Surrogate markers of progression in Parkinson disease: Correlation between clinical features and neuroimaging.

by

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Abstract

Parkinson disease is a progressive neurodegenerative disorder affecting approximately 100,000 Canadians. Loss of dopaminergic neurons in the substantia nigra of the basal ganglia is the prominent pathology associated with many of the motor features that characterise Parkinson disease. Increased levels of iron in the substantia nigra are seen in Parkinson disease although the mechanistic significance is uncertain. It is becoming increasingly clear that the disability associated with Parkinson disease extends beyond motor features and that changes in cognition are prominent features, even in early disease. The debilitating phenomenon "freezing of gait" (FOG) emerges in some with Parkinson disease, often accompanying this decline in cognitive function.

The purpose of this research was to test the hypothesis that Parkinson disease is associated with longitudinal changes in motor function and cognition which can be quantified clinically and with brain imaging (magnetic resonance imaging and/or spectroscopy). We hypothesized that imaging markers of abnormal brain structure and function will correlate with these clinical changes and serve as biomarkers of disease progression. In order to investigate this, we evaluated the evolving relationship between clinical manifestations, reflected in changes of gait and cognition, and in vivo structural and functional changes as measured by high field magnetic resonance imaging and spectroscopy in early, untreated individuals with Parkinson disease over a period of 36 months.

In the first study (Chapter 2), we evaluated changes in midbrain iron content in a group of early untreated Parkinson disease subjects compared to a control group and correlated evolving motor dysfunction with these changes. Results

suggested that the change in midbrain iron content, as estimated by R_2*, correlated with a change in motor symptoms over three years in the Parkinson disease group. This provides evidence that this measure may have utility as an imaging marker for Parkinson disease progression.

In the second study (Chapter 3), we investigated changes in this same iron-rich midbrain region to determine if changes were associated differently between a cohort of early, untreated Parkinson disease subjects who developed FOG over three years compared to one who did not. While the measured R_2* values of both groups changed over time, the FOG group showed more rapid deterioration in motor function and SNc changes suggestive of increased iron content. This result suggests a difference in midbrain pathology between these groups, with increased nigral iron content appearing to be closely associated with severity of motor features in Parkinson disease. To our knowledge, this is the first longitudinal study to observe differences in midbrain iron content, as measured R_2* values, to differentiate subjects in whom FOG emerged from those with similar disease duration who did not.

In the third study (Chapter 4), we examined the relationship of cognitive changes and clinical motor features, including FOG, with a magnetic resonance spectroscopy maker of regional neuronal loss, NAA/Cr ratio, in the pre-supplementary motor area. We compared a group of early, untreated Parkinson disease to controls and, separately, a group who developed FOG to one that did not, over 36 months. Significant cognitive deficits associated with frontal lobe dysfunction were present in patients with early, untreated Parkinson disease but impaired cognition was not predictive of the development of FOG. In contrast,

individuals who developed FOG had worse motor function at baseline and more rapid decline in motor scores over 36 months indicating an association between disease burden and the development of FOG.

Together, these results suggest that measures of neuronal loss in the pre-supplementary motor area, reflected in NAA/Cr ratios, are a poor correlate of impaired cognitive and/or motor function, at least in this study cohort with early disease. Midbrain iron content, estimated by R_2* values, however, may be a marker of disease progression and may help differentiate those with Parkinson disease who are destined to develop FOG from those who are not.

PREFACE

This thesis is an original work by Marguerite Wieler. The research project, of which this thesis is a part, received research ethics approval from the University of Alberta Research Ethics Board, Project Name "Biomarkers of Disease Progression in Parkinsonism: Magnetic resonance imaging and spectroscopy." No. 4574, March 14, 2003.

Some of the research conducted for this thesis forms part of a project, led by Dr. W.R. Wayne Martin at the University of Alberta. Dr. Martin (Principal Investigator) and Dr. Richard Camicioli (Co-Investigator) were responsible for the original study design and securing funding from the Canadian Institutes of Health Research. Marguerite Wieler took primary responsibility for subject recruitment, data collection and database management for all studies presented in this thesis. Imaging for all three studies was performed by Dr. Myrlene Gee and Dr. Chris Hanstock, with voxel and slice placement confirmed by Marguerite Wieler. Image processing and analysis was performed by Dr. Myrlene Gee and Dr. Chris Hanstock. A version of the work presented in Chapter 2 of this thesis has been accepted for publication in *Parkinsonsim and Related Disorders*. Chapters 3 and 4 provide the basis for two further manuscripts currently in preparation.

Chapter 2: Wieler M, Gee M, Martin WRW. Longitudinal Midbrain Changes in Early Parkinson's Disease: Iron Content Estimated from R_2*/MRI (submitted) M. Wieler was responsible for manuscript composition. M. Gee was responsible for image analysis and contributed to manuscript edits. WRW Martin contributed to manuscript edits. All were involved with concept formation for the paper and for interpretation of the results.

Chapter 3: Wieler M, Gee M, Camicioli R, Martin WRW. Freezing of Gait in Early Parkinson's Disease: Nigral Iron Content Estimated from MRI. (in preparation) M. Wieler was responsible for manuscript composition. M. Gee was responsible for image analysis and contributed to manuscript edits. R Camicioli contributed to manuscript edits. WRW Martin contributed to manuscript edits. All were involved with concept formation for the paper and for interpretation of the results.

Chapter 4: Wieler M, Gee M, Hanstock C, Camicioli R, Martin WRW. Emergence of Freezing of Gait in Parkinson disease: Are there predictors? (in preparation) M. Wieler was responsible for manuscript composition and analysis. M. Gee was responsible for image analysis and contributed to manuscript edits. C. Hanstock contributed to analysis. R Camicioli contributed to manuscript edits. WRW Martin contributed to manuscript edits. All were involved with concept formation for the paper and for interpretation of the results.

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The confidence that I am capable of achieving my goals was instilled in me by my parents through their love and support. Thank you. My family, immediate and extended, have encouraged, supported and prodded me in different (and sometime creative!) ways over the years to reach this milestone. Thank you. "Anna the Great ", you know your invaluable contribution to the completion of this tome! Thank you. Felix and Forrest, your "punny" presence added much needed distraction. Thank you. I include in my extended family Pam and Cecil who fed and supported me. Thank you. Friends, near and far, have also played their part in keeping me on track to get this work done. Thank you. Janice, your PhD was an inspiration. Thank you. Allyson, urging me to think about my future beyond the lifespan of the Movement Disorders Program was a necessary nudge. Thank you.

None of this work could have been achieved without the gracious and generous contribution of time and energy from the participants, both those living with Parkinson disease and the controls. Thank you.

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List of Abbreviations

AC	anterior commissure
ADL	activities of daily living
ANOVA	repeated measures analysis of variance
ATP	adenosine triphosphate
BBS	Berg Balance Scale
BDI	Beck Depression Inventory
BOLD	blood oxygenation level dependent
Cho	choline
CIHR	Canadian Institutes of Health Research
COMT	catechol-O-methyl transferase
COWAT	Controlled Oral Word Association Test
Cr	creatine
CSF	cerebral spinal fluid
CVLT	California Verbal Learning Test
DAT	dopamine-transporter
DBS	deep brain stimulation
DTI	diffusion tensor imaging
EMG	electromyography
FA	fractional anisotropy
FAB	Frontal Assessment Battery
F-DOPA	fluorodopa
fMRI	functional magnetic resonance imaging
FOG	freezing of gait
FOG	freezing of gait
GABA	gamma-aminobutyric acid
GM	gray matter
GP	globus pallidus
GPe	globus pallidus externus
GPi	globus pallidus internus
JOL	Judgment of Line Orientation
MAO-B	monoamine oxidase type B
MC	motor cortex
MCI	mild cognitive impairment
MD	mean diffusivity
MLR	midbrain locomotor region
MMSE	Mini-Mental State Exam
MPRAGE	Magnetization Prepared RApid Gradient Echo
MR	magnetic resonance
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy

NAA	N-acetylaspartate
nFOG	no freezing of gait
PIGD	postural instability gait disorder
PC	posterior commissure
PDQ	Parkinson Disease Questionnaire
PET	positron emission tomography
PMC	premotor cortex
PRESS	Point Resolved Spectroscopy Sequence
Pu	putamen
r2	Pearson product-moment correlation squared
R_2*	transverse relaxation rate
rANOVA	repeated measures analysis of variance
RN	red nucleus
ROI	region-of-interest
SD	standard deviation
SMA	supplementary motor area
SNc	substantia nigra pars compacta
SNr	substantia nigra pars reticulata
STN	subthalamic nucleus
TCS	transcranial sonography
TE	echo time
TI	inversion time
TR	repetition time
TUG	Timed Up and Go
UPDRS	Unified Parkinson's Disease Rating Scale
VBM	voxel-based morphometry
VL	ventrolateral
VMAT	vesicular monoamine transporter
WM	white matter

Chapter 1: Background

Introduction

Idiopathic Parkinson disease is a common neurodegenerative disorder currently affecting close to 100,000 Canadians (Guttman, Slaughter et al. 2003, Lai, Schulzer et al. 2003, Svenson, Platt et al. 1993, Jones, Martin et al. 2012). Of those diagnosed, 85% are over age 65, an age group predicted to grow dramatically over the next 30 years, significantly increasing the expected prevalence of Parkinson disease (Dorsey, Constantinescu et al. 2007). Complications of this chronic progressive disease have significant negative consequences from both a human and economic perspective, with increased healthcare utilization and lost productivity (Findley 2007, Hassan, Wu et al. 2013).

Etiology of Parkinson Disease

The underlying causes for the vast majority of cases of Parkinson disease are unknown. Exposure to environmental toxins such as pesticides and herbicides, rural living and drinking well water (Jenner 2001) have been associated with the development of Parkinson disease, while cigarette smoking and caffeine consumption have putative protective properties (Crosiers, Theuns et al. 2011, Hernan, Takkouche et al. 2002). The interval between exposure and development of Parkinson disease symptoms makes a causal link to environmental factors difficult. The contribution of genetic causes to the development of Parkinson disease remains to be clarified. Genome-wide studies have shown specific mutations such as LRRK2, PARK2, PARK7, PINK1 or SNCA to be linked to the approximately 15% of people with Parkinson disease who have a family history of Parkinson disease as well as a correlation of other genetic markers with idiopathic Parkinson disease (Crosiers, Theuns et al. 2011, Dauer, Przedborski 2003). The

development of Parkinson disease is thought to be a result of a complex interaction between genetic and environmental (some of them not-yet-elucidated) risk factors. The contribution of α-synuclein-containing Lewy bodies (a pathologic hallmark of Parkinson disease) to disease processes, including cognitive impairment, also remains unclear (Libow, Frisina et al. 2009).

Pathogenesis of Parkinson Disease

Progressive and gradual degeneration of the dopamine-producing neurons in the substantia nigra, leading to decreased levels of striatal dopamine and decreased dopamine levels in the frontal lobes and limbic circuits, is the primary neurochemical abnormality underlying many of the motor features in Parkinson disease (Obeso, Rodriguez-Oroz et al. 2008). At the onset of symptoms in Parkinson disease, ~80% of striatal dopamine (most pronounced in the dorsolateral putamen) (Marsden 1992) has been depleted and ~60% of dopaminergic neurons in the substantia nigra pars compacta (SNc) have already been lost (Fearnley, Lees 1991). This long prodromal period provides a potential opportunity to intervene with neuroprotective strategies. Lateral SNc neuronal loss has been reported to be particularly correlated with the clinical motor features of Parkinson disease (Greffard, Verny et al. 2006, Lee, Schulzer et al. 1994).

