural instability and gait dominant (PIGD). This analysis compared stimulation (Lateral vs Medial vs Sham) and PD Subtype (Tremor Dominant (TD) vs Postural Instability Gait Impaired Dominant (PIGD)) and one within factor (Pre-assessment vs Post-assessment). This analysis was run for both hands combined, as well as the most affected hand by PD symptoms separately, to account for the ipsilateral effects expected from lateral stimulation of the cerebellum.

With the expectation that tremor symptoms would only improve in tremor-dominant participants, a second mixed repeated measures ANOVA comparing the effects of stimulation (Lateral vs Medial vs Sham) from pre-assessment to post-assessment using only TD patients (except for the Sham group) was run. Any significant findings were further examined with Tukey's HSD post hoc procedure.

Results

Tremor outcome data was not included from three participants, therefore statistical analysis was run on 19 participants who received medial stimulation, 20 participants who received lateral stimulation and 8 participants who received sham stimulation (total 47 participants). Data was found to be normally distributed.

The two-way repeated measures ANOVA did not produce any statistically significant interactions, however the analysis did demonstrate a clear advantage for taking into account the presence of tremor as an independent factor. Graphical representation of the results of resting tremor showed no change in the PIGD group, meanwhile there appears to be a trend towards improvement in both rest and postural tremor in the TD participants regardless of whether the

medial or lateral stimulation was applied. This result was consistent for analyses looking at the results from both hands combined (See Figures 1 & 2), as well as for the hand most affected by PD tremor (See Figure 3).

A mixed repeated measures ANOVA, including only TD participants, showed a near-significant time x stimulation interaction ($F(2,25)=2.89$, $p=0.07$), demonstrating a decrease in tremor frequency at post-assessment for both the medial and lateral stimulation groups (Figure 3). Post hoc analysis revealed the medial stimulation group to have improved tremor by 29.1% ($p=0.024$) and lateral stimulation to have improved by 37.5% ($p=.033$). Importantly, there was no change in the sham stimulation group, demonstrating this effect could not be attributed to placebo effects. Given there were only 5 participants in the sham group who were classified as tremor dominant, this group was not divided based on PD subtype to preserve statistical power. This would not have influenced the results since the previous ANOVA (using tremor dominance as an independent factor) did not indicate any placebo effect of sham stimulation on TD or PIGD participants.

Discussion

The aim of the current study was to use a low frequency rTMS protocol to transiently inhibit activity in the cerebellum of individuals with PD to gain an understanding of how localized over-activity in the cerebellum may contribute to tremor symptoms. The effects of stimulation were assessed based upon changes in tremor symptoms, where a change or improvement following inhibitory stimulation might suggest the involvement of cerebellar over-activity in the generation of tremor.

To date, this is the first cerebellar rTMS protocol which indicated improvements specifically in resting tremor in individuals with PD. Our results suggest that the severity of resting tremor was reduced in individuals regardless of whether stimulation was applied over the medial or lateral cerebellum (although it appears that the lateral cerebellum was the more effective target). This effect was specific to individuals with PD who were tremor-dominant, suggesting involvement of the cerebellum in the generation of resting tremor. This is in contrast to previous work which utilized continuous theta burst stimulation and found no change to resting tremor frequency, concluding the cerebello-thalamo-cortical circuit to have no involvement at all in the generation of resting tremor [47]. Another study which utilized a paired-pulse stimulation paradigm, suggested the isolated involvement of the cerebellum in postural tremor only, and not resting tremor [33]. This might suggest the generation of resting and postural tremor to be from different pathways. Findings from the current study, however, provide evidence which supports the theory that hyperactivity in the cerebellum contributes to resting tremor in PD. Key differences in the current study such as an objective measure of tremor, the comparison between medial and lateral stimulation, and analysis by tremor-dominance, may have enabled these findings.

Given that a low frequency, inhibitory stimulation was applied, the trend towards improvement in resting tremor may be attributed to the potential normalization of the activation level in the cerebellar circuitry. The cerebello-thalamo-cortical pathway might allow transmission of cerebellar excitability changes to the thalamus, basal ganglia and M1. Thus, there are two pathways in which the cerebellum may interrupt the ongoing tremor drive: i) cerebellum-thalamus-primary motor cortex, or ii) cerebellum-thalamus-basal ganglia loop-primary motor cortex. Interestingly, inhibitory stimulation applied over the medial or lateral cerebellum both

benefit resting tremor by supressing the output from either the dentate or fastigial nucleus in PD (though results suggest lateral stimulation to be slightly more effective). It is important to note the inhibitory effects of stimulation may not have reached the deep cerebellar nuclei, and thus tremor improvements could alternatively be the result of modulation of cerebellar cortex activity. Given the net inhibitory output of the Purkinje cells in the cerebellum, modulation at the level of the cerebellar cortex may have indirectly affected activity of the deep cerebellar nuclei. Alternatively, the spread of TMS current could have stimulated the interpositus nuclei (which lies between the fastigial and dentate nuclei) and thus the effects could have been the result of modulation of spinocerebellar tracts. However, since differential effects between stimulation applied to the medial versus lateral cerebellum were found (where the lateral stimulation target appeared to be more effective) the spread of TMS current can be ruled out. Only if the effects were found to be identical following stimulation of either cerebellar target could the spread of TMS current be thought to have contributed to tremor modulation. Knowing that tremor has previously been shown to be unrelated to levels of striatal dopamine depletion [34], this study supports the theory that the pathophysiology of tremor-dominant PD may be less associated with dysfunction of the basal ganglia and more related to over-activity in the cerebello-thalamo-cortical circuit [27].

To build upon the current evidence, an important extension of this study would be to recruit a larger sample, consisting of only tremor-dominant PD participants. This would provide adequate power to assess exactly how significant the improvement might be in this PD sub-group. Further, it is important to understand what other effects the stimulation may have had on the PIGD sub-group. Since there was no improvements in tremor expected in the PIGD sub-group, an assessment of how the stimulation may effect gait and balance symptoms should be

considered. The assessment of changes in gait and balance outcome measures would also be important for the TD sub-group, since the cerebellum is an important subcortical structure implicated in the control of gait and balance. Understanding of the more global effect this stimulation may have on less-dopaminergic responsive PD symptoms, encompassing tremor, gait and balance would help to determine the therapeutic potential of low frequency inhibitory stimulation.

Overall, this study suggests the involvement of the medial and lateral cerebellum in the generation of resting tremor in PD. These single sessions of inhibitory stimulation over either the medial or lateral cerebellum provide evidence to suggest that long-term application of the inhibitory protocol, consisting of multiple rTMS sessions, could potentially produce longer lasting benefits. Understanding the mechanisms underlying the cerebellar pathophysiology in PD has the potential for developing new treatments for symptoms which are less responsive to dopaminergic replacement.

Tables & Figures:

	Medial	Lateral	Sham
N	20 (4F, 16M)	20 (6F, 14M)	10 (3F, 7M)
Age (years)	69.4	66.8	71.1
UPDRS-III	22.7	23.1	19.5

Table 1: Participant demographics; no significant differences.

	Medial, Tremor	Medial, PIGD	Lateral, Tremor	Lateral, PIGD	Sham
N	12 (3F, 9M)	8 (1F, 7M)	8 (3F, 5M)	12 (3F, 9M)	10 (3F, 7M)
Age (years)	71.4 (6.9)	66.7 (10.8)	59.1 (12.8)*	71.3 (9.0)	71.1 (8.7)
UPDRS-III	24.8 (6.7)	19.5 (9.9)	25.4 (12.5)	21.5 (10.3)	19.5 (8.0)

Table 2: Participant demographics following stratification by tremor dominance or gait dominance.

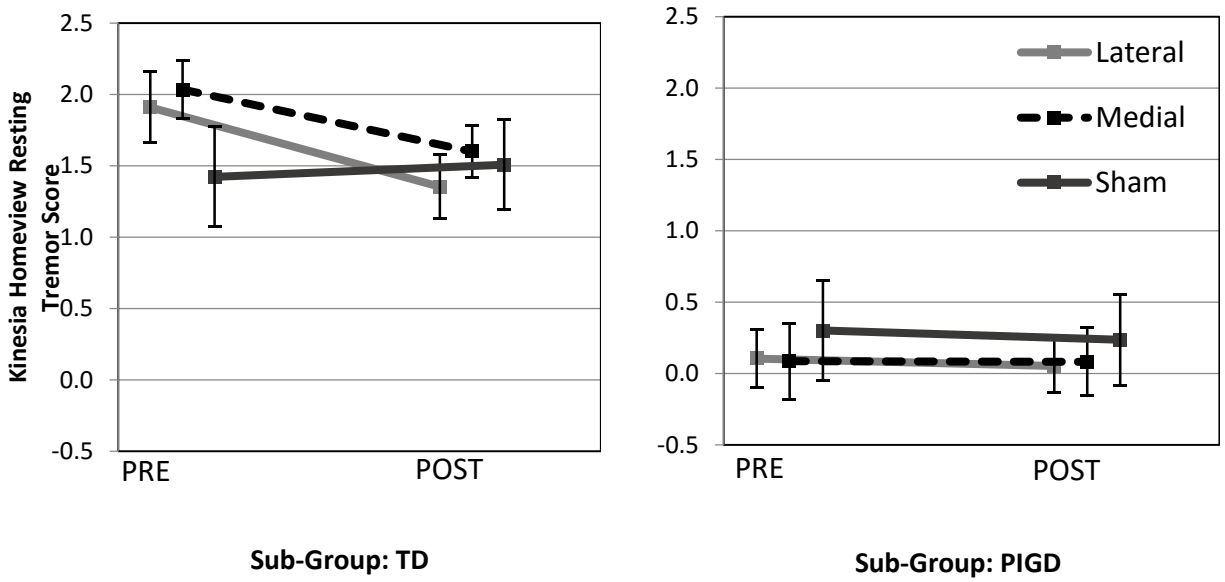

Figure 1: Resting tremor measures for both sides combined. Standard error of each measure represented by vertical bars.

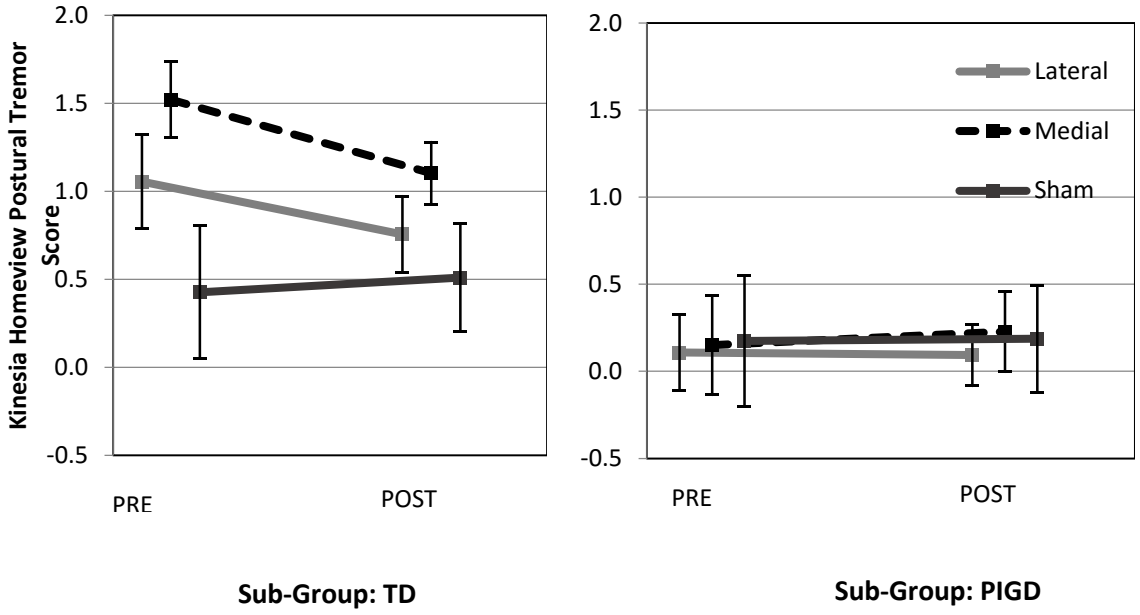

Figure 2: Postural tremor measures for both sides combined. Standard error of each measure represented by vertical bars.