One theory of pathogenesis of Parkinson disease proposes that the earliest stage of Parkinson disease is characterized by Lewy pathology in the non-dopaminergic structures of medulla oblongata or olfactory bulb, and that only when this synuclein pathology reaches the SNc from the brainstem at mid-stage disease do the cardinal motor features of Parkinson disease emerge. As the pathology continues to spread, it affects the limbic and neocortical regions, at which point

behavioural and cognitive impairment emerge (Braak, Del Tredici et al. 2003b). This conceptual framework does not fully account for many features of Parkinson disease, particularly as the Braak stages have no relationship to clinical severity of Parkinson disease. This theory, however, has been widely accepted and has guided thinking with respect to how diagnosis of early-stage Parkinson disease is regarded, potential biomarkers, and "best" animal models for the disease (Burke, Dauer et al. 2008).

Although degeneration of dopaminergic nigral cells is a prominent underlying pathologic feature of Parkinson disease, selective lesions in non-dopaminergic areas, such as the noradrenergic locus coeruleus neurons, cholinergic neurons in the pedunculopontine nuclei and glutamatergic projections neurons of the pre-supplementary motor cortex, are also affected and are responsible for dopamine non-responsive features of Parkinson disease (Halliday, Barker et al. 2010). The underlying mechanisms of the degeneration of these neurons are not clear, but mitochondrial dysfunction, misfolding and aggregation of proteins and oxidative stress have been implicated (Dauer, Przedborski 2003).

Diagnosis and Symptoms of Parkinson Disease

The emerging evidence of genetic variations and the heterogeneity of clinical presentation and disease progression have led to a debate as to the definition of Parkinson disease, with some suggesting that "Parkinson disease" should be considered an umbrella term for a spectrum of disorders that share common clinical, pathologic and genetic features (Jenner, Morris et al. 2013). Currently, however, the diagnosis of Parkinson disease is based on clinical criteria: (Hughes, Daniel et al. 1992) the presence of bradykinesia and at least one of rigidity, rest

tremor or postural instability, in the absence of any other identifiable cause of these symptoms. (Imaging of the dopamine transporter system has recently been approved in some countries to help distinguish the diagnosis of Parkinson disease from essential tremor but at best it only provides evidence of dopaminergic dysfunction without providing a definite diagnosis of Parkinson disease.) The classic motor features that characterize Parkinson disease are varying combinations of bradykinesia, rest tremor, rigidity, and balance/gait impairment, often with unilateral onset.

While Parkinson disease is classically defined as a movement disorder, non-motor disturbances are increasingly recognized as having a significant negative impact on the lives of those living with the disease, their care partners and family, often further impairing quality of life. The impact of non-motor symptoms is also linked to increased caregiver burden, high rates of moving to institutionalized care, and increased morbidity and mortality (Findley 2007, Chaudhuri, Healy et al. 2006, Hely, Reid et al. 2008, Aarsland, Larsen et al. 2000). Common non-motor features include disturbances of mood (depression, apathy and anxiety), cognition, autonomic function (orthostatic hypotension, urinary frequency, constipation), and sleep (excessive daytime sleepiness and disrupted night time sleep) as well as pain and fatigue, and these features do not respond well to dopaminergic therapy (Chaudhuri, Healy et al. 2006). Despite the negative impact posed by non-motor features, they are often not reported, are poorly recognized or are inadequately treated (Chaudhuri, Prieto-Jurcynska et al. 2010, Shulman, Taback et al. 2002). Depression is common and may result from disruption of dopaminergic,

serotonergic and noradrenergic pathways (Tandberg, Larsen et al. 1997). Impaired cognition is also common and is associated with significant motor dysfunction.

Differences in rate of progression and clinical picture appear to be related to the subtypes distinguished below. Those with symptom onset before the age of 40 tend to have slower disease progression (Sato, Hatano et al. 2006), later onset of cognitive decline and falls (Selikhova, Williams et al. 2009), (but earlier time to freezing of gait) (Contreras, Grandas 2012), a higher risk for and shorter time until the development of dyskinesia and wearing off (Sato, Hatano et al. 2006), and a higher risk for anxiety (Burn, Landau et al. 2012). Those with more tremor, compared to those with more prominent postural instability and axial symptoms, tend to have a more benign course of disease (Williams-Gray, Evans et al. 2009). Tremor is associated with a poorer response to levodopa and slower disease progression (Selikhova, Williams et al. 2009, van de Berg, Hepp et al. 2012), lower risk for depression and mood impairments (Burn, Landau et al. 2012), and lower risk of dementia (Roos, Jongen et al. 1996). Postural instability and gait impairment (PGID) as the more prominent features tend to relate to a higher prevalence of dementia (Selikhova, Williams et al. 2009, van de Berg, Hepp et al. 2012) and severity of depressive symptoms (Burn, Landau et al. 2012, Starkstein, Petracca et al. 1998). Features associated with a more rapid progression of disease without dementia include older age at onset, early depressive symptoms and tremor-dominant symptoms (Selikhova, Williams et al. 2009). This clinical variability is consistent with the concept that what is often referred to as Parkinson disease is more of a syndrome, i.e. a constellation of clinical manifestations potentially of multiple etiologies.

Treatment Approaches

Across the spectrum of disease, the main approach to management of Parkinson disease is through pharmacological interventions, aimed primarily at reducing motor symptoms. Non-motor symptoms have proven to remain largely resistant to medication treatment. There is currently no disease-modifying therapy available to retard or halt progression of Parkinson disease, though this is a major focus of current research.

Currently, the most effective treatment strategies for Parkinson disease attempt to improve the motor impairment resulting from dopamine reduction; the primary goal is to minimize the disability associated with impaired motor function. Levodopa (in combination with carbidopa or benserazide) remains the single most effective drug for the symptomatic treatment of Parkinson disease, with virtually all patients with Parkinson disease experiencing a clinically significant benefit (Hughes, Ben-Shlomo et al. 1992). Long-term use of levodopa therapy commonly results in side effects that include motor fluctuations, "wearing off" and dyskinesia. Several other classes of drugs are used, either as monotherapy or in conjunction with levodopa, primarily to reduce the motor symptoms in Parkinson disease and to lessen their impact on health-related quality of life. Dopamine agonists (including pramipexole, ropinirole, and rotigotine) directly stimulate dopamine receptors in the brain. They can be used as monotherapy, particularly in early disease, or as an adjunct to levodopa. Monoamine oxidase B inhibitors (including selegiline and rasagiline) slow the breakdown of dopamine in the brain and have a mild symptomatic benefit as well as showing some weak evidence for neuroprotection in early disease. They can be used as monotherapy or as an adjunct to levodopa.

Catechol-O-methyl transferase inhibitors (entacapone), used in combination with levodopa, increase its bioavailability. Anticholinergics are used occasionally to reduce tremor and rigidity by restoring a more normal balance between dopamine and acetylcholine. The negative effects on cognition, however, severely restrict the use of anticholinergic medication in Parkinson disease. Amantadine may be helpful in the treatment of dyskinesia associated with levodopa therapy (Martin, Wieler 2003).

Medication-Based Approaches

Medication strategies in early Parkinson disease are designed to provide symptomatic benefit to maximize motor function. The focus of treatment in patients with more advanced disease may be to minimize the motor and/or behavioural and cognitive treatment-related complications. The major motor complications of levodopa use include fluctuations in motor response and dyskinesia (abnormal involuntary movements occurring in response to levodopa administration). Motor fluctuations consist of alternating periods of relatively good response to medication ("on" periods) and periods of significantly impaired motor function ("off" periods) with a suboptimal response to medication. With disease progression, response to a single dose of levodopa becomes progressively shorter and parkinsonian symptoms re-appear before the next dose (end-of-dose deterioration, or "wearing off"). Some patients experience unpredictable and rapid fluctuations between "on" and "off" periods unrelated to the timing of antiparkinsonian medication intake. Additional side effects shared by all anti-parkinsonian medications, due to their direct action on dopamine receptors, include hallucinations, postural hypotension, dystonia, excessive daytime somnolence

(including sudden onset of sleep, "sleep attacks") (Frucht, Rogers et al. 1999), and, most commonly seen with dopamine agonist use, impulse control disorders (Weintraub, Siderowf et al. 2006). Complicated medication regimes, unpredictable response and medication side effects, particularly postural hypotension and excessive daytime somnolence, can make it difficult to reliably plan daily activities.

Neurosurgical Approaches

While pharmacological therapies remain the mainstay of symptomatic treatment in Parkinson disease, neurosurgical approaches are increasingly a part of the management of Parkinson disease. The most frequent surgical intervention is deep brain stimulation via electrodes placed in various targets including the globus pallidus interna, subthalamic nucleus and occasionally the ventral intermediate nucleus of the thalamus (for tremor suppression alone). Deep brain stimulation (DBS) may improve many motor symptoms associated with Parkinson disease including tremor, rigidity, stiffness, slowed movement, bradykinesia, but it has variable effects on speech, cognition, balance and gait (specifically freezing) (Krack, Batir et al. 2003, Johnsen, Mogensen et al. 2009, Weaver, Follett et al. 2009, Volkmann 2007, Piper, Abrams et al. 2005, Beuter, Modolo 2009).

Rehabilitation-Based Approaches

An essential complement to symptomatic treatment modalities, rehabilitation-based approaches in Parkinson disease focus on the maintenance of physical function, independence and psychosocial health (Morris, Martin et al. 2010, Dibble, Addison et al. 2009, Hackney, Earhart 2009b, Goodwin, Richards et al. 2008, Keus, Bloem et al. 2007, Kwakkel, de Goede et al. 2007). A comprehensive

team that includes physical and occupational therapists, speech language pathologists, psychologists and dieticians can provide support for many of the challenges facing people as they learn to live with a chronic progressive disorder that affects all aspects of life.

Physical Therapy

Physical therapy employs a variety of intervention strategies across the continuum of impairments and disability to address complex gait disturbance, balance and posture. In addition to interventions to improve overall muscular strength, aerobic capacity, flexibility and balance, specific strategies are used to overcome gait impairments in Parkinson disease. Application of motor learning principles combined with the use of external visual or auditory cues may improve aspects of the spatial and temporal disturbances of gait as well as freezing of gait (Nieuwboer, Dom et al. 2001, Marchese, Bove et al. 2003). These approaches tap into alternative neural circuits to generate walking by shifting motor control from the usual automatic mode to a more external and attention-demanding mode of control. Cognitive training and motor learning strategies may be used in gait re-education, freezing of gait and falls as well as for activities of daily living and transfers. Frequent coincident deficits, however, in motor planning, learning, executive function and attention may impede the use of cognitive strategies to compensate for impaired locomotion.

Impaired mobility, together with the non-motor features of Parkinson disease, leads to decreased physical activity and may result in additional chronic conditions associated with inactivity, including cardiorespiratory disorder, osteoporosis and depression (Ravina, Elm et al. 2009). There is accumulating data

in animal models of Parkinson disease for a beneficial role of exercise (Al-Jarrah, Jamous et al. 2010, Pothakos, Kurz et al. 2009, Petzinger, Fisher et al. 2010) and emerging evidence that "exercise" may retard progression of and disability related to Parkinson disease in humans, though its specific role in disease modification remains speculative at this point (Petzinger, Fisher et al. 2010, Fisher, Wu et al. 2008, Hirsch, Farley 2009).

While there is growing evidence for the benefits of physical therapy interventions, sustained benefit after treatments ends, from any approach, remains less than three months (Tomlinson, Patel et al. 2012). Improved therapies, based on the emerging understanding of the pathophysiology of both the motor and non-motor features of Parkinson disease, hold promise for long-term efficacious interventions targeted at disability during all stages of the disease.

Interdisciplinary Approach

The complex nature of disability seen in Parkinson disease demands an interdisciplinary approach to treatment (Nutt et al. 2014). Advances in the understanding of the underlying pathophysiology, combined with markers of disease progression, will further the ability to identify which interventions provided at which point in disease progression are most appropriate to provide the best chance of improving the quality of life of those touched by this disease.