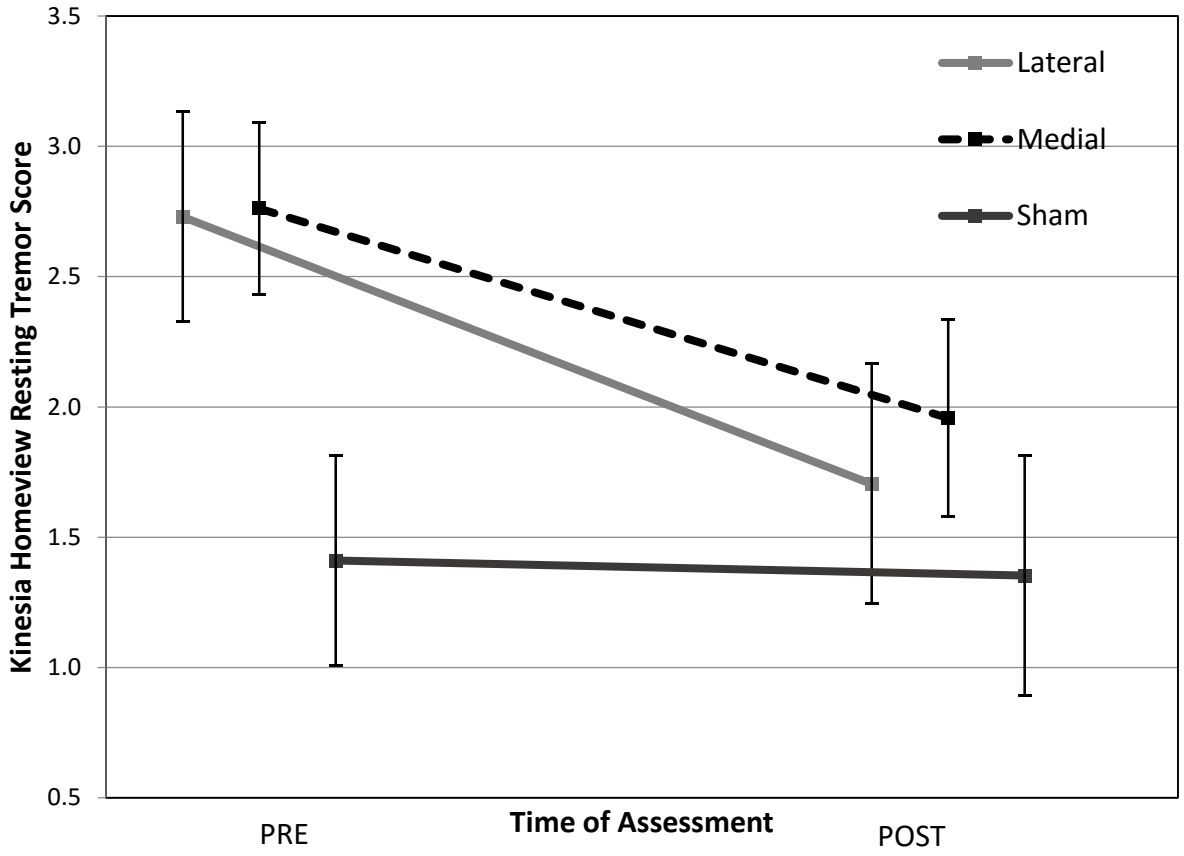

Figure 3: Resting tremor measures for the side most affected by PD demonstrate near-significant group x time interaction ($F(2,25)=2.89$, $p=0.074$). Standard error of each measure represented by vertical bars.

References

[1] Wirdefeldt K, Adami H-O, Cole P, Trichopoulos D, Mandel J. Epidemiology and etiology of Parkinson's disease: a review of the evidence. Eur J Epidemiol 2011;26:S1–58.

[2] Thanvi B, Lo N, Robinson T. Levodopa-induced dyskinesia in Parkinson's disease: clinical features, pathogenesis, prevention and treatment. Postgrad Med J 2007;83:384–8.

[3] Pahwa R, Lyons KE. Levodopa-related wearing-off in Parkinson's disease: identification and management. Curr Med Res Opin 2009;25:841–9.

[4] Almeida QJ, Hyson HC. The Evolution of Pharmacological Treatment for Parkinson ' s Disease. Recent Pat CNS Drug Discov 2008;3:50–4.

[5] Rothwell J. Transcranial magnetic stimulation as a method for investigating the plasticity of the brain in Parkinson ' s Disease and dystonia. Parkinsonism Relat Disord 2007;13:417–20.

[6] Lomarev MP, Kanchana S, Bara-Jimenez W, Iyer M, Wassermann EM, Hallett M. Placebo-controlled study of rTMS for the treatment of Parkinson's disease. Mov Disord 2006;21:325–31.

[7] Hamada M, Ugawa Y, Tsuji S. High-frequency rTMS over the supplementary motor area for treatment of Parkinson's disease. Mov Disord 2008;23:1524–31.

[8] Arias P, Vivas J, Grieve KL, Cudeiro J. Controlled trial on the effect of 10 days low-frequency repetitive transcranial magnetic stimulation (rTMS) on motor signs in Parkinson's disease. Mov Disord 2010;25:1830–8.

[9] Khedr EM, Farweez HM, Islam H. Therapeutic effect of repetitive transcranial magnetic stimulation on motor function in Parkinson's disease patients. EurJNeurol 2003;10:567–72.

[10] Lefaucheur J-P, Drouot X, Von Raison F, Ménard-Lefaucheur I, Cesaro P, Nguyen J-P. Improvement of motor performance and modulation of cortical excitability by repetitive transcranial magnetic stimulation of the motor cortex in Parkinson's disease. Clin Neurophysiol 2004;115:2530–41.

[11] Benninger, D.H., Berman, B.D., Houdayer, E., Pal, N., Luckenbaugh, D.A., Schneider, L., Miranda, S. & Hallett M. Intermittent theta-burst transcranial magnetic stimulation for treatment of Parkinson disease. Neurology 2011;76:601–9.

[12] Arias-Carrión O. Basic mechanisms of rTMS: Implications in Parkinson's disease. Int Arch Med 2008;1:2.

[13] Hallett M. Transcranial magnetic stimulation: a primer. Neuron 2007;55:187–99.

[14] Kobayashi M, Pascual-leone A. Transcranial magnetic stimulation in neurology. Lancet Neurol 2003;2:145–56.

[15] Cantello R, Tarletti R, Civardi C. Transcranial magnetic stimulation and Parkinson ' s disease. Brain Res Rev 2002;38:309–27.

[16] Fregni F, Simon DK, Wu a, Pascual-Leone a. Non-invasive brain stimulation for Parkinson's disease: a systematic review and meta-analysis of the literature. J Neurol Neurosurg Psychiatry 2005;76:1614–23.

[17] Rossini PM, Rossi S. Transcranial magnetic stimulation: diagnostic, therapeutic, and research potential. Neurology 2007;68:484–8.

[18] Madison D V, Malenka RC, Nicoll R a. Mechanisms underlying long-term potentiation of synaptic transmission. Annu Rev Neurosci 1991;14:379–97.

[19] Malenka RC, Bear MF. LTP and LTD: an embarrassment of riches. Neuron 2004;44:5–21.

[20] Massey P V, Bashir ZI. Long-term depression: multiple forms and implications for brain function. Trends Neurosci 2007;30:176–84.

[21] Linden DJ. Long-term synaptic depression in the mammalian brain. Neuron 1994;12:457–72.

[22] Siebner HR, Rothwell J. Transcranial magnetic stimulation: new insights into representational cortical plasticity. Exp Brain Res 2003;148:1–16.

[23] Filipović SR, Rothwell JC, Bhatia K. Slow (1 Hz) repetitive transcranial magnetic stimulation (rTMS) induces a sustained change in cortical excitability in patients with Parkinson's disease. Clin Neurophysiol 2010;121:1129–37.

[24] DeLong MR, Wichmann T. Circuits and circuit disorders of the basal ganglia. Arch Neurol 2007;64:20–4.

[25] Smith Y, Bevan MD, Shink E, Bolam JP. Microcircuitry of the direct and indirect pathways of the basal ganglia. Neuroscience 1998;86:353–87.

[26] Strafella AP, Vanderwerf Y, Sadikot AF. Transcranial magnetic stimulation of the human motor cortex influences the neuronal activity of subthalamic nucleus. Eur J Neurosci 2004;20:2245–9.

[27] Lewis MM, Galley S, Johnson S, Stevenson J, Huang X, Mckeown MJ. The Role of the Cerebellum in the Pathophysiology of Parkinson ' s Disease. Can J Neurol Sci 2013;40:299–306.

[28] Wu T, Hallett M. The cerebellum in Parkinson's disease. Brain 2013;136:696–709.

[29] Carrillo F, Palomar FJ, Conde V, Diaz-corrales FJ, Porcacchia P, Fernández-del-olmo M, et al. Study of Cerebello-Thalamocortical Pathway by Transcranial Magnetic Stimulation in Parkinson ' s Disease. Brain Stimul 2013;6:582–9.

[30] Kishore A, Meunier S, Popa T. Cerebellar influence on motor cortex plasticity : behavioral implications for Parkinson ' s disease. Front Neurol 2014;5:1–8.

[31] Hurley MJ, Mash DC, Jenner P. Markers for dopaminergic neurotransmission in the cerebellum in normal individuals and patients with Parkinson's disease examined by RT-PCR. Eur J Neurosci 2003;18:2668–72.

[32] Yu H, Sternad D, Corcos DM, Vaillancourt DE. Role of hyperactive cerebellum and motor cortex in Parkinson ' s disease. Neuroimage 2007;35:222–33.

[33] Ni Z, Pinto AD, Lang AE, Chen R. Involvement of the Cerebellothalamocortical Pathway in Parkinson Disease. Ann Neurol 2010;68:816–24.

[34] Helmich RC, Janssen MJR, Oyen WJG, Bloem BR, Toni I. Pallidal Dysfunction Drives a Cerebellothalamic Circuit into Parkinson Tremor. Ann Neurol 2011;69:269–81.

[35] Foreman KB, Wisted C, Addison O, Marcus RL, Lastayo PC, Dibble LE. Improved Dynamic Postural Task Performance without Improvements in Postural Responses: The Blessing and the Curse of Dopamine Replacement. Parkinsons Dis 2012;2012:692150.

[36] Rochester L, Baker K, Nieuwboer A, Burn D. Targeting dopa-sensitive and dopa-resistant gait dysfunction in Parkinson's disease: selective responses to internal and external cues. Mov Disord 2011;26:430–5.

[37] Lord S, Baker K, Nieuwboer A, Burn D, Rochester L. Gait variability in Parkinson's disease: an indicator of non-dopaminergic contributors to gait dysfunction? J Neurol 2011;258:566–72.

[38] Almeida QJ, Frank JS, Roy E a, Patla AE, Jog MS. Dopaminergic modulation of timing control and variability in the gait of Parkinson's disease. Mov Disord 2007;22:1735–42.

[39] Tan T, Almeida QJ, Rahimi F. Proprioceptive deficits in Parkinson's disease patients with freezing of gait. Neuroscience 2011;192:746–52.

[40] Caudron S, Guerraz M, Eusebio A, Gros J, Azulay J, Vaugoyeau M. Evaluation of a visual biofeedback on the postural control in Parkinson ' s disease. Clin Neurophysiol 2014;44:77–86.

[41] Vaugoyeau M, Hakam H, Azulay J. Proprioceptive impairment and postural orientation control in Parkinson ' s disease. Hum Mov Sci 2011;30:405–14.

[42] Tagliabue M, Ferrigno G, Horak F. Effects of Parkinson's disease on proprioceptive control of posture and reaching while standing. Neuroscience 2009;158:1206–14.

[43] Horak FB. Postural orientation and equilibrium: what do we need to know about neural control of balance to prevent falls? Age Ageing 2006;35 Suppl 2:ii7–11.

[44] Jacobs J V, Horak FB. Abnormal proprioceptive-motor integration contributes to hypometric postural responses of subjects with Parkinson's disease. Neuroscience 2006;141:999–1009.

[45] Muller MLTM, Albin RL, Kotagal V, Koeppe RA, Scott PJH, Frey KA, et al. Thalamic cholinergic innervation and postural sensory integration function in Parkinson ' s disease. Brain 2013;136:3282–9.

[46] Popa T, Velayudhan B, Hubsch C, Pradeep S, Roze E, Vidailhet M, et al. Cerebellar Processing of Sensory Inputs Primes Motor Cortex Plasticity. Cereb Cortex 2013;23:305–14.