Basal Ganglia

The basal ganglia are a group of interconnected subcortical grey matter structure with close connections to the cerebral cortex and thalamus. These nuclei include the putamen, caudate, globus pallidus internus (GPi) and externus (GPe),

subthalamic nucleus (STN), and substantia nigra which is subdivided into the pars reticulata (SNr) and pars compacta (SNc). The basal ganglia are intimately involved in the regulation and organization of motor behaviour, particularly the preparation, initiation and execution of learned motor programs as well as in the suppression of unwanted movements. The major input structure of the basal ganglia is the striatum (caudate and putamen) which receives widespread input from the cerebral cortex, brainstem and thalamus. The basal ganglia also receive cortical inputs indirectly from the STN. The major output structures are the GPi and SNr, projecting to the thalamus, which in turn has extensive connections to the frontal lobe and the striatum as well as to the brainstem, including the pedunculopontine nuclei. The development of the understanding in recent years of basal ganglia connections to brainstem nuclei, particularly to include the pedunculopontine nucleus, has implications for the control of gait, posture and balance. In spite of the absence of a direct connection to the spinal motor neurons, inputs from the motor cortex, the thalamus and other areas of the cortex are processed by the basal ganglia and thus indirectly influence motor output systems. There are extensive interconnections with both inhibitory and excitatory pathways within the basal ganglia.

Basal Ganglia Circuits

Numerous neurotransmitters, including dopamine, glutamate, acetylcholine, enkephalin, and gamma-aminobutyric acid (GABA), exert inhibitory or excitatory influences on multiple pathways involving the basal ganglia. The balanced action of these neurotransmitters ensures normal functioning of the basal ganglia with a net inhibitory effect on the thalamus. The SNr contains GABAerigc neurons, the SNc

has dopamine-containing neurons projecting primarily to the striatum, and the STN contains glutaminergic and cholinergic neurons (DeLong, Wichmann 2010).

Direct and Indirect Pathways

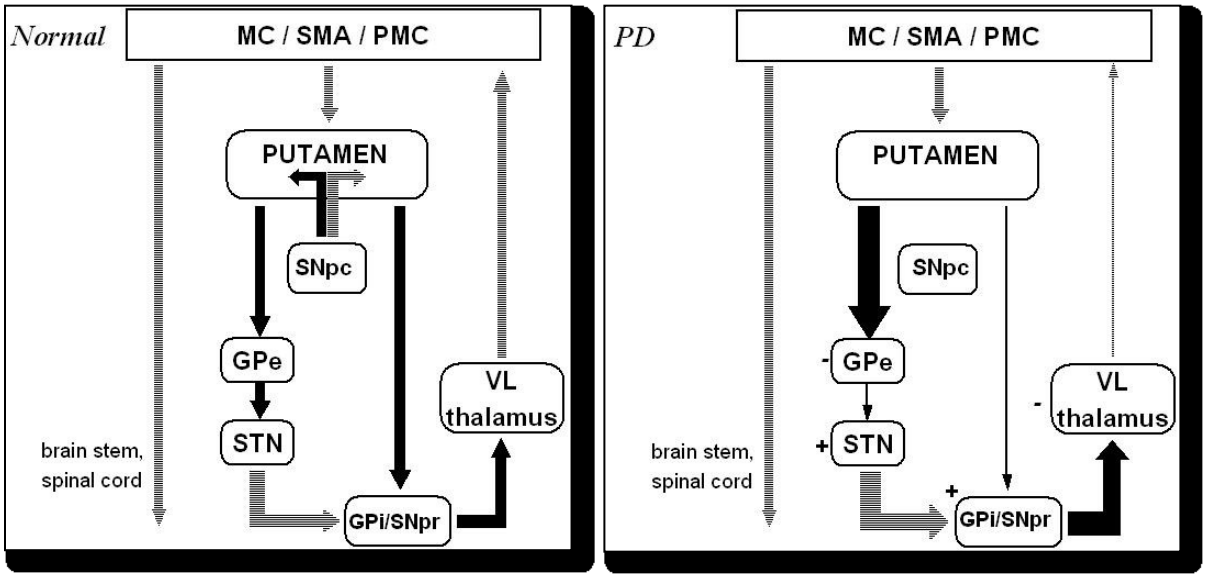
The understanding of the complex circuitry of the basal ganglia continues to evolve, aided by new insights provided by anatomic, physiologic and neuroimaging studies (DeLong, Wichmann 2010, Lang, Lozano 1998, Albin, Young et al. 1989). Traditionally, circuit models have been proposed to explain both normal motor control and the abnormal motor control seen in Parkinson disease. (See Figure 1.1) Neurons in the striatum receive excitatory projections from the cortex. These neurons then give rise to two separate pathways - *direct* and *indirect* - that connect the striatum to the output nuclei of the basal ganglia, the GPi and the SNr.

The *direct* pathway, with D1 receptors, projects directly from the putamen to the GPi/SNr. These neurons provide a direct inhibitory effect on the GPi/SNr. The GPi/SNr then exerts an inhibitory influence on the thalamus, which in turn projects to the cortex and brainstem (Alexander, Crutcher et al. 1990). Excitatory input from the SNc and cortex results in net inhibition of the GPi. The resulting disinhibition of the thalamus increases excitation of the precentral motor and supplementary motor regions in the cortex that may facilitate cortically-initiated movement (Albin, Young et al. 1989).

The *indirect* pathway, with D2 receptors, involves the putamen providing inhibitory input to the GPe which, in turn, sends inhibitory projections to the STN, which then sends excitatory projections to the GPi/SNr. Stimulation of this indirect pathway exerts a net inhibitory effect on the excitatory output of the STN on the

GPi/SNr. This results in an overall decrease in the inhibitory influence of the GPi on the thalamus and facilitates initiation of movement. The net output of basal ganglia activity is regulated by the inhibitory influence of the direct pathway and the opposing excitatory influence of the indirect pathway (Alexander, DeLong et al. 1986).

Figure 1.1: Model of Basal Ganglia Circuits (adapted from Albin et al., 1989)



The **solid** line indicates an inhibitory pathway. A **hatched** line indicates an excitatory pathway. In **normal** striatal circuitry, the output from the GPi exerts a chronic inhibitory effect on the thalamocortical neurons (VL thalamus). Activation of the direct pathway inhibits firing of the GPi, whereas activation of the indirect pathway (through the GPe and STN) stimulates it. Dopamine from the SNpc acts on the direct and indirect pathways. In **Parkinson's disease**, decreased striatal dopaminergic input, secondary to degeneration of SNpc neurons, reduces the inhibition of the GPi by the direct pathway and increases excitation of the GPi via the indirect pathway. The net result is an excessive inhibition of VL thalamus and subsequent diminished output to the cortical motor regions.

Abbreviations: **MC** - motor cortex; **SMA** - supplementary motor area; **PMC** - premotor cortex; **VL** - ventrolateral, **SNpc** - substantia nigra pars compacta.

Parallel Loop Models

More recent conceptualizations (DeLong, Wichmann 2010, Redgrave, Rodriguez et al. 2010) of the basal ganglia circuitry suggest a more complicated

organization with more extensive connections of the *direct* pathway with the GPe and also reciprocal connections in the *indirect* pathway between the striatum, GPe and STN. New concepts include a role for abnormal circuit-wide oscillations, synchrony and burst discharges with a differential effect of dopamine on the firing of basal ganglia neurons (DeLong, Wichmann 2010). Rather than a concept where the basal ganglia and cerebellar circuits converge on the thalamus which then connects to the motor cortex, the basal ganglia can be thought of as connecting to external structures via multiple parallel, partially segregated motor and non-motor loops (DeLong, Wichmann 2010). These include a motor, oculomotor, prefrontal and limbic loop that are both anatomically subdivided into functional regions and topographically organized with connections to external structures that contribute to emotional, associative and sensorimotor functions (DeLong, Wichmann 2010, Alexander, DeLong et al. 1986, Redgrave, Rodriguez et al. 2010).

Impact of Loss of Nigral Neurons

The abnormal motor control seen in Parkinson's disease is due, in part, to loss of dopaminergic neurons in the SNc resulting in overactivity of the GPi and STN. The GPi primarily exerts an inhibitory effect with major projections to the thalamus and brainstem. In Parkinson disease, decreased striatal dopamine in the basal ganglia causes under-activity of the *direct* pathways and over-activity of the *indirect* pathways. This results in a net increase in the inhibition of thalamic output and subsequent reduction of activity in the cortex (Albin, Young et al. 1989). The pattern of degeneration within the basal ganglia may impact the clinical presentation seen in Parkinson disease. The loss of dopaminergic neurons in the lateral SNc and its projections to the posterior putamen, a region of major motor

connectivity, is consistent with the appearance of the classic motor features of early Parkinson disease. It has been postulated that this area also underlies the ability to select automatic movement and that, if there is dysfunction in this area, the ability to perform these movements would require more volitional control, relying on more cognitive processes (Redgrave, Rodriguez et al. 2010). There is also evidence that decreased striatal dopamine facilitates an alteration in the spatial segregation between different cortico-striatal loops which may underlie dysfunction in sensorimotor integration in Parkinson disease (Helmich, Derikx et al. 2010). The susceptibility of transient improvement or worsening of the symptoms of Parkinson disease to general arousal and stress, as well as impairment of the ability to perform dual tasks (Marchese, Bove et al. 2003, O'Shea, Morris et al. 2002) may also be related to a reduction of the spatial segregation of motor, cognitive and limbic loops (Bronfeld, Bar-Gad 2011).

Normal function of the basal ganglia depends on a balance of multiple neurotransmitters and connections. Disruption of this balance results from the pathology seen in Parkinson disease with implications for altered motor, cognitive and limbic function.

Supplementary Motor Cortex

The supplementary motor cortex, located in the medial aspect of the frontal lobe, is active during voluntary movement, and has been implicated in the control of gait (Wai, Wang et al. 2012b, Hanakawa, Katsumi et al. 1999). Much of our information about the anatomical connections of the supplementary motor cortex comes from non-human primate and cat studies, but, with the availability of

imaging modalities that can capture brain activity, confirmation of these connections in humans is now emerging.

The supplementary motor cortex is divided into two parts with distinct and different cortical and subcortical connections. The supplementary motor area is somatotopically organised (Fried, Katz et al. 1991) with direct projections to the spinal cord (He, Dum et al. 1995, Luppino, Matelli et al. 1994) and reciprocal connections to the primary motor cortex (Luppino, Matelli et al. 1993). The pre-supplementary area, on the other hand, has widespread pre-frontal connectivity (Luppino, Matelli et al. 1993). An imaging study in humans has confirmed the association of the supplementary motor area with motor cortex connectivity and the pre-supplementary motor area with prefrontal regions (Johansen-Berg, Behrens et al. 2004). Both areas project to the striatum (pre-supplementary motor area rostral to the supplementary motor area) which act on the GPi both directly and indirectly, (Lehericy, Ducros et al. 2004, Inase, Tokuno et al. 1999) pathways that are important in the cortico-subcortical loop. In addition, the supplementary motor area has connections to the subthalamic nucleus through the "hyper-direct" path which may be important in the modulating the cortical-basal ganglia circuits (Frank, Samanta et al. 2007). Monitoring dopamine release with positron emission tomography during a motor-learning task, there was an increase in dopamine release in the pre-supplementary motor area and a corresponding reduction in release of dopamine from the GPi, demonstrating the functional interconnectedness of the basal ganglia and frontal cortical areas (Garraux, Peigneux et al. 2007). Imaging studies in Parkinson disease, show the supplementary and pre-supplementary motor areas display dysfunction in cognitive tasks (Jahanshahi,

Jenkins et al. 1995, Hikosaka, Sakai et al. 1996) and during imagined gait and freezing of gait and other motor tasks (Shine, Moustafa et al. 2013, Peterson, Pickett et al. 2014b, Rushworth, Hadland et al. 2002, Jacobs, Lou et al. 2009).

The supplementary and pre-supplementary motor areas are implicated in a variety of functions of movement and cognition. Leading up to a movement, a *Bereitschaftspotential* (part of pre-planning for a movement) is seen in the supplementary motor cortex (Deecke, Kornhuber 1978). The *Bereitschaftspotential* is greater during self-initiated movements compared to externally triggered movements in healthy individuals, (Jahanshahi, Jenkins et al. 1995) particularly in the pre-supplementary motor area (Nachev, Rees et al. 2005, Nachev, Wydell et al. 2007, Jenkins, Jahanshahi et al. 2000, Deiber, Passingham et al. 1991, Deiber, Honda et al. 1999) This is decreased in Parkinson disease (Jahanshahi, Jenkins et al. 1995). These areas are also involved in aspects of multiple sequential movements, specifically in ordering individual movements with correct timing (Tanji 2001). During tests of cognitive function during imaging studies, the pre-supplementary motor area is implicated in response selection and inhibition as well as in set-shifting (Rushworth, Hadland et al. 2002, Wager, Jonides et al. 2004, Simmonds, Pekar et al. 2008) and response is altered in Parkinson disease (Cools, Barker et al. 2003, Obeso, Wilkinson et al. 2011).

Iron

Iron plays a critical role in multiple brain metabolic processes (Todorich, Pasquini et al. 2009) including DNA synthesis, mitochondrial adenosine triphosphate (ATP) generation, myelination (Todorich, Pasquini et al. 2009), and neurotransmission (Zecca, Youdim et al. 2004). Particularly relevant to Parkinson

disease, iron is a co-factor for the enzyme tyrosine hydroxylase, a crucial factor in the pathway for dopamine synthesis. Iron is present in olygodendrocytes, neurons and microglia, in certain substructures of the basal ganglia (with a significant amount sequestered in the neuromelanin granules in dopaminergic granules of the SNc, (Zecca, Tampellini et al. 2001)) and in the noradrenergic neurons of the locus coeruleus (Zecca, Shima et al. 1996). Normal aging is associated with the accumulation of non-heme iron, contained in ferritin, in the globus pallidus, red nucleus, substantia nigra and putamen (Drayer, Burger et al. 1986, Hallgren 1958, Haacke, Cheng et al. 2005, Aquino, Bizzi et al. 2009). In Parkinson disease, compared to healthy controls, significant iron accumulation has been identified in the substantia nigra (Graham, Paley et al. 2000, Kosta, Argyropoulou et al. 2006, Martin, Wieler et al. 2008a, Vymazal, Righini et al. 1999) and caudate (Haacke, Cheng et al. 2005, Wallis, Paley et al. 2008, Antonini, Leenders et al. 1993). Evidence for iron accumulation in the putamen (Haacke, Cheng et al. 2005, Graham, Paley et al. 2000, Wallis, Paley et al. 2008, Dexter, Carayon et al. 1991) and basal ganglia (Dexter, Carayon et al. 1991, Griffiths, Dobson et al. 1999, Zhang, Zhang et al. 2010) is equivocal in Parkinson disease with reports showing an increase in iron load, a decrease in iron load or no change. When iron is stored in compounds such as ferritin, it does not appear to have detrimental effects. The release of free iron in the brain, however, may result from the breakdown of heme by heme-oxygenease-1 which can have toxic consequences through free radical mediated mechanisms (Schipper 2004).

The precise role of iron in neurodegeneration remains unclear, but the increase of iron found in the striatum in Parkinson disease suggests that iron-

related oxidative stress may be an important risk factor for nigrostriatal neuronal degeneration in Parkinson disease (Sofic, Riederer et al. 1988, Riederer, Sofic et al. 1989, Dexter, Sian et al. 1994, Loeffler, Connor et al. 1995). The presence of increased iron content in the substantia nigra in Parkinson disease remains unclear - does it have a role in the pathogenesis of Parkinson disease or is it simply an epiphenomenon? There is, however, post-mortem evidence suggesting increased iron content in the substantia nigra in Parkinson disease (Sofic, Riederer et al. 1988, Dexter, Wells et al. 1989), with the changes most marked in severe disease (Youdim, Ben-Shachar et al. 1990). This association of increased measures of regional iron content in the SNc with more severe disease may provide an indication of the pathological severity of the disease.

Imaging and Parkinson Disease

Brain imaging allows for an objective, non-invasive assessment of the structure and function of cortical and subcortical areas, providing the opportunity to gain insights into underlying pathological processes. A non-invasive tool with the potential to monitor and provide an objective marker of disease progression from a very early stage could have profound impact on the management of Parkinson disease and provide an important tool in the evaluation of the efficacy of new therapeutic approaches.

Magnetic Resonance Methods

Conventional structural brain magnetic resonance imaging (MRI) is sensitive to biophysical properties of local brain tissues and allows for evaluation of brain structure. In Parkinson disease, the loss of dopamine-producing cells in the

substantia nigra projecting to the striatum is a major source of disability. Despite the loss of up to 80% of striatal dopamine (Bohnen, Albin et al. 2006) and 50% of nigral neurons (Greffard, Verny et al. 2006) prior to the emergence the typical motor features of Parkinson disease, conventional structural MRI is not sensitive to the anatomical and functional changes seen in the disease. Even in disease of longer duration, structural brain MRI may report normal findings (Schrag, Good et al. 2000). The continuing evolution of new techniques for imaging and analysis, together with higher field strength systems, has produced significant advances in our understanding of the changes in brain structure and function associated with Parkinson disease. These magnetic resonance-based approaches, including non-conventional structural imaging and analysis (susceptibility-based imaging, voxel-based morphometry (VBM), diffusion tensor imaging (DTI), cortical thickness mapping), magnetic resonance spectroscopy (MRS), functional MRI (fMRI), and resting state fMRI, have improved the quantification of neuronal damage and altered function seen in Parkinson disease. Together with higher field strength MRI that offers high spatial resolution and a high degree of anatomical definition, these approaches offer the possibility of measuring brain status and altered activity with greater accuracy and precision, improving our understanding of the pathological neural substrates correlated with the motor, cognitive and behaviours changes and progression seen in Parkinson disease.

Susceptibility-Based Imaging

Increased nigral iron is evident *ex vivo* in Parkinson disease (Sofic, Riederer et al. 1988, Dexter, Wells et al. 1989, Earle 1968) and it is possible to evaluate brain non-heme iron deposition using MRI. Conventional T_2-weighted images (T_2 -

transverse relaxation) show reduced signal in nigra, RN, and pallidum (Drayer, Burger et al. 1986), presumably related to a local increase in iron. The increase may be related to disease severity as there is little difference between controls and mildly affected patients, but a marked increase in severe Parkinson disease (Youdim, Ben-Shachar et al. 1990). T_2 quantitation, however, has produced contradictory results in Parkinson disease, (Antonini, Leenders et al. 1993, Ryvlin, Broussolle et al. 1995) leading to the exploration of other methods to assess brain structure.

Ferritin shortens T_2 more in high magnetic fields than in low fields. This field-dependent change has been used to show an age-related increase in striatal iron (Bartzokis, Mintz et al. 1994). This unwieldy method, however, requires independent studies in the same patients at different field strengths. Other methods to quantify T_2* changes relate to local field inhomogeneities induced by changes in brain iron. (T_2*, and its reciprocal R_2*, = *apparent* transverse relaxation in the presence of field inhomogeneity.) A T_2*-based technique was used to show increased nigral iron in Parkinson disease (Ordidge, Gorell et al. 1994), correlating with motor performance (Gorell, Ordidge et al. 1995). Use of a multiple echo sequence yielding both T_2* and T_2 data showed increased nigral iron in Parkinson disease (Graham, Paley et al. 2000). A method using a high field (3 tesla) gradient echo sequence can capture the influence of magnetic field inhomogeneity produced by the presence of paramagnetic ions in tissue to more accurately reflect midbrain iron content (Ye, Allen et al. 1996). This approach showed correlation between age and striatal iron (Martin, Ye et al. 1998) and an

increase in pallidal and putamenal iron correlating with symptom severity in Parkinson disease (Ye. Allen, et al. 1998).

Voxel-Based Morphometry

Voxel-based morphometry is a complementary method for assessing structural change from high-resolution T_1-weighted images. It provides an automated, objective, observer-independent method to identify relatively subtle differences in regional gray or white matter density amongst specific patient populations by comparing tissue volumes or concentration differences on a voxel-by-voxel basis. Measurements of structural volume have produced inconsistent results in Parkinson disease. There are some reports of decreases in the volumes of putamen, pallidum, and prefrontal cortex in Parkinson disease (O'Neill, Schuff et al. 2002, Lisanby, McDonald et al. 1993). Others show conflicting data where striatal, cerebellar and brainstem volumes were not different from control subjects (Schulz, Skalej et al. 1999). Reported brain volume loss in temporal (Martin, Wieler et al. 2009, Mak, Zhou et al. 2014), frontal and parietal-occipital regions are correlated with cognitive decline in non-demented Parkinson disease patients (Hu, Taylor-Robinson et al. 1999, Ashburner, Friston 2000, Lee, Sen et al. 2013, Ellfolk, Joutsa et al. 2014). A recent meta-analysis provided evidence of significant grey matter atrophy in the medial temporal lobe and basal ganglia in Parkinson disease dementia (Pan, Shi et al. 2013). At 3 tesla, changes in the substantia nigra and basal ganglia have been demonstrated with atrophy of the putamen, correlating with severity of clinical findings (Geng, Li et al. 2006).

Cortical Thickness Mapping

Cortical thickness mapping is an alternate approach to MRI analysis with the capability of showing changes in cortical thickness associated with neuronal loss. Surface-based cortical thickness analysis of T_1-weighted images (Fischl, Dale 2000) is sensitive to early grey matter changes. Focal cortical thinning has been shown in patients with early Parkinson disease, correlating with cognitive function (Jubault, Gagnon et al. 2011, Biundo, Calabrese et al. 2013, Zarei, Ibarretxe-Bilbao et al. 2013).

Diffusion Tensor Imaging

Diffusion-weighted images can be used as a surrogate marker to study the connectivity and integrity of white matter tracts. They are sensitive to the diffusion of water within an area, providing information including mean diffusivity (MD - degree or magnitude of molecular movement of water within tissue) and fractional anisotropy (FA - directionality of movement). Within the central nervous system, the direction of water molecule travel is greatest along white matter tracts. Reduction in measured FA equates to disruption of tracts (Beaulieu 2002). Tractography allows the topography of axonal projections in the brain to be derived from diffusion data (Mori, Zhang 2006). Decreased FA has been demonstrated in regions thought to correspond to ascending nigrostriatal tracts in early Parkinson disease (Yoshikawa, Nakata et al. 2004), in the substantia nigra in *de novo* Parkinson disease (Vaillancourt, Spraker et al. 2009), nigral thalamic path (Menke, Scholz et al. 2009) and other areas (Planetta, McFarland et al. 2014) reflecting, perhaps, changes in white matter integrity or functional connectivity. Probabilistic tractography considers the uncertainty associated with estimates of fibre direction

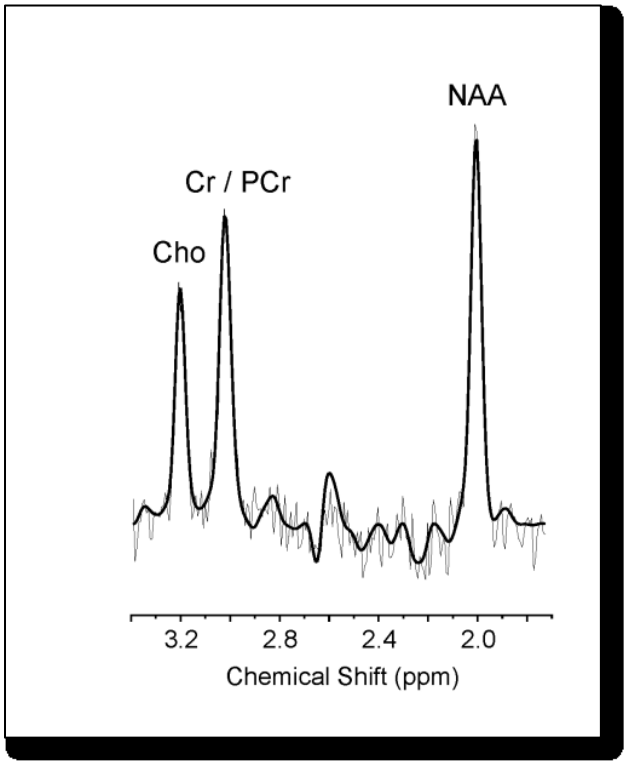
and is useful for demonstrating pathways in and near grey matter (Behrens, Johansen-Berg et al. 2003). DTI supports the expected lack of involvement of middle and superior cerebellar peduncles in Parkinson disease (Blain, Barker et al. 2006) but may show nigrostriatal tract abnormalities (Yoshikawa, Nakata et al. 2004). Diffusion tractography has been used to trace pedunculopontine nucleus connections in normal subjects (Muthusamy, Aravamuthan et al. 2007, Aravamuthan, Muthusamy et al. 2007) and abnormal pedunculopontine nucleus connectivity has been reported in patients with Parkinson disease and "on" freezing (Fling, Cohen et al. 2013).