[47] Bologna M, Di Biasio F, Conte A, Iezzi E, Modugno N, Berardelli A. Effects of cerebellar continuous theta burst stimulation on resting tremor in Parkinson's disease. Parkinsonism Relat Disord 2015;21:1–6.

[48] Minks E, Mareček R, Pavlík T, Ovesná P, Bareš M. Is the cerebellum a potential target for stimulation in Parkinson's disease? Results of 1-Hz rTMS on upper limb motor tasks. Cerebellum 2011;10:804–11.

[49] Koch G, Brusa L, Carrillo F, Lo Gerfo E, Torriero S, Oliveri M, et al. Cerebellar magnetic stimulation decreases levodopa-induced dyskinesias in Parkinson disease. Neurology 2009;73:113–9.

[50] Popa T, Russo M, Meunier S. Long-lasting inhibition of cerebellar output. Brain Stimul 2010;3:161–9.

[51] Fierro B, Giglia G, Palermo A, Pecoraro C, Scalia S, Brighina F. Modulatory effects of 1 Hz rTMS over the cerebellum on motor cortex excitability. Exp Brain Res 2007:440–7.

[52] Langguth B, Eichhammer P, Zowe M, Landgrebe M, Binder H, Sand P, et al. Modulating cerebello-thalamocortical pathways by neuronavigated cerebellar repetitive transcranial stimulation (rTMS). Clin Neurophysiol 2008;38:289–95.

[53] Hardwick RM, Lesage E, Miall RC. Cerebellar transcranial magnetic stimulation: The role of coil geometry and tissue depth. Brain Stimul 2014;7:643–9.

[54] Ugawa Y, Uesaka Y, Terao Y, Hanajima R, Kanazawa I. Magnetic stimulation over the cerebellum in humans. Ann Neurol 1995;37:703–13.

[55] Giuffrida JP, Riley DE, Maddux BN, Heldmann D a. Clinically deployable kinesia technology for automated tremor assessment. Mov Disord 2009;24:723–30.

[56] Heldman D a., Giuffrida JP, Chen R, Payne M, Mazzella F, Duker AP, et al. The modified bradykinesia rating scale for Parkinson's disease: Reliability and comparison with kinematic measures. Mov Disord 2011;26:1859–63.

[57] Heldman D a., Espay AJ, LeWitt P a., Giuffrida JP. Clinician versus machine: Reliability and responsiveness of motor endpoints in Parkinson's disease. Park Relat Disord 2014;20:590–5.

[58] Ilg W, Giese M a, Gizewski ER, Schoch B, Timmann D. The influence of focal cerebellar lesions on the control and adaptation of gait. Brain 2008;131:2913–27.

[59] Manto M, Bower JM, Conforto AB, Delgado-García JM, Da Guarda SNF, Gerwig M, et al. Consensus paper: Roles of the cerebellum in motor control-the diversity of ideas on cerebellar involvement in movement. Cerebellum 2012;11:457–87.

[60] Horak FB. Clinical assessment of balance disorders. Gait Posture 1997;6:76–84.

[61] Benninger DH, Iseki K, Kranick S, Luckenbaugh D a., Houdayer E, Hallett M. Controlled Study of 50-Hz Repetitive Transcranial Magnetic Stimulation for the Treatment of Parkinson Disease. Neurorehabil Neural Repair 2012;26:1096–105.

[62] Von Papen M, Fisse M, Sarfeld AS, Fink GR, Nowak D a. The effects of 1 Hz rTMS preconditioned by tDCS on gait kinematics in Parkinson's disease. J Neural Transm 2014;121:743–54.

[63] Bohnen NI, Müller ML, Dauer WT, Albin RL. Parkinson's disease: what role do pedunculopontine cholinergic neurons play? Future Neurol 2014;9:5–8.

[64] Tard C, Delval a., Devos D, Lopes R, Lenfant P, Dujardin K, et al. Brain metabolic abnormalities during gait with freezing in Parkinson's disease. Neuroscience 2015;307:281–301.

[65] Brugger F, Abela E, Hagele-Link S, Bohlhalter S, Galovic M, Kagi G. Do executive dysfunction and freezing of gait in Parkinson's disease share the same neuroanatomical correlates? J Neurol Sci 2015;356:184–7.

[66] Göttlich M, Münte TF, Heldmann M, Kasten M, Hagenah J, Krämer UM. Altered Resting State Brain Networks in Parkinson's Disease. PLoS One 2013;8.

[67] Helmich RC, Derikx LC, Bakker M, Bloem BR, Toni I. Spatial Remapping of Cortico-striatal Connectivity in Parkinson ' s Disease. Cereb Cortex 2010;20.

[68] Rubino A, Assogna F, Piras F, Di Battista ME, Imperiale F, Chiapponi C, et al. Does a volume reduction of the parietal lobe contribute to freezing of gait in Parkinson's disease? Park Relat Disord 2014;20:1101–3.

[69] Chan HF, Kukkle PL, Merello M, Lim SY, Poon YY, Moro E. Amantadine improves gait in PD patients with STN stimulation. Park Relat Disord 2013;19:316–9.

[70] Théoret H, Haque J, Pascual-Leone A. Increased variability of paced finger tapping accuracy following repetitive magnetic stimulation of the cerebellum in humans. Neurosci Lett 2001;306:29–32.

[71] Miall RC, Christensen LOD. The effect of rTMS over the cerebellum in normal human volunteers on peg-board movement performance. Neurosci Lett 2004;371:185–9.

Chapter 3: Does cerebellar over-activity contribute to gait and balance deficits in PD?

Submitted to: Gait & Posture

Abstract

Parkinson's disease (PD) symptoms which respond poorly to dopaminergic replacement, such as gait and balance deficits, point to an alternative pathology to dopaminergic dysfunction within the basal ganglia. For example, decreased cholinergic innervation of the pedunculopontine nucleus (PPN) has been related to poor balance control in PD. A second possibility may be that over-activity of the cerebellum contributes to balance problems, since other hyperkinetic motor symptoms have been linked to cerebellar dysfunction. The cerebellum and PPN both relay sensory information to the thalamus, however it is unclear how cerebellar over-activity might be affecting gait and balance deficits. Thus, the aim of the current study was to assess how cerebellar over-activity may be affecting gait and balance deficits in PD by inhibiting cerebellar activity using repetitive transcranial magnetic stimulation (rTMS). Fifty PD participants were randomized to receive stimulation over the medial (n=20) or lateral cerebellum (n=20) or sham stimulation (n=10). 900 pulses at 1Hz were delivered at an intensity of 120% resting motor threshold determined from the first dorsal interosseous muscle representation in the primary motor cortex. Balance was measured with objective, computerized protocols: modified clinical test of sensory integration and balance (m-CTSIB) and postural stability testing (PST). Spatiotemporal gait was measured quantitatively during self-paced walking. All assessments were performed before and after real or sham stimulation. Inhibition of the cerebellar nuclei did not result in modulation of gait and balance outcome measures. Hence, dysfunction of the cerebellum may not be a contributing factor to gait and balance deficits in PD.

Introduction

It is well known that motor symptoms in Parkinson's disease (PD) are related to dopaminergic dysfunction. However, gait and balance deficits have been shown to be poorly responsive to dopaminergic replacement, suggesting these deficits may have a pathology alternative to dopaminergic dysfunction. Over-activity of the cerebellum has been identified in individuals with PD [28–30,32], however the over-activity has not been localized to a specific area of the cerebellum. Given that the cerebellum is an important subcortical structure to the control of gait and balance, one obvious possibility may be that dysfunction in the cerebellum contributes to these symptoms. The dentate nucleus is the main output of the lateral cerebellum, and is thought to be responsible for using sensory information to control limb movements, while the fastigial nucleus is the main output of the medial cerebellum and is thought to control muscle tone and upright stance [58,59]. However, it is not understood how over-activity of the cerebellum might affect gait and balance deficits in PD. Thus, it would be advantageous to modulate the over-activity in the cerebellar nuclei and evaluate how this might influence gait and balance deficits.

Low frequency repetitive transcranial magnetic stimulation (rTMS) is one method which can be used to inhibit synaptic activity within the cerebellum. Temporary inhibition in the current study would enable the assessment of how gait and balance symptoms might be affected in the absence of altered input from cerebellar nuclei. A direct comparison between the effects of inhibitory stimuli applied over the medial (fastigial nucleus) versus the lateral cerebellum (dentate nucleus) is essential to help disentangle how the cerebellar nuclei might be differentially affecting gait and balance deficits. Functionally, the lateral cerebellum is primarily associated with limb movements and gait, while the medial cerebellum is associated with upright stance

[58,59]. From an anatomical perspective, these stimulation targets provide the opportunity to modulate two separate output pathways; while output from both nuclei relays first in the thalamus, the key difference is output from the dentate nucleus (but not the fastigial nucleus) is additionally relayed in the basal ganglia before reaching the motor cortex. Given the close proximity of the stimulation areas, an advantage of comparing both targets from a technical perspective is the additional level of control to account for the potential spread of TMS current. Hence, the aim of the current study was to evaluate how over-activity in the medial and lateral cerebellum might be influencing gait and balance deficits in PD.

It is hypothesized that temporary inhibition of the medial cerebellum will result in improved static balance, characterized by decreased postural sway during assessments. In addition, it is hypothesized that the non-dopaminergic responsive gait parameters, such as single and double limb support time, will improve following lateral cerebellar stimulation. It is anticipated that stimulation applied to the medial cerebellum will demonstrate greater improvement of symptoms since the output from the fastigial nucleus does not relay in the dysfunctional basal ganglia. Understanding the influence of cerebellar over-activity in PD has the potential to uncover new knowledge regarding mechanisms underlying symptoms which are poorly responsive to dopaminergic replacement, such as gait and balance deficits.

Methods

Participants

Fifty individuals diagnosed with idiopathic PD were recruited for participation from the Movement Disorders Research & Rehabilitation Centre database (Wilfrid Laurier University, Waterloo, Ontario, Canada). For participant demographics, see Table 1. For safety, individuals

who had received deep brain stimulation, implanted aneurysm clips or cochlear implants were excluded from participation. Previous history of seizures or the prescription of medications which lower the seizure threshold were also criterion for exclusion. Given the nature of the assessments, participants were required to be able to walk five metres, in addition to standing for five minutes unassisted. Eligible participants were blinded and randomized into three stimulation groups: Medial target (n=20), Lateral target (n=20) or Sham (n=10). This study was approved by the research ethics board at Wilfrid Laurier University, with all participants providing informed consent.

Stimulation Protocol

Stimulation was delivered using a Magstim Rapid2 with 70mm double air film coil (Magstim, UK) guided by TMS Manager navigation (Northern Digital Inc., Waterloo, Ontario). Stimulation intensity was based upon the resting motor threshold (RMT) of the right first dorsal interosseous (FDI) muscle hot spot in the left primary motor cortex (M1), in accordance with Ni et al [33]. RMT was determined by decreasing stimulator output in 1% intervals and defined as the lowest stimulus intensity which produced a motor evoked potential of 50-100μV in amplitude in at least 5 out of 10 consecutive responses with the hand relaxed [15,29,50]. RMT allows standardization of stimulus intensity to be reflective of inter-individual variability in motor thresholds and cortical excitability across participants.

The stimulation protocol consisted of one single session, where 900 pulses at 1Hz were applied at 120% RMT. Previous studies have shown 900 pulses to be sufficient for cerebellar suppression [50,51]. The higher stimulation intensity was used to account for the increased distance between the coil and the cerebellum in comparison to the motor cortex [52]. The stimulation target was either the cerebellar vermis (medial group) or lateral cerebellum (lateral

group). The vermis is located directly beneath the inion, and was located through surface palpation. The lateral cerebellum, or dentate nucleus, is located three centimetres lateral and one centimetre inferior to the vermis. Lateral cerebellar stimulation was applied to the side of the participant that was most affected by PD symptoms. Side affected was determined by self-report and confirmed by clinician assessment. All stimulation (both real and sham) was applied while participants were seated with their face resting on a padded surface, creating both a comfortable position for the participants, while at the same time minimizing head and neck movement. Sham stimulation was employed by angling the coil at 90 degrees to the participants' scalp, following the same protocol as real stimulation. All stimulation (real and sham) was delivered while participants were in their ON medication state.

Outcome Measures

The Unified Parkinson's Disease Rating Scale motor sub-section (UPDRS-III) scores for each participant were assessed by a blinded movement disorders specialist prior to any assessments or stimulation. Participants next performed baseline gait and balance assessments in a randomized order. Participants then received either sham or real rTMS (medial or lateral cerebellar target) in accordance with group randomization. Immediately following stimulation, participants were again assessed on gait and balance outcome measures, with the total visit taking 1.5 hours. Spatiotemporal gait parameters were measured and assessed using a Zeno Walkway System (ProtoKinetics, Havertown, PA, USA) with ProtoKinetics Movement Analysis Software (PKMAS) version 507c7c. Participants were instructed to begin walking on the experimenters command, starting one metre back from the edge of the carpet to account for acceleration, and to walk at least one meter beyond the end of the carpet to account for deceleration. Alongside a spotter, participants completed 5 walking trials before stimulation and

again after receiving stimulation. Gait outcome measures included: step length, step time, step width, swing time, cadence, velocity, stance time and percentage of time spent in single limb and double limb support. These measures included gait parameters known to be responsive and/or non-responsive to dopaminergic treatment, with the intention to disentangle which specific parameters might be modulated with cerebellar stimulation.

Balance control and postural stability were measured using the Biodex Balance System SD (Biodex Medical Systems Inc., Shirley, New York), in accordance with the postural stability testing (PST) and the modified clinical test of sensory integration and balance (m-CTSIB) protocols. Given that proprioceptive and sensory integration deficits are known to affect balance control in PD [39–43], it is important to assess balance under varying sensory conditions. The PST protocol averages centre of pressure deviations from three quiet balance trials, each lasting thirty seconds. The equipment provided scores, termed as stability index, which measured overall postural stability, in addition to scores in the medio-lateral and anterior-posterior planes. The m-CTSIB protocol has been validated for quantifying an individual's sensory organization during the performance of balance tasks [60]. In this protocol, balance control was measured during twenty second trials in each of four sensory conditions: 1) eyes open firm surface, 2) eyes closed firm surface, 3) eyes open foam surface and 4) eyes closed foam surface. The equipment generates a sway score, which was produced from the variability (standard deviation) of postural deviations from a central reference point. For both the m-CTSIB and the PST protocols, high scores were indicative of poor balance control.

Analysis:

A mixed repeated measures ANOVA was run for each of the gait and balance outcome measures which compared the effects of stimulation (Medial vs Lateral vs Sham) across time

(pre-assessment vs post-assessment). Any significant findings were further analyzed with Tukey's HSD post hoc procedure.

Results

Gait

Data for two participants was compromised, thus analyses were done on a total of 48 participants (20 medial, 19 lateral, 9 sham). Gait analysis revealed no significant effects of stimulation on any of the spatiotemporal gait parameters measured (see Table 2).

Balance

There were no significant effects of stimulation found in any of the PST outcome measures (see Table 3). Similarly, there were no significant effect of the stimulation on m-CTSIB outcome measures; however, there was a main effect of vision ($F(1,45)=137.79$, $p=0.000$) demonstrating significantly worse balance overall in the eyes closed conditions, as well as a main effect of surface ($F(1,45)=64.97$, $p=0.000$) showing balance to be significantly worse for all participants on the foam surface. These findings were further supported by a significant vision x surface interaction ($F(1,45)=15.92$, $p=0.00001$), where the worst balance overall was found with the eyes closed on the foam surface.

Discussion

This was the first study to target the cerebellum using low-frequency rTMS to evaluate the influence of cerebellar over-activity on gait and balance deficits in PD. Systematic evaluations of gait and balance were performed to directly compare the effects of rTMS on medial versus lateral cerebellum, in contrast to sham stimulation. The main finding was that spatiotemporal gait parameters and static balance were not affected by stimulation applied to

either cerebellar target. The current findings are in contrast to previous studies which found improved gait following various rTMS protocol which targeted the primary motor cortex (M1) in individuals with PD. Improvements in walking speed were found following 5 Hz rTMS [9], 0.5 Hz rTMS [10] and 25 Hz rTMS [6], but not following single or multiple sessions of 50 Hz intermittent theta burst stimulation [11,61]. Given that inhibitory stimulation applied to the cerebellum has previously been shown to decrease M1 excitability[52], and influence gait parameters in healthy individuals [6,9,10,62], improvements in gait following cerebellar inhibition during the current study might have been expected. However, regardless of medial or lateral cerebellar stimulation, spatiotemporal gait parameters and static balance were not affected, suggesting cerebellar over-activity may not contribute to gait and balance deficits in PD.

Gait parameters such as velocity, stride length and stride time demonstrate improvements after dopaminergic replacement, suggesting these deficits might be primarily related to the dysfunctional basal ganglia. Meanwhile, other gait parameters such as step frequency and double limb support time have proven to be unresponsive to dopaminergic treatment [36–38], suggesting an additional mechanism to basal ganglia dysfunction must also be involved in gait deficits. Given that proprioceptive deficits have previously been well established in PD [39–43], alternative mechanisms to consider might involve poor sensory processing.

One alternative mechanism is cholingeric denervation in the PPN, which has been demonstrated by previous research to negatively affect sensory processing, leading to gait and balance deficits in PD [45,63]. One way to conceptualize this idea is to think about the sensory inputs relaying in the thalamus; both the PPN and the cerebellum relay sensory information in the ventrolateral nucleus of the thalamus, however, both provide poor inputs in PD. The PPN

input may be negatively affected by decreased cholinergic innervation [45,63], and the cerebellar input may be negatively affected by over-activity in the cerebellar nuclei. Therefore, there could be two sources of "broken" input for sensory integration which are relaying in the thalamus in PD; the combination of these two inputs may compound to result in poor sensory integration, and thus, gait and balance deficits. If this was true, then inhibition of the cerebellar output (via rTMS) may have reduced the load on the thalamus by reducing the number of "broken" inputs to only one input, that being the input coming from the denervated PPN. Importantly, there was no change or improvement in gait or balance symptoms following transient inhibition of the cerebellum, suggesting that PPN cholinergic denervation, and the "broken" signals which result, may have prevailed. This theory would suggest that cerebellar over-activity must not compound the effects of PPN denervation and thus, further impair sensory integration at the level of the thalamus. Hence, cholinergic denervation as opposed to over-activity in the cerebellum might be one contributor to gait and balance deficits in PD.

The influence of the dysfunctional basal ganglia on gait and balance symptoms cannot be ignored. Comparing the effects of stimulation targeted to the medial versus lateral cerebellum would enable indirect assessment of basal ganglia involvement (since medial cerebellar output output does not relay in the basal ganglia). It should be noted the current experimental paradigm cannot directly assess basal ganglia involvement, however, this consideration might further support the idea of PPN involvement in gait and balance deficits. The theoretically "normalized" output from the cerebellum must first relay in the thalamus, whereupon the output from the cholinergic dennervated PPN could simultaneously "damage" this cerebellar output. Thus, "broken" output from the lateral cerebellum (after relaying in the thalamus) would reach the dysfunctional basal ganglia and likely be further impaired. Since dysfunction of the basal

ganglia has also been related to abnormal sensory processing in PD, it might then be suggested that both the basal ganglia and the PPN influence gait and balance deficits.

Within the sensory system, another alternative mechanism that may be influencing gait and balance deficits in PD is dysfunction of the parietal cortex. Individuals with PD who have severe gait deficits demonstrate increased activity in the parietal cortex [64], an area which is important for sensory processing. This increased activation of the parietal areas during gait has been suggested to reflect abnormal sensory processing. This has been suggested to lead to gait deficits in PD [64–66], as a result of the inability of individuals to process relative sensory information from the environment. Striatal dopamine degeneration has also been linked to a shift in coupling of the inferior parietal cortex from the posterior putamen to the anterior putamen [67], which suggests these parietal cortex changes to be PD-specific. A reduction in gray matter volume, and selective parietal cortex atrophy has also been found in PD [68]. Given the parietal cortex is an important structure in sensory processing during gait and balance, it might be suggested that dysfunction of this area, as opposed to cerebellar over-activity, may also be contributing to these deficits in PD.

The findings from the current study do not support the involvement of the cerebellum in gait and balance deficits in PD, however it does support previous research which suggests that cholinergic denervation in the PPN may be responsible for poor sensory integration at the level of the thalamus, and thus, poor balance control. These findings also support the notion that rTMS protocols targeting the primary motor cortex, as opposed to the cerebellum, may provide greater clinical benefit. Further research aimed at understanding the mechanisms by which PPN denervation is affecting sensory integration at the level of the thalamus may have the potential to lead to novel treatments for gait and balance deficits in PD.

References

[1] Wirdefeldt K, Adami H-O, Cole P, Trichopoulos D, Mandel J. Epidemiology and etiology of Parkinson's disease: a review of the evidence. Eur J Epidemiol 2011;26:S1–58.

[2] Thanvi B, Lo N, Robinson T. Levodopa-induced dyskinesia in Parkinson's disease: clinical features, pathogenesis, prevention and treatment. Postgrad Med J 2007;83:384–8.

[3] Pahwa R, Lyons KE. Levodopa-related wearing-off in Parkinson's disease: identification and management. Curr Med Res Opin 2009;25:841–9.

[4] Almeida QJ, Hyson HC. The Evolution of Pharmacological Treatment for Parkinson ' s Disease. Recent Pat CNS Drug Discov 2008;3:50–4.

[5] Rothwell J. Transcranial magnetic stimulation as a method for investigating the plasticity of the brain in Parkinson ' s Disease and dystonia. Parkinsonism Relat Disord 2007;13:417–20.

[6] Lomarev MP, Kanchana S, Bara-Jimenez W, Iyer M, Wassermann EM, Hallett M. Placebo-controlled study of rTMS for the treatment of Parkinson's disease. Mov Disord 2006;21:325–31.

[7] Hamada M, Ugawa Y, Tsuji S. High-frequency rTMS over the supplementary motor area for treatment of Parkinson's disease. Mov Disord 2008;23:1524–31.

[8] Arias P, Vivas J, Grieve KL, Cudeiro J. Controlled trial on the effect of 10 days low-frequency repetitive transcranial magnetic stimulation (rTMS) on motor signs in Parkinson's disease. Mov Disord 2010;25:1830–8.

[9] Khedr EM, Farweez HM, Islam H. Therapeutic effect of repetitive transcranial magnetic stimulation on motor function in Parkinson's disease patients. EurJNeurol 2003;10:567–72.

[10] Lefaucheur J-P, Drouot X, Von Raison F, Ménard-Lefaucheur I, Cesaro P, Nguyen J-P. Improvement of motor performance and modulation of cortical excitability by repetitive transcranial magnetic stimulation of the motor cortex in Parkinson's disease. Clin Neurophysiol 2004;115:2530–41.

[11] Benninger, D.H., Berman, B.D., Houdayer, E., Pal, N., Luckenbaugh, D.A., Schneider, L., Miranda, S. & Hallett M. Intermittent theta-burst transcranial magnetic stimulation for treatment of Parkinson disease. Neurology 2011;76:601–9.

[12] Arias-Carrión O. Basic mechanisms of rTMS: Implications in Parkinson's disease. Int Arch Med 2008;1:2.

[13] Hallett M. Transcranial magnetic stimulation: a primer. Neuron 2007;55:187–99.

[14] Kobayashi M, Pascual-leone A. Transcranial magnetic stimulation in neurology. Lancet Neurol 2003;2:145–56.

[15] Cantello R, Tarletti R, Civardi C. Transcranial magnetic stimulation and Parkinson ' s disease. Brain Res Rev 2002;38:309–27.

[16] Fregni F, Simon DK, Wu a, Pascual-Leone a. Non-invasive brain stimulation for Parkinson's disease: a systematic review and meta-analysis of the literature. J Neurol Neurosurg Psychiatry 2005;76:1614–23.

[17] Rossini PM, Rossi S. Transcranial magnetic stimulation: diagnostic, therapeutic, and research potential. Neurology 2007;68:484–8.

[18] Madison D V, Malenka RC, Nicoll R a. Mechanisms underlying long-term potentiation of synaptic transmission. Annu Rev Neurosci 1991;14:379–97.

[19] Malenka RC, Bear MF. LTP and LTD: an embarrassment of riches. Neuron 2004;44:5–21.

[20] Massey P V, Bashir ZI. Long-term depression: multiple forms and implications for brain function. Trends Neurosci 2007;30:176–84.

[21] Linden DJ. Long-term synaptic depression in the mammalian brain. Neuron 1994;12:457–72.