Magnetic Resonance Spectroscopy

Magnetic resonance spectroscopy allows for non-invasive analysis of the neurochemical milieu of the living brain. MRS is based on the behaviour of specific nuclei within a magnetic field and the general principle that the resonant frequency of a nucleus depends on its immediate chemical environment. The majority of clinical studies deal with the behaviour of native nuclei such as hydrogen. With MRS, the major hydrogen-containing compounds can be quantified non-invasively. (See Figure 1.2) These compounds include N-acetylaspartate (NAA), a putative neuronal marker; creatine (Cr), a molecule found in all types of neuronal tissue and often used as a concentration reference; choline (Cho), associated with neuronal membrane breakdown; lactate, a marker of anaerobic metabolism; and myo-inositol, a putative glial marker. In neurodegenerative disease, including Parkinson disease, most MRS studies have utilized a single voxel technique. In this method, spectroscopic data are acquired from a single predefined volume of interest,

typically containing a combination of grey matter, white matter, and cerebrospinal fluid.

Figure 1.2 Representative ^{1}H-MRS spectra from healthy control



Spectra from a 2 x 2 x 2 cm^3 voxel located on the midline regions of the pre-supplementary motor area. The data shown highlight the metabolic peaks from *N*-acetylaspartate (NAA), Creatine plus phosphocreatine (Cr/PCr), and choline-containing compounds (Cho) and illustrate both the experimental data (black) and the LCModel fit (gray).

Loss of dopaminergic neurons in the substantia nigra is a major pathologic feature underlying idiopathic Parkinson disease. A decrease in NAA, as a marker of functional neuronal integrity, would be expected in this structure in Parkinson disease. This, however, is a challenging structure to study with MRS. Not only is it a relatively small structure, but it has been shown to have high iron content. As

such, abnormalities in the substantia nigra in Parkinson disease have not been reproducibly demonstrated with MRS (Choe, Park et al. 1998, Holshouser, Komu et al. 1995).

There is increasing recognition that Parkinson disease is a multisystem disorder with neuronal dysfunction extending beyond the dopaminergic pathways and basal ganglia. Consistent with this, a reduced NAA/Cr ratio has been reported in motor cortex (Lucetti, Del Dotto et al. 2001), temporoparietal cortex (Hu, Taylor-Robinson et al. 1999, Taylor-Robinson, Turjanski et al. 1999), putamen (Abe, Terakawa et al. 2000) and posterior cingulate cortex (Camicioli, Korzan et al. 2004). At high field strength (3 tesla), non-demented Parkinson disease patients, had normal NAA/Cr ratio in the posterior cingulate and those with dementia had a significant reductions in NAA/Cr ratio (Griffith, den Hollander et al. 2008). In order to more fully understand the role of NAA/Cr ratio as a marker for cognitive changes in Parkinson disease, further studies are necessary in larger patient groups using carefully standardized MR and clinical methodology.

There are a number of important factors that may contribute to the variability of results that have been reported in these MRS studies. One major issue is the methodology used to quantify metabolite concentrations (Tofts, Wray 1988). The standard approach used by many investigators has been to express the data as a ratio between the metabolite of interest and an endogenous metabolite (such as Cr) that is presumed to remain constant. The accuracy of these ratio-based methods is therefore directly related to the assumption that the chosen endogenous standard is indeed constant in a given pathological situation. Most studies have reported only a small number of patients, often with fewer than ten

subjects in each group. The patient groups themselves have varied somewhat from study to study, with some patients having early, untreated Parkinson disease, and others having more advanced disease requiring treatment. Variability in the MR technique itself, for example in the choice of echo time, may lead to heterogeneous results. Lastly, the high iron content in the basal ganglia has a significant impact on the ability to obtain reproducible high resolution spectra from this tissue volume and may impact on the accuracy of quantitative results extracted from the spectra from these regions.

Functional Magnetic Resonance Imaging

Functional MRI provides an indirect measure of brain activity, relying on the oxygen-dependent magnetic state of hemoglobin to provide a signal. During a motor or cognitive task, there are changes in local oxygen consumption, blood flow and blood volume related to neuronal metabolic demands. This results in a decrease in the concentration of deoxyhemoglobin and the resultant localized magnetic field change is seen as a blood oxygenation level dependent (BOLD) signal, detectable on T_2* weighted sequences. The BOLD signal is used to assess functional connectivity and activation patterns by comparing the task-related signal to baseline activity. With high spatial and temporal resolution (Sabatini, Boulanouar et al. 2000), this technique can provide a window into the mechanisms underlying a range of motor and non-motor impairment in Parkinson disease. Abnormal basal ganglia activations have been reported in a variety of paradigms including motor (simple hand movements (Rowe, Friston et al. 2002, Cerasa, Hagberg et al. 2006, Wu, Wang et al. 2010)) and cognitive tasks (working memory and planning (Rottschy, Kleiman et al. 2013, Ekman, Eriksson et al. 2012), set

shifting and feedback (Monchi, Petrides et al. 2004, Monchi, Petrides et al. 2007), and temporal processing (Jahanshahi, Jones et al. 2010, Harrington, Castillo et al. 2011)). Abnormal activation in Parkinson disease compared to controls with motor tasks has been identified in supplementary motor area, prefrontal cortex, putamen (Prodoehl, Spraker et al. 2010, Disbrow, Sigvardt et al. 2013); and with imagined gait in supplementary motor area, pre-supplementary motor area, somatosensory cortex, pons, pedunculopontine nucleus and cerebellar locomotor region (Peterson, Pickett et al. 2014b, Iseki, Hanakawa et al. 2008, Jahn, Deutschlander et al. 2004, Jahn, Deutschlander et al. 2008a). Additionally, differences in Parkinson disease freezing of gait (FOG) compared with no FOG have been reported in cortical and subcortical regions (Tattersall, Stratton et al. 2014, Peterson, Pickett et al. 2014a, Shine, Matar et al. 2013c, Snijders, Leunissen et al. 2011a). These findings, among others, illustrate the dense and complex interconnections found between near and remote brain regions associated with changes in movement, cognition and behaviours in Parkinson disease. Interpretation of these changes, however, may be confounded by the fact that patients with Parkinson disease have difficulty performing motor tasks.

Resting State Functional Magnetic Resonance Imaging

Resting state fMRI is a powerful method for evaluating functional interactions between brain regions in a subject at rest, i.e. not performing an explicit task (Fox, Raichle 2007), thus providing an alternate way to get an index of activity without asking participants to do potentially difficult motor tasks. The technique allows identification of functional interactions between remote brain areas through correlated low frequency spontaneous fluctuations using MRI data acquisition

sensitive to the BOLD signal. A number of characteristic patterns of functional connectivity "resting state networks"" in healthy controls have been identified including the somatosensory, visual, auditory, executive-attention networks (Rosazza, Minati 2011). Altered neural activity in these networks has been demonstrated in Parkinson disease, particularly in those that include basal ganglia-thalamic-cortical loops, correlating with disease severity and partially normalized in the short term by levodopa administration (Wu, Wang et al. 2012). The striatal-midbrain connectivity changes correspond to the well-documented gradient of striatal dopaminergic function loss in Parkinson disease.

Different studies in Parkinson disease show a marked reduction in functional connectivity between a variety of regions including, for example, the striatum and the thalamus, midbrain, pons and cerebellum (Hacker, Perlmutter et al. 2012) and between putamen and supplementary motor area (Yu, Liu et al. 2013). Altered activity has also been seen in networks associated with apathy, depression (Skidmore, Yang et al. 2013) and, associated with the development of FOG, disruption of executive-attention and visual networks (Tessitore, Amboni et al. 2012b). It is not known if altered patterns of activation are a direct result of Parkinson-specific changes in function or are due to compensatory mechanisms. A recent study, however, in drug-naive Parkinson disease patients, demonstrated widespread decreased functional integration across neural networks involving striatum, mesolimbic cortex, and sensorimotor region (Luo, Song et al. 2014) and reduced connectivity with the cerebellum and particularly brainstem (Hacker, Perlmutter et al. 2012). This baseline neural activity in multiple circuits is important in the integration of information from internal and external sources,

forming functional networks. The relationship of these resting state networks to structural and functional connectivity remains to be fully explored, but alteration from patterns seen in healthy controls gives important clues to pathophysiology when differences in resting state networks are correlated to clinical measures of Parkinson disease.

Positron Emission Computed Tomography

Functional imaging of the nigrostriatal dopamine system with positron emission computed tomography (PET) provides valuable *in vivo* information about the function of dopamine neurons. Specific radioligands can measure different components of this system: DOPA decarboxylase (a measurement of dopamine synthesis), dopamine-transporter (DAT - a measure of density of functioning dopaminergic terminals), vesicular monoamine transporter (VMAT-2 – an index of the integrity of presynaptic neuronal function), and post-synaptic D2 receptors. The integrity of the pre- and/or post-synaptic components of the dopaminergic system is reflected as changes in radiotracer receptor binding.(Patel, Lee et al. 2008)

In Parkinson disease, markers of DAT, VMAT2 and DOPA decarboxylase consistently show more involvement of the posterior putamen, with relative preservation of the caudate and declining uptake with disease progression (Stoessl 2011). Despite a report suggesting bradykinesia correlates best with makers of nigrostriatal dopaminergic deficits (Vingerhoets, Schulzer et al. 1997), as Parkinson disease progresses, to date, no significant correlation with changes in clinical measures of motor function and changes in imaging markers has been identified (Stoessl 2011). The promise of evolving dopaminergic dysfunction, as measured by

these techniques, as a biomarker of disease progression has not been borne out. Potential explanations include methodological issues, the effect of medication (despite over-night withdrawal of all dopaminergic therapy) and/or the contribution of non-dopaminergic mechanisms to continued impairment of function (Stoessl 2011).

While the techniques outlined provide valuable information regarding the brain in Parkinson disease, they have not undergone sufficiently rigorous evaluation to reach the level of routine implementation in the clinical management of Parkinson disease.

Gait

Normal Control of Locomotion

Human gait involves complex interactions of central processing and peripheral sensorimotor inputs: visual, vestibular and proprioceptive. It requires the ability to assume an upright posture and support the body against gravity; the ability to generate, initiate and maintain a basic locomotor pattern: and the ability to adapt its pattern to meet the demands of both the environment and the individual's goals. Locomotion can be considered as a well-learned, highly automatic movement program where the relative timing of muscle activation is fairly fixed, while the speed, force production and muscle selection is variable. Increasing evidence from imaging studies confirm that complex neural networks are involved in the control of locomotion, showing activation in frontal motor regions, prefrontal cortices, subcortical areas and projections to the spinal cord (Hanakawa, Katsumi et al. 1999, Jahn, Deutschlander et al. 2008a, Jahn, Zwergal 2010).

Conditions that affect one or more of the systems involved in locomotor control may lead to impairments of mobility with significant functional consequences.