[22] Siebner HR, Rothwell J. Transcranial magnetic stimulation: new insights into representational cortical plasticity. Exp Brain Res 2003;148:1–16.

[23] Filipović SR, Rothwell JC, Bhatia K. Slow (1 Hz) repetitive transcranial magnetic stimulation (rTMS) induces a sustained change in cortical excitability in patients with Parkinson's disease. Clin Neurophysiol 2010;121:1129–37.

[24] DeLong MR, Wichmann T. Circuits and circuit disorders of the basal ganglia. Arch Neurol 2007;64:20–4.

[25] Smith Y, Bevan MD, Shink E, Bolam JP. Microcircuitry of the direct and indirect pathways of the basal ganglia. Neuroscience 1998;86:353–87.

[26] Strafella AP, Vanderwerf Y, Sadikot AF. Transcranial magnetic stimulation of the human motor cortex influences the neuronal activity of subthalamic nucleus. Eur J Neurosci 2004;20:2245–9.

[27] Lewis MM, Galley S, Johnson S, Stevenson J, Huang X, Mckeown MJ. The Role of the Cerebellum in the Pathophysiology of Parkinson ' s Disease. Can J Neurol Sci 2013;40:299–306.

[28] Wu T, Hallett M. The cerebellum in Parkinson's disease. Brain 2013;136:696–709.

[29] Carrillo F, Palomar FJ, Conde V, Diaz-corrales FJ, Porcacchia P, Fernández-del-olmo M, et al. Study of Cerebello-Thalamocortical Pathway by Transcranial Magnetic Stimulation in Parkinson ' s Disease. Brain Stimul 2013;6:582–9.

[30] Kishore A, Meunier S, Popa T. Cerebellar influence on motor cortex plasticity : behavioral implications for Parkinson ' s disease. Front Neurol 2014;5:1–8.

[31] Hurley MJ, Mash DC, Jenner P. Markers for dopaminergic neurotransmission in the cerebellum in normal individuals and patients with Parkinson's disease examined by RT-PCR. Eur J Neurosci 2003;18:2668–72.

[32] Yu H, Sternad D, Corcos DM, Vaillancourt DE. Role of hyperactive cerebellum and motor cortex in Parkinson ' s disease. Neuroimage 2007;35:222–33.

[33] Ni Z, Pinto AD, Lang AE, Chen R. Involvement of the Cerebellothalamocortical Pathway in Parkinson Disease. Ann Neurol 2010;68:816–24.

[34] Helmich RC, Janssen MJR, Oyen WJG, Bloem BR, Toni I. Pallidal Dysfunction Drives a Cerebellothalamic Circuit into Parkinson Tremor. Ann Neurol 2011;69:269–81.

[35] Foreman KB, Wisted C, Addison O, Marcus RL, Lastayo PC, Dibble LE. Improved Dynamic Postural Task Performance without Improvements in Postural Responses: The Blessing and the Curse of Dopamine Replacement. Parkinsons Dis 2012;2012:692150.

[36] Rochester L, Baker K, Nieuwboer A, Burn D. Targeting dopa-sensitive and dopa-resistant gait dysfunction in Parkinson's disease: selective responses to internal and external cues. Mov Disord 2011;26:430–5.

[37] Lord S, Baker K, Nieuwboer A, Burn D, Rochester L. Gait variability in Parkinson's disease: an indicator of non-dopaminergic contributors to gait dysfunction? J Neurol 2011;258:566–72.

[38] Almeida QJ, Frank JS, Roy E a, Patla AE, Jog MS. Dopaminergic modulation of timing control and variability in the gait of Parkinson's disease. Mov Disord 2007;22:1735–42.

[39] Tan T, Almeida QJ, Rahimi F. Proprioceptive deficits in Parkinson's disease patients with freezing of gait. Neuroscience 2011;192:746–52.

[40] Caudron S, Guerraz M, Eusebio A, Gros J, Azulay J, Vaugoyeau M. Evaluation of a visual biofeedback on the postural control in Parkinson ' s disease. Clin Neurophysiol 2014;44:77–86.

[41] Vaugoyeau M, Hakam H, Azulay J. Proprioceptive impairment and postural orientation control in Parkinson ' s disease. Hum Mov Sci 2011;30:405–14.

[42] Tagliabue M, Ferrigno G, Horak F. Effects of Parkinson's disease on proprioceptive control of posture and reaching while standing. Neuroscience 2009;158:1206–14.

[43] Horak FB. Postural orientation and equilibrium: what do we need to know about neural control of balance to prevent falls? Age Ageing 2006;35 Suppl 2:ii7–11.

[44] Jacobs J V, Horak FB. Abnormal proprioceptive-motor integration contributes to hypometric postural responses of subjects with Parkinson's disease. Neuroscience 2006;141:999–1009.

[45] Muller MLTM, Albin RL, Kotagal V, Koeppe RA, Scott PJH, Frey KA, et al. Thalamic cholinergic innervation and postural sensory integration function in Parkinson ' s disease. Brain 2013;136:3282–9.

[46] Popa T, Velayudhan B, Hubsch C, Pradeep S, Roze E, Vidailhet M, et al. Cerebellar Processing of Sensory Inputs Primes Motor Cortex Plasticity. Cereb Cortex 2013;23:305–14.

[47] Bologna M, Di Biasio F, Conte A, Iezzi E, Modugno N, Berardelli A. Effects of cerebellar continuous theta burst stimulation on resting tremor in Parkinson's disease. Parkinsonism Relat Disord 2015;21:1–6.

[48] Minks E, Mareček R, Pavlík T, Ovesná P, Bareš M. Is the cerebellum a potential target for stimulation in Parkinson's disease? Results of 1-Hz rTMS on upper limb motor tasks. Cerebellum 2011;10:804–11.

[49] Koch G, Brusa L, Carrillo F, Lo Gerfo E, Torriero S, Oliveri M, et al. Cerebellar magnetic stimulation decreases levodopa-induced dyskinesias in Parkinson disease. Neurology 2009;73:113–9.

[50] Popa T, Russo M, Meunier S. Long-lasting inhibition of cerebellar output. Brain Stimul 2010;3:161–9.

[51] Fierro B, Giglia G, Palermo A, Pecoraro C, Scalia S, Brighina F. Modulatory effects of 1 Hz rTMS over the cerebellum on motor cortex excitability. Exp Brain Res 2007:440–7.

[52] Langguth B, Eichhammer P, Zowe M, Landgrebe M, Binder H, Sand P, et al. Modulating cerebello-thalamocortical pathways by neuronavigated cerebellar repetitive transcranial stimulation (rTMS). Clin Neurophysiol 2008;38:289–95.

[53] Hardwick RM, Lesage E, Miall RC. Cerebellar transcranial magnetic stimulation: The role of coil geometry and tissue depth. Brain Stimul 2014;7:643–9.

[54] Ugawa Y, Uesaka Y, Terao Y, Hanajima R, Kanazawa I. Magnetic stimulation over the cerebellum in humans. Ann Neurol 1995;37:703–13.

[55] Giuffrida JP, Riley DE, Maddux BN, Heldmann D a. Clinically deployable kinesia technology for automated tremor assessment. Mov Disord 2009;24:723–30.

[56] Heldman D a., Giuffrida JP, Chen R, Payne M, Mazzella F, Duker AP, et al. The modified bradykinesia rating scale for Parkinson's disease: Reliability and comparison with kinematic measures. Mov Disord 2011;26:1859–63.

[57] Heldman D a., Espay AJ, LeWitt P a., Giuffrida JP. Clinician versus machine: Reliability and responsiveness of motor endpoints in Parkinson's disease. Park Relat Disord 2014;20:590–5.

[58] Ilg W, Giese M a, Gizewski ER, Schoch B, Timmann D. The influence of focal cerebellar lesions on the control and adaptation of gait. Brain 2008;131:2913–27.

[59] Manto M, Bower JM, Conforto AB, Delgado-García JM, Da Guarda SNF, Gerwig M, et al. Consensus paper: Roles of the cerebellum in motor control-the diversity of ideas on cerebellar involvement in movement. Cerebellum 2012;11:457–87.

[60] Horak FB. Clinical assessment of balance disorders. Gait Posture 1997;6:76–84.

[61] Benninger DH, Iseki K, Kranick S, Luckenbaugh D a., Houdayer E, Hallett M. Controlled Study of 50-Hz Repetitive Transcranial Magnetic Stimulation for the Treatment of Parkinson Disease. Neurorehabil Neural Repair 2012;26:1096–105.

[62] Von Papen M, Fisse M, Sarfeld AS, Fink GR, Nowak D a. The effects of 1 Hz rTMS preconditioned by tDCS on gait kinematics in Parkinson's disease. J Neural Transm 2014;121:743–54.

[63] Bohnen NI, Müller ML, Dauer WT, Albin RL. Parkinson's disease: what role do pedunculopontine cholinergic neurons play? Future Neurol 2014;9:5–8.

[64] Tard C, Delval a., Devos D, Lopes R, Lenfant P, Dujardin K, et al. Brain metabolic abnormalities during gait with freezing in Parkinson's disease. Neuroscience 2015;307:281–301.

[65] Brugger F, Abela E, Hagele-Link S, Bohlhalter S, Galovic M, Kagi G. Do executive dysfunction and freezing of gait in Parkinson's disease share the same neuroanatomical correlates? J Neurol Sci 2015;356:184–7.

[66] Göttlich M, Münte TF, Heldmann M, Kasten M, Hagenah J, Krämer UM. Altered Resting State Brain Networks in Parkinson's Disease. PLoS One 2013;8.

[67] Helmich RC, Derikx LC, Bakker M, Bloem BR, Toni I. Spatial Remapping of Cortico-striatal Connectivity in Parkinson ' s Disease. Cereb Cortex 2010;20.

[68] Rubino A, Assogna F, Piras F, Di Battista ME, Imperiale F, Chiapponi C, et al. Does a volume reduction of the parietal lobe contribute to freezing of gait in Parkinson's disease? Park Relat Disord 2014;20:1101–3.

[69] Chan HF, Kukkle PL, Merello M, Lim SY, Poon YY, Moro E. Amantadine improves gait in PD patients with STN stimulation. Park Relat Disord 2013;19:316–9.

[70] Théoret H, Haque J, Pascual-Leone A. Increased variability of paced finger tapping accuracy following repetitive magnetic stimulation of the cerebellum in humans. Neurosci Lett 2001;306:29–32.

[71] Miall RC, Christensen LOD. The effect of rTMS over the cerebellum in normal human volunteers on peg-board movement performance. Neurosci Lett 2004;371:185–9.

Tables & Figures:

	Medial	Lateral	Sham
N	20 (4F, 16M)	20 (6F, 14M)	10 (3F, 7M)
Age (years)	69.4 (9.1)	66.8 (12.1)	71.1 (8.7)
UPDRS-III	22.7 (8.6)	23.1 (11.4)	19.5 (8.0)

Table 1: Participant demographics; no significant differences.

Gait Parameter	Time	Group		
		Medial	**Lateral**	**Sham**
Step Length (cm)	*Pre*	60.4 (10.7)	63.8 (16.1)	59.5 (15.6)
	Post	60.1 (9.3)	64.9 (15.7)	59.9 (15.6)
Step Length Variability (cv)	*Pre*	5.7 (2.9)	8.2 (8.9)	6.9 (5.1)
	Post	5.7 (2.8)	6.2 (4.4)	6.6 (4.5)
Step Time (s)	*Pre*	0.54 (0.05)	0.57 (0.07)	0.56 (0.04)
	Post	0.53 (0.05)	0.55 (0.06)	0.55 (0.05)
Step Time Variability (cv)	*Pre*	4.42 (1.2)	5.78 (5.5)	4.61 (2.0)
	Post	4.47 (1.27)	4.61 (2.1)	4.68 (2.1)
Stance Time (s)	*Pre*	0.73 (0.08)	0.76 (0.1)	0.74 (0.09)
	Post	0.71 (0.07)	0.72 (0.09)	0.74 (0.09)
Stance Percent Time	*Pre*	66.2 (3.3)	65.3 (3.7)	65.5 (3.0)
	Post	66.3 (2.9)	64.8 (2.9)	65.6 (3.4)
Swing Time (s)	*Pre*	0.37 (0.04)	0.39 (0.04)	0.39 (0.03)
	Post	0.36 (0.04)	0.39 (0.05)	0.38 (0.03)
Swing Percent Time	*Pre*	33.8 (3.3)	34.7 (3.7)	34.5 (3.0)
	Post	33.7 (2.9)	35.2 (2.9)	34.4 (3.4)
Single Support Time (s)	*Pre*	0.37 (0.04)	0.39 (0.04)	0.39 (0.03)
	Post	0.36 (0.04)	0.39 (0.05)	0.38 (0.03)
Single Support Percent Time	*Pre*	26.9 (2.9)	27.8 (2.5)	27.7 (2.4)
	Post	33.6 (2.8)	35.1 (2.9)	34.4 (3.3)
Total Double Support Time (s)	*Pre*	0.35 (0.08)	0.36 (0.14)	0.35 (0.09)
	Post	0.35 (0.07)	0.33 (0.08)	0.35 (0.09)
Double Support Percent Time	*Pre*	32.2 (6.5)	30.5 (7.4)	30.8 (6.1)
	Post	32.6 (5.8)	29.6 (5.9)	31.1 (6.7)
Velocity (cm/s)	*Pre*	111.7 (22.3)	114.0 (33.4)	107.9 (30.1
	Post	113.2 (18.9)	118.4 (29.9)	109.3 (30.1)

Table 2: Gait Outcome Measures

		Medial	Lateral	Sham
m-CTSIB	**Eyes Open, Firm**			
	Pre	0.83 (0.31)	0.92 (0.39)	0.84 (0.25)
	Post	0.86 (0.28)	1.07 (0.38)	0.94 (0.49)
	Eyes Closed, Firm			
	Pre	1.78 (0.74)	1.46 (0.53)	1.04 (0.35)
	Post	1.94 (0.62)	1.29 (0.51)	1.32 (0.39)
	Eyes Open, Foam			
	Pre	1.58 (0.64)	2.24 (1.22)	1.46 (0.23)
	Post	1.57 (0.66)	2.12 (1.28)	3.24 (5.66)
	Eyes Closed, Foam			
	Pre	3.42 (1.1)	3.54 (0.68)	3.02 (1.19)
	Post	3.17 (0.72)	3.58 (1.13)	2.89 (0.91)
Postural Stability Test (PST)	**Overall**			
	Pre	0.875 (0.65)	1.02 (0.82)	1.32 (0.40)
	Post	0.71 (0.48)	1.08 (1.73)	0.87 (0.64)
	Anteroposterior			
	Pre	0.66 (0.61)	0.69 (0.56)	0.9 (0.83)
	Post	0.49 (0.28)	0.71 (0.92)	0.78 (0.59)
	Mediolateral			
	Pre	0.43 (0.32)	0.56 (0.79)	0.7 (0.78)
	Post	0.4 (0.33)	0.65 (1.37)	0.26 (0.16)

Table 3: Balance Outcome Measures

Chapter 4: Grand Discussion

Given that the cerebellum demonstrates over-activity in PD [28,30,32], an inhibitory rTMS protocol was used in the current thesis to modulate this activity and assess the effects on motor symptoms. Of specific interest, was the response of non-dopaminergic responsive motor symptoms, such as tremor, gait and balance since these have been suggested to result from cerebellar dysfunction. Measuring the effects of the stimulation on these specific symptoms was done with the intention to further understand how the cerebellum might be involved in PD. The most influential finding from this study was that 1Hz stimulation delivered over the cerebellum reduced resting tremor of the hand most-affected by PD but did not influence gait or balance outcome measures. The improvement in resting tremor was specific to tremor-dominant individuals with PD, and was demonstrated regardless of whether stimulation was applied medially or laterally to the cerebellum. It must be noted that no changes in the sham group were found for any of the symptom outcome measures indicating that any effects found were specific to the stimulation effects and not placebo effects. These results suggest the dentate and fastigial nuclei may be involved in the generation of resting tremor, but not gait and balance deficits in PD.

Tremor, Gait, Balance and Cerebellar Over-activity

The involvement of the cerebellum in PD tremor is not an entirely new concept. Previous studies have debated the influence of the cerebello-thalamo-cortical circuit on tremor, where some studies support the theory and other studies suggest there to be no involvement of the cerebellum in PD tremor. Using a paired-pulse stimulation paradigm, Ni et al. (2010) demonstrated the reset of only postural tremor following stimulation of the cerebellum. While no

change in resting tremor was found following stimulation of the cerebellum, this same group found a reset of both resting and postural tremor when the pulse was applied over the motor cortex [33]. This group concluded the cerebellum to have isolated involvement in postural, but not resting tremor. A separate study, using continuous theta burst stimulation, found no change to resting tremor frequency and concluded that the cerebellum was not involved in PD resting tremor[47]. The current findings are in contrast to both of these studies, and suggest that the cerebello-thalamo-cortical circuit may in fact be influencing resting tremor in PD.

Increased activation of the cerebellum, motor cortex and putamen has been correlated with clinical tremor ratings [28], which provides the rationale as to why an inhibitory stimulation protocol was chosen. In the same light, the improvements in resting tremor might suggest the reduction of cerebellar activity to be therapeutic. It is possible that the reduction in resting tremor may be attributed to a temporary normalization of synaptic activity in the over-active cerebellar nuclei. Synaptic activity in this study would have been modulated by long term depression, which is characterized by a decrease in synaptic strength. The induction of long term depression in the cerebellum requires the activation of both parallel and climbing fibres, or direct activation of Purkinje neurons[19–21]. The stimulation having an effect on motor symptoms might suggest that long term depression may have been induced for a short period of time in the cerebellum.

The two stimulation targets in this study were the dentate nucleus (lateral cerebellum) and the fastigial nucleus (medial cerebellum). The lateral cerebellum is primarily associated with upper and lower limb movements and gait, while the medial cerebellum is associated with regulating extensor muscle tone and maintaining upright stance [58,59]. Inhibitory stimulation applied to each cerebellar nuclei has the potential to modulate activity through the output pathways to the motor cortex (See Figure 1). Output from the dentate nucleus relays first in the

thalamus and is then projected to both the striatum and globus pallidus externus of the basal ganglia before reaching the motor cortex [28–30]. Outputs from the fastigial nucleus relay only in the thalamus before projecting straight to the motor cortex. Thus, there were two pathways that were studied with the use of rTMS in this thesis. While both pathways relay first in the thalamus, the key difference between output of the dentate and fastigial nuclei, is that output from the dentate nucleus also relays in the basal ganglia. This is important because it allows the assessment of the effects of inhibitory stimulation applied to the cerebellar nuclei, but also considers what likely ensues when this modulated output from the cerebellum reaches the dysfunctional basal ganglia.

Inhibitory stimulation targeted at either the dentate or fastigial nucleus would have theoretically resulted in a more normalized output leaving the cerebellum to relay in the thalamus. However, once the output from the dentate nucleus reaches the basal ganglia (which is well known to be dysfunctional in PD) there are two ways in which the output may have been affected. First, if the dysfunctional basal ganglia were involved in tremor generation, then any normalization of the output coming from the dentate nucleus (due to rTMS) could be compromised when this output relays through the basal ganglia loops. Alternatively, if the basal ganglia were not involved in tremor, then the normalized output from the dentate nucleus would not be affected upon relay in the basal ganglia. Interestingly, stimulation applied over both the medial and lateral cerebellum had similar effects on resting tremor, however the improvement was slightly greater when stimulation was targeted to the lateral cerebellum. These results suggest that cerebellar over-activity is a factor contributing to resting tremor generation in PD. Importantly, the differential improvement between stimulation targeted to the dentate and fastigial nuclei suggest some level of basal ganglia involvement in resting tremor generation. The

improvement in resting tremor was greater following stimulation targeted at the dentate nucleus, whereupon the output would relay in the basal ganglia. One explanation, is the possibility that the normalized output from the dentate nucleus may have in some way improved the function of the basal ganglia. Given that the improvement in resting tremor was diminished by only 8% when the basal ganglia were involved, this theoretical framework does not deny involvement of the basal ganglia. However, the findings do support the idea that tremor in PD may be more related to dysfunction at the level of the cerebellum, than at the level of the basal ganglia[27,28,34].

Regardless of whether inhibitory stimulation was targeted at the dentate or fastigial nucleus, no improvement in gait or balance measures were found. Since the lateral cerebellum is thought to be responsible for integrating sensory information to control limb movements [58,59], it might have been expected that stimulation may have improved gait parameters. Meanwhile, the medial cerebellum is thought to control muscle tone and upright stance [58,59], so balance control improvements may have been expected following medial cerebellar stimulation. In contrast, these results might suggest that over-activity of the cerebellum may not be contributing to gait and balance deficits in PD. Importantly, the improvement in resting tremor demonstrates that cerebellar inhibition (both at the dentate and fastigial nuclei) was successful, meaning that potentially normalizing the cerebellar output must not have similar therapeutic effects on gait and balance deficits. One possible explanation may lie in previous research which has shown that individuals with PD who have severe postural instability have significantly decreased cholinergic innervation of the pedunculopontine nucleus (PPN)[45,63]. It has been suggested that this cholinergic denervation may result in poor integration and relay of sensory information at the thalamus, resulting in balance deficits. Importantly, cholinergic PPN neurons innervate a large

proportion of the thalamus, including relays for cerebellar nuclei [45,63]. Since the thalamus is the initial point of relay for output coming from both the dentate and fastigial nuclei, it is possible that the cholinergic denervation had a negative effect on the "normalized" output following stimulation. This might suggest that the PPN input (which is detrimental to gait and balance) had a greater influence on thalamus output than did the effects of normalizing the output of cerebellar nuclei. Thus, output contributing to poor gait and balance control was propagated from the thalamus to the motor cortex. Hence, it might be suggested that PPN cholinergic denervation and not over-activity of the cerebellum contributes to gait and balance deficits in PD.

Additionally, the involvement of basal ganglia dysfunction in gait and balance impairments in PD must be considered [36–38,69]. Some gait parameters, such as velocity, stride length and stride time, demonstrate improvement following dopaminergic replacement therapy, indicating basal ganglia pathology [36–38]. Knowing that the dentate nucleus pathway relays in the basal ganglia, it might be expected that if cerebellar over-activity was involved in gait and balance deficits, then a greater improvement in symptoms would result following stimulation of the fastigial nucleus (a pathway which does not relay in the dysfunctional basal ganglia). Importantly, this study found no improvement following stimulation of either cerebellar target. It must be noted that this experimental paradigm cannot directly assess the involvement of the basal ganglia, however, this does further support the theory of PPN involvement. Regardless of the cerebellar target, the potentially normalized output following stimulation must first relay in the thalamus, which is simultaneously receiving poor input from the cholinergic denervated PPN. Since the input from the PPN is detrimental to gait and balance, then output from either of the cerebellar nuclei might be "damaged" after relaying in the thalamus, regardless of modulation at

the level of the cerebellum. In this case, “damaged” dentate nucleus output (after relaying in the thalamus) would reach the dysfunctional basal ganglia and likely be further impaired. Hence, it is likely that both the basal ganglia and the PPN are playing a role in gait and balance deficits in PD.

It must be noted that stimulation targeted to the medial cerebellum may not have directly influenced the fastigial nucleus, likewise stimulation of the lateral cerebellum may not have directly influenced the dentate nucleus. If this was the case, modulation resulting from the stimulation may have instead occurred at the level of the cerebellar cortex (whether medial or lateral). Instead, activity within the deep cerebellar nuclei may be indirectly affected through modulation of Purkinje cell activity within the cerebellar cortex. It is possible that the inhibitory stimulation protocol may have induced long term depression in the Purkinje fibres, whereupon a decrease in the net inhibitory drive from the Purkinje fibres may have indirectly modulated activity in the deep cerebellar nuclei.

Clinical Implications

Dopaminergic loss in the striatum and dysfunction of the basal ganglia have been found to be more strongly linked with symptoms of bradykinesia and rigidity, and less associated with the generation of tremor[27]. The addition of the current evidence, suggests that over-activity in the cerebellum may be involved in resting tremor in PD[27,28,34] but not influencing gait and balance deficits, may help to explain why these symptoms are not very responsive to dopaminergic replacement therapy. Given that dopaminergic replacement is the current gold standard for treating motor symptoms in PD[2–4], a better understanding of how to manage symptoms that are not responsive to pharmaceutical therapy is crucial.