Neural Substrates Underlying Gait Control

Complexly organized spinal interneurons known as "central pattern generators" contain the fundamental mechanisms to generate rhythmic, alternating limb movements that make up the basic locomotor pattern, but are, on their own, insufficient to control bipedal gait (Calancie, Needham-Shropshire et al. 1994, Dietz, Colombo et al. 1994). The basal ganglia and brainstem structures, including the pedunculopontine nucleus and cerebellar locomotor region, influence equilibrium, balance and gait by generating postural responses and modulating inputs from higher cortical areas. These higher cortical areas include the visual cortex, prefrontal cortices and frontal motor regions (primary motor area, supplementary motor area, pre-supplementary motor area) (Iansek, Bradshaw et al. 1995, Fukuyama, Ouchi et al. 1997, Miyai, Tanabe et al. 2001, Jahn, Deutschlander et al. 2008b).

The supplementary motor area, in particular, is involved in movement initiation, while the pre-supplementary motor area is involved in the selection and preparation of specific movements. The basal ganglia are involved in a variety of activities including preparation, initiation and execution of motor programs, posture and perception and cognition. The basal ganglia influence the ability to initiate and modulate the gait pattern to meet the appropriate demands of a changing environment (Iansek, Bradshaw et al. 1995, Cunnington, Iansek et al. 1999, Visser, Bloem 2005). The midbrain locomotor region (MLR), and its major constituent nuclei, the pedunculopontine nucleus, appear to play a significant role in the control

of gait. The pedunculopontine nucleus, with both cholinergic and non-cholinergic neurons, is densely interconnected with many structures, including the basal ganglia, cerebellum and cortical regions (Martinez-Gonzalez, Bolam et al. 2011, Nandi, Aziz et al. 2002, Lee, Rinne et al. 2000). It, too, is thought to be important in initiation, modulation and execution of gait (Karachi, Grabli et al. 2010). The precise role of the pedunculopontine nucleus in human locomotion is only now beginning to be elucidated (Lee, Rinne et al. 2000, Mazzone, Lozano et al. 2005, Nandi, Stein et al. 2002, Pahapill, Lozano 2000).

Parkinson Disease and Gait

Gait disturbance in Parkinson disease is a common and major source of functional disability, and is related to a decrease in reported quality of life (Muslimovic, Post et al. 2008, Perez-Lloret, Negre-Pages et al. 2014). Although gait dysfunction is more common in advanced stages of the disease, 3.5% - 18% of individuals diagnosed with Parkinson disease have a gait complaint as the presenting feature (Hoehn, Yahr 1967). The symptoms of bradykinesia, akinesia, rigidity and deficits of postural control all contribute to decreased gait performance. The degree of mobility impairment can fluctuate in response to medication and extremes of gait disturbance can be observed in the same individual depending on whether they are in the "off" state (when symptomatic effects of antiparkinsonian medications are absent or ineffective) or "on" (state when the severity of motor symptoms are ameliorated). Gait difficulties in Parkinson disease increase with disease progression as medication responses become less effective and less predictable. In early Parkinson disease, gait impairment is largely dopamine-responsive, characterized by features such as decreased stride length and velocity,

leading to a shuffling gait. With disease progression, however, dopamine non-responsive features, such as impaired gait initiation and postural control emerge, further impairing mobility. There is evidence that gait changes occur even in *de novo* patients (Wieler, Cooke et al. 2008, Baltadjieva, Giladi et al. 2006a, Ebersbach, Heijmenberg et al. 1999).

Spatio-Temporal Changes in Parkinson Disease Gait

Changes in Parkinson disease gait reflect a general reduction in the scaling and speed of movements (hypokinesia). The inability to control momentum may lead to uncontrolled movement (festination) and falls, while the inability to generate momentum results in disrupted gait with impaired initiation of movement, evidenced in the extreme by FOG. When these gait difficulties are coupled with impairment of postural control, falling becomes a real threat to safe mobility (Bloem, Hausdorff et al. 2004, Grimbergen, Munneke et al. 2004, Ashburn, Stack et al. 2008, Bohnen, Cham 2006).

Specific changes observed in Parkinson disease gait include reduced or absent arm swing and trunk rotation, decreased velocity (Iansek, Bradshaw et al. 1995), reduced stride length (Morris, Iansek et al. 1996), increased step frequency (Iansek, Huxham et al. 2006), decreased left-right bilateral limb coordination, increased left-right gait asymmetry and a tendency to turn *en bloc* (Plotnik, Giladi et al. 2005, Plotnik, Giladi et al. 2005, Plotnik, Hausdorff 2008, Plotnik, Giladi et al. 2009). As well, there is an inability to consistently produce a steady gait rhythm with the resultant increase in stride-to-stride variability. This may be seen even in those with early Parkinson disease who are naïve to dopaminergic therapy (Baltadjieva, Giladi et al. 2006a, Frenkel-Toledo, Giladi et al. 2005, Hausdorff,

Cudkowicz et al. 1998, Hausdorff 2009, Blin, Ferrandez et al. 1990). A measure of increased variability of gait is related to postural instability and fall risk in Parkinson disease and is higher in fallers versus non-fallers (Hausdorff 2009). These gait abnormalities are exacerbated when individuals with Parkinson disease are challenged with complex gait tasks, including turning and walking backwards (Hackney, Earhart 2009a, Spildooren, Vercruysse et al. 2010, Schaafsma, Giladi et al. 2003), when greater performance demands are placed on an impaired system.

Basal Ganglia and Parkinson Disease Gait

The basal ganglia have numerous reciprocal connections, including those between basal ganglia and the midbrain pedunculopontine nucleus, between basal ganglia and the rostral striatum and pre-supplementary motor area and between basal ganglia and the posterior striatum and supplementary motor area. Basal ganglia dysfunction in Parkinson disease disrupts initiation and execution of internally-generated, skilled motor sequences including speech, posture, gait and upper limb manual dexterity/manipulation (Nieuwboer, Vercruysse et al. 2009a). The basal ganglia closely interact with the supplementary motor area in the execution of well-learned motor programs such as locomotion (Iansek, Bradshaw et al. 1995). Brain imaging in cases of severe impairment of initiating gait and making turns (gait ignition failure pointed to lesions in the supplementary motor area (Nadeau 2007, Della Sala, Spinnler et al. 2004)). Basal ganglia dysfunction may disrupt motor set for whole movement sequences or disturb the internal cue for sub-movement preparation resulting in an under-scaling of successive steps (Iansek, Huxham et al. 2006, Bartels, Leenders 2008, Morris, Iansek et al. 2008). Evidence that stride length and velocity are influenced by external cues suggests

that the interrupted internal cuing mechanism of the basal ganglia and the supplementary motor area that normally controls the performance of motor sequences, can be by-passed (Morris, Iansek et al. 1996). Employing cognitive, visual, auditory or motor tricks or cues may allow the individual to abandon the automatic gait mode and switch to a more cortically mediated gait program (Morris, Iansek et al. 1996, Willems, Nieuwboer et al. 2006, Nieuwboer, Kwakkel et al. 2007, Morris, Iansek et al. 1994a). Unfortunately, in Parkinson disease where attentional resources are often limited, the use of attentional or cognitive strategies or any dual task may cause deterioration in the gait pattern (Spildooren, Vercruysse et al. 2010, Camicioli, Oken et al. 1998, Hausdorff, Balash et al. 2003).

Pre-supplementary Motor Area and Parkinson Disease Gait

Functional imaging studies in Parkinson disease show that the pre-supplementary motor area is underactive (Sabatini, Boulanouar et al. 2000, Fukuda, Mentis et al. 2001). While this under-activity may be secondary to decreased excitatory drive from the thalamus, which in turn results from decreased dopaminergic input to the putamen (Cunnington, Egan et al. 2001, Braak, Del Tredici et al. 2003a), direct cell counts show a loss of pyramidal interneurons from pre-supplementary motor area in Parkinson disease (MacDonald, Halliday 2002). Changes in pre-supplementary motor area with MR spectroscopy, consistent with this neuronal loss, have been reported (Camicioli, Hanstock et al. 2007). Suggestive of an attempt to compensate for impaired supplementary motor area function, the premotor cortex has been shown to have enhanced activation in Parkinson disease compared to healthy controls (Hanakawa, Fukuyama et al. 1999). These data support a role for selective loss of pre-supplementary motor

area neurons in generating the motor features of Parkinson disease, and may be relevant to the clinical observation that not all motor features are dopamine-responsive.

Pedunculopontine Nucleus and Parkinson Disease Gait

Increased inhibitory flow from basal ganglia to pedunculopontine nucleus (Karachi, Grabli et al. 2010) and/or neuronal loss within pedunculopontine nucleus may affect posture and gait in Parkinson disease (Snijders, Leunissen et al. 2011a, Karachi, Grabli et al. 2010, Pahapill, Lozano 2000, Schweder, Hansen et al. 2010). Human data suggest a relationship between Parkinson disease severity and loss of cholinergic pedunculopontine nucleus neurons (Karachi, Grabli et al. 2010, Zweig, Jankel et al. 1989, Bohnen, Frey et al. 2014, Bohnen, Albin 2009), indicating, with disease progression, the increasing involvement of non-dopaminergic systems. Reports that deep brain stimulating electrodes in human MLR/pedunculopontine nucleus improve some dopamine resistant axial and gait symptoms in Parkinson disease support a role for this midbrain region in human locomotion (Mazzone, Lozano et al. 2005).

Imaging and Imagined Walking

Given logistical limitations, imaging techniques used to observe disrupted brain activity during Parkinson disease gait rely primarily on motor imagery. There is evidence of overlap between actual motor performance and imagined activity (Deiber, Ibanez et al. 1998, Porro, Francescato et al. 1996, Jeannerod, Decety 1995), giving confidence that changes seen in Parkinson disease gait using these methods may be representative of underlying neural network changes. Studies

using imagined or actual movement in individuals with Parkinson disease have shown altered activation, when compared to healthy controls, in supplementary motor area/pre-motor areas (Hanakawa, Katsumi et al. 1999, Peterson, Pickett et al. 2014b, Snijders, Leunissen et al. 2011b, Wai, Wang et al. 2012a), MLR (Snijders, Leunissen et al. 2011a, Karachi, Grabli et al. 2010, Cremers, D'Ostilio et al. 2012), basal ganglia (Peterson, Pickett et al. 2014b, Prodoehl, Spraker et al. 2010, Bruck, Aalto et al. 2006, Spraker, Prodoehl et al. 2010, Kish, Shannak et al. 1988), and cerebellum (Hanakawa, Katsumi et al. 1999, Schweder, Hansen et al. 2010, Cremers, D'Ostilio et al. 2012).

Supraspinal control of gait is flexible, allowing initiation of gait, stopping, turning and obstacle avoidance. As previously noted, changes seen in Parkinson disease gait can be thought of in two general categories (Hausdorff 2009). Continuous changes describe alterations in the gait pattern that are fairly consistent from one step to the next, including slowed velocity, decreased/absent arm swing, decreased stride length, increased cadence, increased double limb support time, impaired control of posture (Blin, Ferrandez et al. 1990, Schaafsma, Balash et al. 2003, Springer, Giladi et al. 2006, Hausdorff, Rios et al. 2001, Morris, Huxham et al. 2001, Morris, Iansek et al. 1994b). Episodic changes, on the other hand, describe alterations in the gait pattern occurring intermittently and apparently at random, include festination, freezing of gait, and start hesitation (Bloem, Hausdorff et al. 2004, Giladi, Balash 2001, Gray, Hildebrand 2000, Bloem, Grimbergen et al. 2001).

Dopaminergic therapy can improve some gait deficits, such as velocity, stride length and movement amplitude (Nieuwboer, Dom et al. 2001, O'Sullivan, Said et

al. 1998, Shan, Lee et al. 2001, Ferrandez, Blin 1991), but is less effective in normalizing swing and stance duration, cadence, gait variability and kinetic changes (Ebersbach, Heijmenberg et al. 1999, O'Sullivan, Said et al. 1998, Blin, Ferrandez et al. 1991).

Although the motor symptoms of Parkinson disease, including some features of gait, are responsive to dopamine replacement, features of on-state FOG and postural instability respond poorly. The pathophysiology of these disturbances remains yet to be clarified, but their later onset and resistance to levodopa has led to the suggestion that they may result from pathology in non-dopaminergic structures involved in locomotion (Pahapill, Lozano 2000, Giladi, McDermott et al. 2001, Bartels, Balash et al. 2003).