The current study provides evidence that an inhibitory rTMS protocol has the potential to improve resting tremor symptoms in tremor-dominant individuals with PD. The advantages of using rTMS as a treatment is that the procedure is non-invasive, not painful and requires little time per visit. This method of stimulation, when applied over repeated sessions, has the ability to induce changes in the cerebellum that last days to weeks beyond the period of stimulation [6,7]. It is also important to note that the improvements in resting tremor did not come at the cost of detriment to other symptoms, specifically gait and balance. Further understanding of the mechanisms underlying the involvement of cerebellar over-activity in tremor provides promise for the development of a treatment for tremor in PD.

Future Directions

With the evidence that stimulation of the lateral and medial cerebellum improved tremor symptoms, a follow-up study with a sample including only tremor-dominant PD participants is warranted. It would be important to limit study participation to tremor-dominant individuals to increase statistical power and make the results more clear; measuring the effects of the stimulation on tremor in an individual which does not experience tremor would not be possible. Replication of results with a larger tremor-dominant sample size would strengthen the evidence supporting cerebellar involvement in resting tremor. It would also be important to assess how the effects of stimulation may differ when participants are in their OFF medication state. Since tremor responds variably to dopaminergic replacement, assessment of individuals in the OFF state would allow the assessment of changes to tremor at an individuals' worst severity. In addition, this would help to disentangle what might happen to the modulated output from the lateral cerebellum upon relay in the basal ganglia. If tremor was improved to the same degree in the OFF medication state, then it may be possible the output from the lateral cerebellum is either

not relaying in the basal ganglia, or dopaminergic loss and basal ganglia dysfunction may not be strongly related to tremor.

Anecdotal evidence from participants in the current study who experienced a relief in tremor reported improved symptoms beyond the period of assessment (some reporting up to 12 hours of improvement). This is an unusual finding, given that stimulation effects are expected to last for a length of time about equal to that of the period of stimulation. The next logical step would be to also investigate the 24-hour effects of stimulation with an accelerometer which could be worn at home. A better understanding of the temporal profile of the stimulation effects would help to increase understanding of how long the stimulation effects are able to modulate synaptic activity in the cerebellum. Knowing how long the stimulation effects last at the subcortical level would aid in the design of a multiple session rTMS protocol. Repeated sessions of stimulation applied within the correct time period would allow for the effects of the stimulation to accumulate. It is possible that the cumulative effects of stimulation may interact with the mechanisms of cortical plasticity, and prolong the period for which LTD, and thus symptom improvement, lasts. A review by Fregni et al., demonstrated effects following an acute session of stimulation to be predictive of long-lasting effects following repeated sessions of stimulation [16]. For example, if there are not immediate motor benefits after stimulation, then effects following repeated sessions should not be expected. Alternatively, when motor benefits do exist following one session of stimulation, cumulative effects can be hypothesized following multiple sessions of stimulation. Given the clear benefits to tremor following the current protocol, when paired with the proper repeated stimulation schedule, it can be hypothesized that long-lasting benefits resting tremor could occur.

The current study was an important step in the direction towards gaining understanding of the contribution of the cerebellum to PD motor symptoms. It also sheds light on the possibility of investigating further rTMS treatments for the improvement of resting tremor, an otherwise poorly dopaminergic responsive motor symptom in PD.

Limitations

One limitation of this study from a statistical perspective, was that randomization was done only to assign participants to groups (i.e. medial, lateral or sham), and thus, there was an unequal number of tremor-dominant and PIGD participants in each group. Block randomization could have been done to ensure an equal distribution of PD sub-types within each group. Nonetheless, groups were matched in terms of symptoms severity (in addition to tremor severity) despite slightly differing in PD sub-type proportions.

The absence of individual magnetic resonance (MR) images for participants was a limitation in the study that was controlled in a manner based upon the best methods used in previous research. There exists neuronavigation software which enables the upload of participant MR images; this would allow TMS targets to account for individual anatomical anomalies. In this case, access to individual MR images was not possible. Neuronavigation was still utilized, and a target was still created in the program to represent the hot spot for the first dorsal interosseous (FDI) muscle. This was a manual process involving systematic movement of the stimulation coil, and monitoring of EMG activity in the FDI muscle. The program was important in providing a target so that the coil could be maintained in the correct location when the resting motor threshold protocol was done.

MRI-guided neuronavigation can also be advantageous for determining cortical and sub-cortical locations that do not evoke motor responses in the same way the motor cortex does. Two stimulation sites were used in this study: the vermis (fastigial nucleus) and the lateral cerebellum (dentate nucleus). Previous studies have shown the vermis to be located beneath the inion, thus, this target was located through surface palpation. The lateral cerebellum has been reliably located to be three centimetres lateral and one centimetre inferior to the inion[50,70]; this location was carefully measured for individuals who received stimulation targeted at the dentate nucleus.

The final way in which individual MR images could have been utilized in this study was to perform depth calculations to determine stimulation intensity. Depth calculations are important because the thickness of the skull is greater over the cerebellar area than it is over the motor cortex. Since the resting motor threshold is determined by applying stimulation over the motor cortex, the depth calculation would serve to account for the greater impedance of the magnetic pulses when passing through the thicker bone above the cerebellum. Previous studies which were not able to perform depth calculations applied rTMS over the cerebellum at an intensity of 120% of the resting motor threshold to ensure the pulses were strong enough to reach the same depth in the cerebellum[52,71]. These principles were also applied in the current study. It is important to note that by using a standardized percentage of resting motor threshold to set the stimulation intensity for the repeated pulses is advantageous, since the intensity is a relative number based upon individual participants' cortical excitability levels.

Though the use of individual MR images would have added an additional layer of precision to the study protocol, the methods used to compensate have been validated in previous

studies. Given that quantifiable effects on tremor were found following rTMS, it can be concluded that the methods utilized were appropriate.

A few limitations exist within the outcome measures that were chosen. In terms of the outcomes measure used for tremor, it would have been beneficial to have the inability to extract kinematic parameters from the motion sensory individually for analysis as opposed to the output of only a composite score. Understanding whether the improvement in resting tremor was driven mainly by a decrease in tremor frequency or a change in tremor amplitude is important from a clinical perspective. Irrespective of decreasing tremor frequency, an increase in the amplitude of the tremor movements might enable greater functional control of the hands during activities of daily living. The Kinesia motion sensor has however been proven to have a strong correlation with clinical tremor ratings, in addition to having the granularity to detect changes in tremor severity that were not distinguishable by clinician assessment [57].

Similarly, the Biodex balance platform records postural sway and outputs a composite score of average degrees of postural sway in the medio-lateral and anterio-posterior directions along with a standard deviation. While this provides a global assessment of postural control changes, measurement of postural stability and balance with use of a force plate would have allowed more in-depth assessment of balance. The ability to trace postural sway over time, or to look at the movement of the centre of pressure might have enabled detection of more finite changes in balance following stimulation.

Conclusion

The current thesis provides evidence to suggest the involvement of the fastigial and dentate nucleus in the generation of resting tremor in PD. Following a single session of

inhibitory rTMS over either the medial or lateral cerebellum, a reduction in resting tremor was found, specifically in tremor-dominant individuals. Concurrent evidence also suggests gait and balance deficits in PD to be unrelated to cerebellar over-activity, and supports previous research suggesting these deficits may be more related to cholinergic denervation of the PPN and basal ganglia dysfunction. Given that tremor, gait and balance symptoms in PD are poorly responsive to dopaminergic replacement therapy, furthering the current understanding of how the cerebello-thalamo-cortical circuit might be influencing these symptoms has the potential to lead to the development of novel treatments in PD.

References

[1] Wirdefeldt K, Adami H-O, Cole P, Trichopoulos D, Mandel J. Epidemiology and etiology of Parkinson's disease: a review of the evidence. Eur J Epidemiol 2011;26:S1–58.

[2] Thanvi B, Lo N, Robinson T. Levodopa-induced dyskinesia in Parkinson's disease: clinical features, pathogenesis, prevention and treatment. Postgrad Med J 2007;83:384–8.

[3] Pahwa R, Lyons KE. Levodopa-related wearing-off in Parkinson's disease: identification and management. Curr Med Res Opin 2009;25:841–9.

[4] Almeida QJ, Hyson HC. The Evolution of Pharmacological Treatment for Parkinson ' s Disease. Recent Pat CNS Drug Discov 2008;3:50–4.

[5] Rothwell J. Transcranial magnetic stimulation as a method for investigating the plasticity of the brain in Parkinson ' s Disease and dystonia. Parkinsonism Relat Disord 2007;13:417–20.

[6] Lomarev MP, Kanchana S, Bara-Jimenez W, Iyer M, Wassermann EM, Hallett M. Placebo-controlled study of rTMS for the treatment of Parkinson's disease. Mov Disord 2006;21:325–31.

[7] Hamada M, Ugawa Y, Tsuji S. High-frequency rTMS over the supplementary motor area for treatment of Parkinson's disease. Mov Disord 2008;23:1524–31.

[8] Arias P, Vivas J, Grieve KL, Cudeiro J. Controlled trial on the effect of 10 days low-frequency repetitive transcranial magnetic stimulation (rTMS) on motor signs in Parkinson's disease. Mov Disord 2010;25:1830–8.

[9] Khedr EM, Farweez HM, Islam H. Therapeutic effect of repetitive transcranial magnetic stimulation on motor function in Parkinson's disease patients. EurJNeurol 2003;10:567–72.

[10] Lefaucheur J-P, Drouot X, Von Raison F, Ménard-Lefaucheur I, Cesaro P, Nguyen J-P. Improvement of motor performance and modulation of cortical excitability by repetitive transcranial magnetic stimulation of the motor cortex in Parkinson's disease. Clin Neurophysiol 2004;115:2530–41.

[11] Benninger, D.H., Berman, B.D., Houdayer, E., Pal, N., Luckenbaugh, D.A., Schneider, L., Miranda, S. & Hallett M. Intermittent theta-burst transcranial magnetic stimulation for treatment of Parkinson disease. Neurology 2011;76:601–9.

[12] Arias-Carrión O. Basic mechanisms of rTMS: Implications in Parkinson's disease. Int Arch Med 2008;1:2.

[13] Hallett M. Transcranial magnetic stimulation: a primer. Neuron 2007;55:187–99.

[14] Kobayashi M, Pascual-leone A. Transcranial magnetic stimulation in neurology. Lancet Neurol 2003;2:145–56.

[15] Cantello R, Tarletti R, Civardi C. Transcranial magnetic stimulation and Parkinson ' s disease. Brain Res Rev 2002;38:309–27.

[16] Fregni F, Simon DK, Wu a, Pascual-Leone a. Non-invasive brain stimulation for Parkinson's disease: a systematic review and meta-analysis of the literature. J Neurol Neurosurg Psychiatry 2005;76:1614–23.

[17] Rossini PM, Rossi S. Transcranial magnetic stimulation: diagnostic, therapeutic, and research potential. Neurology 2007;68:484–8.

[18] Madison D V, Malenka RC, Nicoll R a. Mechanisms underlying long-term potentiation of synaptic transmission. Annu Rev Neurosci 1991;14:379–97.

[19] Malenka RC, Bear MF. LTP and LTD: an embarrassment of riches. Neuron 2004;44:5–21.

[20] Massey P V, Bashir ZI. Long-term depression: multiple forms and implications for brain function. Trends Neurosci 2007;30:176–84.

[21] Linden DJ. Long-term synaptic depression in the mammalian brain. Neuron 1994;12:457–72.

[22] Siebner HR, Rothwell J. Transcranial magnetic stimulation: new insights into representational cortical plasticity. Exp Brain Res 2003;148:1–16.

[23] Filipović SR, Rothwell JC, Bhatia K. Slow (1 Hz) repetitive transcranial magnetic stimulation (rTMS) induces a sustained change in cortical excitability in patients with Parkinson's disease. Clin Neurophysiol 2010;121:1129–37.

[24] DeLong MR, Wichmann T. Circuits and circuit disorders of the basal ganglia. Arch Neurol 2007;64:20–4.

[25] Smith Y, Bevan MD, Shink E, Bolam JP. Microcircuitry of the direct and indirect pathways of the basal ganglia. Neuroscience 1998;86:353–87.

[26] Strafella AP, Vanderwerf Y, Sadikot AF. Transcranial magnetic stimulation of the human motor cortex influences the neuronal activity of subthalamic nucleus. Eur J Neurosci 2004;20:2245–9.

[27] Lewis MM, Galley S, Johnson S, Stevenson J, Huang X, Mckeown MJ. The Role of the Cerebellum in the Pathophysiology of Parkinson ' s Disease. Can J Neurol Sci 2013;40:299–306.

[28] Wu T, Hallett M. The cerebellum in Parkinson's disease. Brain 2013;136:696–709.

[29] Carrillo F, Palomar FJ, Conde V, Diaz-corrales FJ, Porcacchia P, Fernández-del-olmo M, et al. Study of Cerebello-Thalamocortical Pathway by Transcranial Magnetic Stimulation in Parkinson ' s Disease. Brain Stimul 2013;6:582–9.

[30] Kishore A, Meunier S, Popa T. Cerebellar influence on motor cortex plasticity : behavioral implications for Parkinson ' s disease. Front Neurol 2014;5:1–8.

[31] Hurley MJ, Mash DC, Jenner P. Markers for dopaminergic neurotransmission in the cerebellum in normal individuals and patients with Parkinson's disease examined by RT-PCR. Eur J Neurosci 2003;18:2668–72.

[32] Yu H, Sternad D, Corcos DM, Vaillancourt DE. Role of hyperactive cerebellum and motor cortex in Parkinson ' s disease. Neuroimage 2007;35:222–33.

[33] Ni Z, Pinto AD, Lang AE, Chen R. Involvement of the Cerebellothalamocortical Pathway in Parkinson Disease. Ann Neurol 2010;68:816–24.

[34] Helmich RC, Janssen MJR, Oyen WJG, Bloem BR, Toni I. Pallidal Dysfunction Drives a Cerebellothalamic Circuit into Parkinson Tremor. Ann Neurol 2011;69:269–81.

[35] Foreman KB, Wisted C, Addison O, Marcus RL, Lastayo PC, Dibble LE. Improved Dynamic Postural Task Performance without Improvements in Postural Responses: The Blessing and the Curse of Dopamine Replacement. Parkinsons Dis 2012;2012:692150.

[36] Rochester L, Baker K, Nieuwboer A, Burn D. Targeting dopa-sensitive and dopa-resistant gait dysfunction in Parkinson's disease: selective responses to internal and external cues. Mov Disord 2011;26:430–5.

[37] Lord S, Baker K, Nieuwboer A, Burn D, Rochester L. Gait variability in Parkinson's disease: an indicator of non-dopaminergic contributors to gait dysfunction? J Neurol 2011;258:566–72.

[38] Almeida QJ, Frank JS, Roy E a, Patla AE, Jog MS. Dopaminergic modulation of timing control and variability in the gait of Parkinson's disease. Mov Disord 2007;22:1735–42.

[39] Tan T, Almeida QJ, Rahimi F. Proprioceptive deficits in Parkinson's disease patients with freezing of gait. Neuroscience 2011;192:746–52.

[40] Caudron S, Guerraz M, Eusebio A, Gros J, Azulay J, Vaugoyeau M. Evaluation of a visual biofeedback on the postural control in Parkinson ' s disease. Clin Neurophysiol 2014;44:77–86.

[41] Vaugoyeau M, Hakam H, Azulay J. Proprioceptive impairment and postural orientation control in Parkinson ' s disease. Hum Mov Sci 2011;30:405–14.

[42] Tagliabue M, Ferrigno G, Horak F. Effects of Parkinson's disease on proprioceptive control of posture and reaching while standing. Neuroscience 2009;158:1206–14.

[43] Horak FB. Postural orientation and equilibrium: what do we need to know about neural control of balance to prevent falls? Age Ageing 2006;35 Suppl 2:ii7–11.

[44] Jacobs J V, Horak FB. Abnormal proprioceptive-motor integration contributes to hypometric postural responses of subjects with Parkinson's disease. Neuroscience 2006;141:999–1009.

[45] Muller MLTM, Albin RL, Kotagal V, Koeppe RA, Scott PJH, Frey KA, et al. Thalamic cholinergic innervation and postural sensory integration function in Parkinson ' s disease. Brain 2013;136:3282–9.

[46] Popa T, Velayudhan B, Hubsch C, Pradeep S, Roze E, Vidailhet M, et al. Cerebellar Processing of Sensory Inputs Primes Motor Cortex Plasticity. Cereb Cortex 2013;23:305–14.

[47] Bologna M, Di Biasio F, Conte A, Iezzi E, Modugno N, Berardelli A. Effects of cerebellar continuous theta burst stimulation on resting tremor in Parkinson's disease. Parkinsonism Relat Disord 2015;21:1–6.

[48] Minks E, Mareček R, Pavlík T, Ovesná P, Bareš M. Is the cerebellum a potential target for stimulation in Parkinson's disease? Results of 1-Hz rTMS on upper limb motor tasks. Cerebellum 2011;10:804–11.

[49] Koch G, Brusa L, Carrillo F, Lo Gerfo E, Torriero S, Oliveri M, et al. Cerebellar magnetic stimulation decreases levodopa-induced dyskinesias in Parkinson disease. Neurology 2009;73:113–9.

[50] Popa T, Russo M, Meunier S. Long-lasting inhibition of cerebellar output. Brain Stimul 2010;3:161–9.

[51] Fierro B, Giglia G, Palermo A, Pecoraro C, Scalia S, Brighina F. Modulatory effects of 1 Hz rTMS over the cerebellum on motor cortex excitability. Exp Brain Res 2007:440–7.

[52] Langguth B, Eichhammer P, Zowe M, Landgrebe M, Binder H, Sand P, et al. Modulating cerebello-thalamocortical pathways by neuronavigated cerebellar repetitive transcranial stimulation (rTMS). Clin Neurophysiol 2008;38:289–95.

[53] Hardwick RM, Lesage E, Miall RC. Cerebellar transcranial magnetic stimulation: The role of coil geometry and tissue depth. Brain Stimul 2014;7:643–9.

[54] Ugawa Y, Uesaka Y, Terao Y, Hanajima R, Kanazawa I. Magnetic stimulation over the cerebellum in humans. Ann Neurol 1995;37:703–13.

[55] Giuffrida JP, Riley DE, Maddux BN, Heldmann D a. Clinically deployable kinesia technology for automated tremor assessment. Mov Disord 2009;24:723–30.

[56] Heldman D a., Giuffrida JP, Chen R, Payne M, Mazzella F, Duker AP, et al. The modified bradykinesia rating scale for Parkinson's disease: Reliability and comparison with kinematic measures. Mov Disord 2011;26:1859–63.

[57] Heldman D a., Espay AJ, LeWitt P a., Giuffrida JP. Clinician versus machine: Reliability and responsiveness of motor endpoints in Parkinson's disease. Park Relat Disord 2014;20:590–5.

[58] Ilg W, Giese M a, Gizewski ER, Schoch B, Timmann D. The influence of focal cerebellar lesions on the control and adaptation of gait. Brain 2008;131:2913–27.

[59] Manto M, Bower JM, Conforto AB, Delgado-García JM, Da Guarda SNF, Gerwig M, et al. Consensus paper: Roles of the cerebellum in motor control-the diversity of ideas on cerebellar involvement in movement. Cerebellum 2012;11:457–87.

[60] Horak FB. Clinical assessment of balance disorders. Gait Posture 1997;6:76–84.

[61] Benninger DH, Iseki K, Kranick S, Luckenbaugh D a., Houdayer E, Hallett M. Controlled Study of 50-Hz Repetitive Transcranial Magnetic Stimulation for the Treatment of Parkinson Disease. Neurorehabil Neural Repair 2012;26:1096–105.

[62] Von Papen M, Fisse M, Sarfeld AS, Fink GR, Nowak D a. The effects of 1 Hz rTMS preconditioned by tDCS on gait kinematics in Parkinson's disease. J Neural Transm 2014;121:743–54.

[63] Bohnen NI, Müller ML, Dauer WT, Albin RL. Parkinson's disease: what role do pedunculopontine cholinergic neurons play? Future Neurol 2014;9:5–8.

[64] Tard C, Delval a., Devos D, Lopes R, Lenfant P, Dujardin K, et al. Brain metabolic abnormalities during gait with freezing in Parkinson's disease. Neuroscience 2015;307:281–301.

[65] Brugger F, Abela E, Hagele-Link S, Bohlhalter S, Galovic M, Kagi G. Do executive dysfunction and freezing of gait in Parkinson's disease share the same neuroanatomical correlates? J Neurol Sci 2015;356:184–7.

[66] Göttlich M, Münte TF, Heldmann M, Kasten M, Hagenah J, Krämer UM. Altered Resting State Brain Networks in Parkinson's Disease. PLoS One 2013;8.

[67] Helmich RC, Derikx LC, Bakker M, Bloem BR, Toni I. Spatial Remapping of Cortico-striatal Connectivity in Parkinson ' s Disease. Cereb Cortex 2010;20.

[68] Rubino A, Assogna F, Piras F, Di Battista ME, Imperiale F, Chiapponi C, et al. Does a volume reduction of the parietal lobe contribute to freezing of gait in Parkinson's disease? Park Relat Disord 2014;20:1101–3.

[69] Chan HF, Kukkle PL, Merello M, Lim SY, Poon YY, Moro E. Amantadine improves gait in PD patients with STN stimulation. Park Relat Disord 2013;19:316–9.

[70] Théoret H, Haque J, Pascual-Leone A. Increased variability of paced finger tapping accuracy following repetitive magnetic stimulation of the cerebellum in humans. Neurosci Lett 2001;306:29–32.

[71] Miall RC, Christensen LOD. The effect of rTMS over the cerebellum in normal human volunteers on peg-board movement performance. Neurosci Lett 2004;371:185–9.

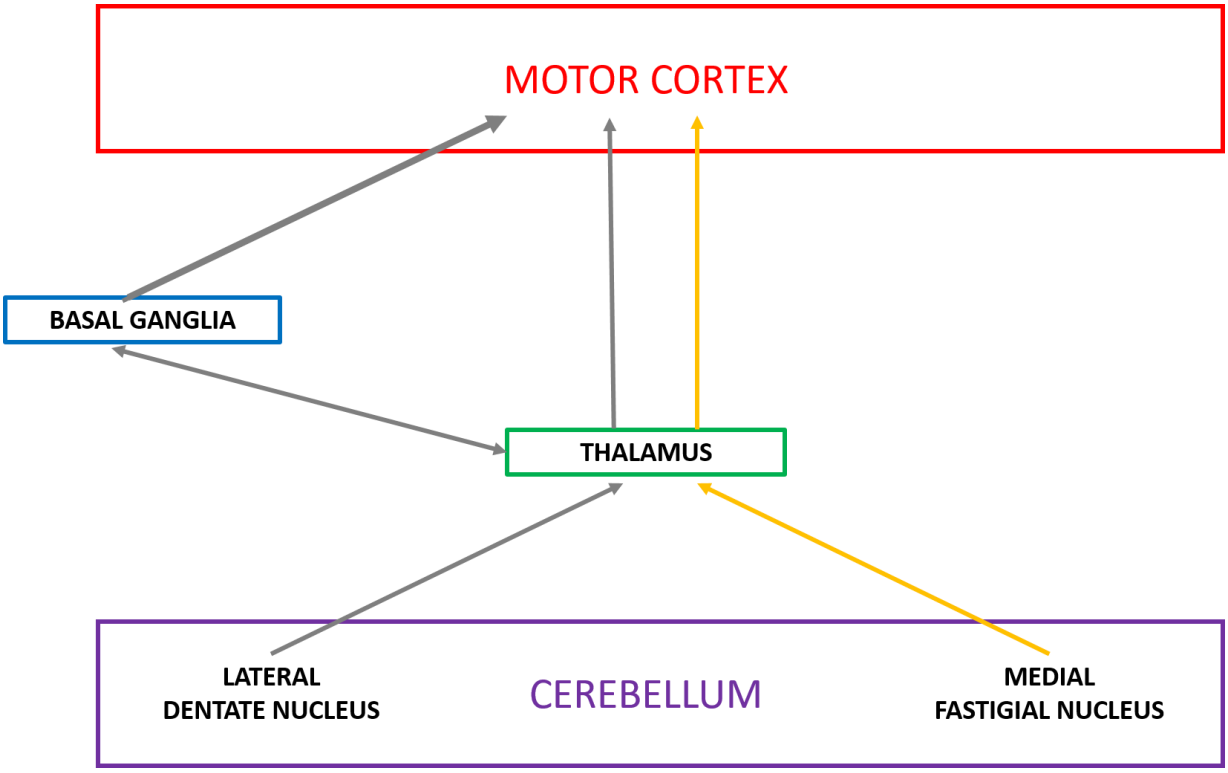

Figure 1: Model of cerebellum in Parkinson's disease