Freezing of Gait in Parkinson Disease

Freezing of gait has been defined as a "brief, episodic absence or marked reduction of forward progression of the feet despite having the intention to walk" (Nutt, Bloem et al. 2011), where individuals state that their feet feel "glued to the floor". Once the episodes, usually lasting 10 seconds or less (though occasionally lasting up to 30 seconds), are finished, a comparatively regular gait pattern is resumed (Schaafsma, Balash et al. 2003, Giladi, Hausdorff 2006a). Freezing of gait in Parkinson disease is a distressing, and often debilitating phenomenon and its unpredictability impacts negatively on social interaction and the ability to perform daily activities (Perez-Lloret, Negre-Pages et al. 2014, Moore, Peretz et al. 2007, Tan, McGinley et al. 2011). When combined with impairment of postural reflexes or impaired cognition, FOG may lead to falls and their consequences, including hip fracture, subdural haematoma, other injuries, fear of falling, and loss of mobility

and independence (Gray, Hildebrand 2000, Bloem, Grimbergen et al. 2001, Adkin, Frank et al. 2003).

This phenomenon is not unique to Parkinson disease and is seen in progressive supranuclear palsy, multiple system atrophy, Lewy body dementia, primary progressive freezing of gait as well as with focal lesions in the basal ganglia, supplementary motor area, subcortical structures, frontal lobes or brainstem (Nutt, Bloem et al. 2011). Freezing of gait is mainly thought of as a feature of longer disease duration with approximately 80% of individuals with more advanced Parkinson disease reporting this troubling feature (Hely, Reid et al. 2008), primarily in the "off" medication state. There is, however, evidence of FOG in *de novo* Parkinson disease (Bloem, Hausdorff et al. 2004, Giladi, McDermott et al. 2001, Garcia-Ruiz 2011), and in approximately 25% of patients in the early stages of Parkinson disease (Giladi, McDermott et al. 2001, Tan, McGinley et al. 2011). The relationship between FOG and dopamine depletion is complex and non-linear (Giladi 2008). Freezing episodes can occur when the patient is "off", i.e. when the clinical benefit from a single intake of levodopa has waned, in which case these episodes may be completely relieved by the next dose of levodopa. In contrast, particularly with more advanced disease, freezing may occur when the patient is "on" and FOG can be induced or exacerbated by levodopa (Espay, Fasano et al. 2012). Freezing of gait may be provoked in a variety of environmental situations, including confined spaces (doorways, narrow halls), turns, initiating gait, and approaching an object (Spildooren, Vercruysse et al. 2010, Spildooren, Vercruysse et al. 2010, Schaafsma, Balash et al. 2003, Nutt, Bloem et al. 2011, Cowie, Limousin et al. 2012, Almeida, Lebold 2010, Snijders, Haaxma et al. 2012,

Cohen, Chao et al. 2011) when attentional demands are increased and the gait pattern needs to be adapted to a changing environment. (Of note, many of these provoking events are the same situations related to supraspinal control of locomotion (Jacobs, Horak 2007a).) Freezing is seen less often when walking in open, uncluttered spaces, tending to be triggered by external circumstances (Schaafsma, Balash et al. 2003, Nutt, Bloem et al. 2011, Nieuwboer, Giladi 2013). Dual-tasking or divided attention also increases the frequency and/or severity of FOG episodes (Snijders, Leunissen et al. 2011a, Bloem, Hausdorff et al. 2004, Spildooren, Vercruysse et al. 2010, Camicioli, Oken et al. 1998, Giladi, Hausdorff 2006b). There is evidence that cueing strategies (visual or auditory) and specific attention may be helpful in overcoming some situations of freezing (Willems, Nieuwboer et al. 2006, Nieuwboer, Kwakkel et al. 2007, Snijders, Nijkrake et al. 2008). In addition to environmental and attention influences on FOG, there also appears to be an emotional or cognitive component as stress levels and heightened anxiety, fear or general arousal may provoke freezing episodes (Fahn 1995, Lieberman 2006, Rahman, Griffin et al. 2011, Moreau C. Defebvre L. Bleuse S. Blatt JL. Duhamel A. Bloem BR. Destée A. Krystkowiak P 2008). Paradoxically, many people who have significant problems with FOG are able to climb stairs or cycle with relative ease compared to walking (Snijders, van Kesteren et al. 2012).

Spatio-temporal Changes in Parkinson Disease and Freezing of Gait

When Parkinson disease gait is compared between "freezers" and "non-freezers", those who freeze have more trouble generating and maintaining appropriate and effective stride amplitude (Nieuwboer, Dom et al. 2001, Iansek, Huxham et al. 2006, Nanhoe-Mahabier, Delval et al. 2012) and have more

variability in timing of gait cycles (Hausdorff 2009, Hausdorff, Schaafsma et al. 2003a, Smulders, van Nimwegen et al. 2013) than non-freezers. There also appears to be a disturbance in inter-limb coordination (Plotnik, Giladi et al. 2005, Hausdorff, Schaafsma et al. 2003a, Plotnik, Giladi et al. 2008, Nieuwboer, Chavret et al. 2007, Nanhoe-Mahabier, Snijders et al. 2013) which suggests that control of walking is abnormal between freezing episodes, particularly in the "off" state.

The steps preceding a freezing episode are abnormal. There is a significant decrease in step length and an increase in both step frequency (perhaps even festination) and in step to step variability (Nieuwboer, Dom et al. 2001, Iansek, Huxham et al. 2006, Chee, Murphy et al. 2009, Moore, MacDougall et al. 2008). Inappropriate lower leg muscle (tibialis anterior and gastrocnemius) electromyography activity (Nieuwboer, Dom et al. 2004) has also been reported prior to the actual cessation of forward movement. Normal stepping is preceded by a single anticipatory postural adjustment to shift weight off the stepping leg (Jacobs, Horak 2007b). In Parkinson disease, patients with FOG have delayed step initiation that may be associated with repetitive anticipatory postural adjustments, seen in alternating leg oscillations (or "trembling in place"), as if they are unable to inhibit postural preparation and release the stepping program appropriately. This may be reflecting inappropriate and ineffective anticipatory postural adjustments (Moore, MacDougall et al. 2008, Jacobs, Nutt et al. 2009).

Documenting Freezing of Gait Episodes

The frequency and duration of FOG is highly variable, making it difficult to quantify, observe and document (Nieuwboer, De Weerdt et al. 1998, Giladi 2001a, Giladi 2001b, Nieuwboer, Giladi 2008). It is also clear that freezing episodes tend

to not occur as frequently in a clinic or lab setting when compared to a person's typical environment. This has led to the development of standardised tasks and protocols to provoke freezing episodes in a laboratory environment in order to more fully characterise them (Spildooren, Vercruysse et al. 2010, Nonnekes, Janssen et al. 2014, Snijders, Weerdesteyn et al. 2010). Questionnaires have also been developed to capture the historical frequency and severity of freezing episodes in an effort to better understand this phenomenon (Giladi, Tal et al. 2009, Nieuwboer, Rochester et al. 2009, Giladi, Shabtaia et al. 2000). Portable sensors and recording devices have also been employed in a research setting to monitor FOG over longer periods in order to better understand the frequency and duration of freezing episodes in a real-life setting (El-Gohary, Pearson et al. 2013, Mitoma, Yoneyama et al. 2010). The lack of a "gold standard" definition leads to confusion in the literature as what one author calls freezing may be quite different from another. In an attempt to bring some clarity to this, a classification system has been proposed where individuals are identified as 1) "self-reported freezer"; 2) "probable freezer" where FOG is confirmed by a third party; and 3) "definite freezer" where FOG is observed in a formal setting (Snijders, Haaxma et al. 2012).

Response of FOG to Therapy

The response of gait disturbance in Parkinson disease to pharmacologic intervention is neither consistent nor does it address all aspects of gait dysfunction. Some spatial temporal features of Parkinson disease gait appear to be responsive to levodopa, such as velocity and stride length (O'Sullivan, Said et al. 1998, Blin, Ferrandez et al. 1991, Chien, Lin et al. 2006, Cioni, Richards et al. 1997, Lubik, Fogel et al. 2006, Bastian, Kelly et al. 2003, Burleigh-Jacobs, Horak et al. 1997);

others, such as cadence, appear to be unresponsive (O'Sullivan, Said et al. 1998, Chien, Lin et al. 2006), and still others have been reported as both responsive and unresponsive, including double support, step time, stride duration (O'Sullivan, Said et al. 1998, Chien, Lin et al. 2006, Lubik, Fogel et al. 2006, Krystkowiak, Blatt et al. 2001, Krystkowiak, Blatt et al. 2003). Freezing of gait has a variable response to levodopa. If FOG occurs in the "off" state, administration of levodopa or a dopamine agonist may ameliorate the frequency and severity of freezing (Schaafsma, Balash et al. 2003, Fietzek, Zwosta et al. 2013). If, however, FOG occurs in the "on" state, it is often resistant to levodopa (Camicioli, Oken et al. 1998, Espay, Fasano et al. 2012, Linazasoro 1996).

Understanding of the underlying neural substrates of FOG in Parkinson disease continues to evolve, complicated by its heterogeneous presentation. Only a subgroup of those with Parkinson disease experience FOG and within this group, its unpredictable presentation with respect to frequency and severity and its variable response to dopaminergic therapy suggests apparent subgroups within the FOG group (Nutt, Bloem et al. 2011). The extensive connectivity implicated in locomotion between the more automatic spinal motor programs of the central pattern generators, brainstem locomotor areas (MLR and cerebellar locomotor region) and higher centres, including the basal ganglia and prefrontal cortex, allows for the initiation, maintenance and modulation of gait through dopaminergic and, likely, other systems (cholinergic, noradrenergic and serotonergic) (Herman, Giladi et al. 2013, Narabayashi, Kondo et al. 1984, Tohgi, Abe et al. 1990). This suggests that the neural substrates underlying FOG are not likely to be located in a single network or structure or as a result of deficiency in one neurotransmitter system.

Gait freezing does not appear to be correlated with tremor, bradykinesia or rigidity, cardinal features of Parkinson disease (Bartels, Balash et al. 2003, Giladi 2001b), but is related to postural instability (Giladi 2001a) and impaired cognition (Yogev-Seligmann, Hausdorff et al. 2008).

Decreased dopamine in nigrostriatal loops is implicated clinically by the response of FOG in some settings to increased doses of levodopa, along with reports of decreased FOG following deep DBS in the STN and altered dopamine and glucose metabolism in the striatum (Schaafsma, Giladi et al. 2003, Ferraye, Debu et al. 2008, Fasano, Herzog et al. 2011, Bartels, de Jong et al. 2006). Evidence following STN DBS suggests that gait parameters that are responsive to levodopa, such as stride length, gait speed, gait kinetics (Ferrarin, Rizzone et al. 2005), are also well treated with STN DBS. Features that do not respond well to dopaminergic therapy do not respond well to STN DBS (Herzog, Volkmann et al. 2003, Lezcano, Gomez-Esteban et al. 2004, Rodriguez-Oroz, Zamarbide et al. 2004). The reported impact of STN DBS on postural stability is conflicting, with some suggesting it is worsened (Krack, Batir et al. 2003, Crenna, Carpinella et al. 2006, Gan, Xie-Brustolin et al. 2007), and others that it is improved (Shivitz, Koop et al. 2006, Bejjani, Gervais et al. 2000). There is also no agreement about other features, with some data supporting improved "on" time, motor function and quality of life, but that risk of falls significantly worsened (Weaver, Follett et al. 2009). The response of FOG to STN DBS is also inconclusive. Some studies suggest an improvement with stimulation (Ferraye, Debu et al. 2008, Moreau, Defebvre et al. 2009, Niu et al. 2012), while other suggest that it does not respond (Davis, Lyons et al. 2006, Stolze, Klebe et al. 2001, Adams et al. 2011), with the caveat that

those who had "off" freezing that responded to levodopa also tend to respond to stimulation.

The loss of automaticity of locomotion points to disruption of the striato-frontal loops, particularly between the supplementary motor area and premotor area, which are considered to be intimately involved in mediating action selection and response inhibition (Coxon, Van Impe et al. 2012).

Imaging and Freezing of Gait in Parkinson Disease

In Parkinson disease, functional and structural imaging show both altered pedunculopontine nucleus function and connectivity. Healthy controls and those with Parkinson disease with no FOG show connectivity between the cerebellum and the pedunculopontine nucleus, connectivity that is altered in those with FOG (Schweder, Hansen et al. 2010). Altered connectivity and function is also seen between the pedunculopontine nucleus and higher cortical regions, including the thalamus and multiple regions of the frontal cortex, some preferentially in the right hemisphere (Fling, Cohen et al. 2013, Peterson, Pickett et al. 2014a, Snijders, Leunissen et al. 2011a, Ba, Wieler et al. 2014, Youn, Cho et al. 2012). The asymmetry seen in the connectivity of the pedunculopontine nucleus showed that the more left hemisphere-lateralized the pedunculopontine nucleus tract volume, the poorer the performance on tasks requiring the cognitive initiation of appropriate actions and/or the inhibition of inappropriate actions, specifically within patients with FOG. These results suggest that breakdowns in structural and functional connectivity of the right hemisphere's locomotor pathway involving the pedunculopontine nucleus may contribute to FOG (Fling, Cohen et al. 2013), though a more recent publication from this group presents a less clear pattern of

lateralisation in the disruption of structural and functional connectivity in individuals with FOG (Fling, Cohen et al. 2014).

Changes in grey matter suggest that FOG is multifactorial, associated with cognitive and emotional state as well as impaired gait. Loss of grey matter in the MLR/pedunculopontine nucleus is also noted to be greater in FOG suggesting degeneration of cholinergic neurons may be related to abnormal gait and posture (Karachi, Grabli et al. 2010, Pahapill, Lozano 2000, Bohnen, Albin 2009, Tykocki, Mandat et al. 2011, Devos, Defebvre et al. 2010). Grey matter atrophy in the left inferior frontal gyrus, precentral gyrus and inferior parietal gyrus is reported to be increased in freezers in one study (Kostic, Agosta et al. 2012) and in the left cuneus, precuneus, lingual gyrus and posterior cingulate cortex (Tessitore, Amboni et al. 2012a) with concomitant impaired executive function. Interestingly, deep brain stimulation in the pedunculopontine nucleus region has been reported to improve gait and reduce the number of freezing episodes (Plaha, Gill 2005, Thevathasan, Cole et al. 2012, Thevathasan, Coyne et al. 2011). One report suggested that the addition of pedunculopontine nucleus stimulation might be indicated in the context of medication and/or STN stimulation-resistant gait dysfunction, specifically FOG (Ferraye, Debu et al. 2011). Double-blind clinical trials have been less positive, showing only marginal benefit from pedunculopontine nucleus stimulation on gait disturbance and FOG (Moro, Hamani et al. 2010, Ferraye, Debu et al. 2010).

Underlying Neural Correlates of Freezing of Gait

An attempt to clarify the complexity of imaging and clinical features associated with FOG is postulated in a recent review of the current literature on the

underlying neural basis of FOG and summarized four explanatory models of this phenomenon (Nieuwboer, Giladi 2013). (See Table 1.1)

Table 1.1: Models for the episodic appearance of Freezing of Gait

Models of FOG	Principle	Prediction of FOG-Episodes
Threshold	Accumulation of motor deficits until threshold is reached and freeze occurs	▪ Increase motor cycle frequency ▪ Decrease amplitude ▪ Increase coordination complexity
Interference	Competition for common central processing resources induces breakdown	▪ Increase number of concurrent tasks ▪ Increase difficulty level tasks ▪ Increase load of external input
Cognitive	Deterioration in processing of response conflict induces block	▪ Increase incongruency level ▪ Increase response speed ▪ Increase load on executive function
Decoupling	Decoupling between motor programs and motor responses induces block	▪ Increase strength startle stimuli ▪ Increase frequency startle stimuli ▪ Increase postural load or instability

Adapted from: Nieuwboer, Giladi, 2013.

The first "threshold model" proposes that those with FOG have greater baseline gait abnormalities with respect to step scaling, gait rhythm and bilateral symmetry and coordination. This, then, predisposes them to a lower threshold of gait pattern deterioration when increasing demands (e.g. turning, dual-tasking) are placed on locomotion (Plotnik, Giladi et al. 2012).

The "interference model" suggests a transient breakdown in the simultaneous processing of cognitive and limbic information during a motor task, resulting in the FOG. A breakdown in the segregation of basal ganglia circuits (oculomotor, sensorimotor, associative and limbic loops) results in inappropriate interaction between the loops which then leads to abnormal activity in GPi with a resultant

inhibition of the pedunculopontine nucleus with FOG emerging. Focussed attention or external cues may "reset" the abnormal interference, allowing a more normal gait pattern to resume (Lewis, Barker 2009a, Shine, Matar et al. 2013b). The more demands, number and complexity of concurrent tasks, placed on the system, the more likely FOG will be provoked (Spildooren, Vercruysse et al. 2010, Cowie, Limousin et al. 2012).

The "cognitive model" suggests that individuals with FOG have deficits in their ability to appropriately select a response as well as to appropriately inhibit automatic and consciously controlled processes. This may be related to impairments in automaticity and executive function seen more prominently in FOG and those without FOG (Vandenbossche, Deroost et al. 2012a).

The final model is the "decoupling model" which points to a decoupling between pre-planned motor programs and the release of a step (gait initiation) (Jacobs, Nutt et al. 2009, Okada, Fukumoto et al. 2011). Those with FOG, in experimental settings, have exhibited inappropriate anticipatory postural adjustments followed by a failure to initiate a step (Jacobs, Horak 2007b). They also show prolonged double-limb support times of the initial step which may reflect a decoupling of balance, postural preparation and movement (Okada, Fukumoto et al. 2011).

These models each explain different features (and sometimes overlapping characteristics) seen in FOG, but a single, unifying theory of the pathophysiology remains elusive. Common to all these models is that FOG is often provoked when complex demands are placed on a compromised system of motor, cognitive and limbic control. Difficulty processing multiple, sometime conflicting, inputs may

result in problems with conflict resolution, and interference may push an unstable motor system beyond the threshold for freezing after which decoupling may prevent recommencement of a more normal gait pattern (Nieuwboer, Giladi 2013). There is emerging evidence that freezing behaviour is not limited to gait. Similar disruptions in upper limb movements point to a more complex disruption of movement control (Vercruysse, Spildooren et al. 2012, Nieuwboer, Vercruysse et al. 2009b).

Summary of Gait in Parkinson Disease

Gait disturbance in Parkinson disease can make walking a challenge. Recent advances in neuroimaging and clinical understanding of the determinants of impaired gait point to a complex interaction between structural, functional and behavioural factors in the emergence and progression of gait dysfunction. The interplay of motor, cognitive and affective contributions remains to be fully understood. Confirming associations between Parkinson disease, FOG, cognitive impairment and altered brain structure and function will continue to reveal relevant neural substrates with the goal of improving management of current mobility issues and the identification of those destined to develop FOG.

Cognition

Cognition describes a broad range of information-processing abilities whereby an individual perceives, registers, stores, retrieves and uses information. Faced with an ever-changing environment, flexible and complex control is needed to address the motor and behavioural demands of life. Cognitive impairment and dementia are common features of Parkinson disease (Hely, Reid et al. 2008,

Aarsland, Andersen et al. 2003) and correlate with a significant negative impact on quality of life (Schrag, Jahanshahi et al. 2000), higher economic burden (Spottke, Reuter et al. 2005) and hastening the need for institutionalised care (Aarsland, Larsen et al. 2000). These cognitive processes are a result of coordinated processing among distributed brain regions. Disruption to communication within and between these circuits and networks has detrimental effects on cognition and, because of close connections with motor processes and motor network, concurrent cognitive and motor dysfunction is often observed. A clearer understanding of the neural correlates underlying cognitive impairment is key to developing strategies to treat this challenging feature of Parkinson disease.

Evidence of significant cognitive decline across a wide range of cognitive function has been shown (Aarsland, Bronnick et al. 2009, Williams-Gray, Foltynie et al. 2007) and performance on neuropsychological testing shows a cognitive gradation in decline from healthy controls to individuals with Parkinson disease who are not demented to those with Parkinson disease dementia, particularly in the areas of executive function, verbal fluency, memory and working memory, visuospatial function, attention and language (Williams-Gray, Foltynie et al. 2007, Compta, Ibarretxe-Bilbao et al. 2012, Taylor, Saint-Cyr et al. 1986, Lewis, Cools et al. 2003, Dalrymple-Alford, Kalders et al. 1994). Neuropsychological tests assessing various domains of cognition commonly impaired in Parkinson disease are listed in Table 1.2. Cognitive disturbance, however, is both heterogeneous in presentation and variable in progression (Aarsland, Bronnick et al. 2009). This wide variation is likely due to a combination of varied cognitive profiles (Litvan, Aarsland et al. 2011), different pathologic changes (Kotzbauer, Cairns et al. 2012,

Halliday, Hely et al. 2008), and the contribution of genetics seen in Parkinson disease (Svenningsson, Westman et al. 2012). The emergence of cognitive dysfunction also varies greatly, with some individuals presenting with these complaints very early in the disease while others become impaired much later in the disease process (Williams-Gray, Foltynie et al. 2007, Foltynie, Brayne et al. 2004).

Table 1.2: Neuropsychological tests

Neuropsychiatric Test	Cognitive Domain	Cognitive Function
Mini-Mental State Exam		▪ global cognitive function
Frontal Assessment Battery	executive functions	▪ screening task
California Verbal Learning Test-II	memory	▪ verbal learning ▪ organization ▪ memory
Verbal Fluency - letters (COWAT)*	language	▪ spontaneous word generation
Trail Making Test A	executive functions	▪ Attention ▪ processing speed
Trail Making Test B	executive functions	▪ set-shifting
Digit Span	executive functions	▪ working memory ▪ attention
Judgment of Line Orientation	visuospatial functions	▪ orientation judgement

*Controlled Oral Word Association Test

Cognitive Impairment in Parkinson disease

The most frequently reported impairments of cognition in Parkinson disease are found in executive functions, attention, working memory and processing speed, retrieval-related memory problems, and visuospatial processing (Aarsland,

Andersen et al. 2003, Kulisevsky, Garcia-Sanchez et al. 2000, Pascual-Leone, Grafman et al. 1995, Saint-Cyr, Taylor et al. 1988, Ballard, Aarsland et al. 2002, Watson, Leverenz 2010, Emre 2003).

Impairment of Executive Function

Executive function is an umbrella term for various higher-level cognitive processes that organize information and regulate action. It includes components of attention, planning, working memory, set-shifting, abstract reasoning, appropriate inhibition and conflict resolution of sensory and motor information, cognitive flexibility, and decision making. It encompasses the concept of goal-directed action control rather than the habitual control (or more automatic actions) attributed to subcortical regions, particularly the basal ganglia (Saint-Cyr, Taylor et al. 1988, Dubois, Pillon 1997, Taylor, Saint-Cyr 1995). Executive function critically depends on an intact frontal cortex, but is mediated by dynamic and flexible frontal cortical and subcortical networks rather than by discrete structures. The cortico-striatal circuit closely links the frontal cortex to striatal structures through the thalamus and globus pallidus, and is dependent on dopaminergic neural transmission (Alexander, Crutcher 1990). The executive dysfunction characteristic of Parkinson disease, and seen even in *de novo* and early patients when the pathology is primarily thought to be confined to the basal ganglia, supports a strong link between cognition and dopaminergic function (Taylor, Saint-Cyr et al. 1986, Owen, James et al. 1992).

Dopaminergic Therapy and Cognition

This link to dopamine is further supported by the effect of dopaminergic therapy on cognition in Parkinson disease which is complex with both beneficial and aggravating effects (Cools 2006, Gotham, Brown et al. 1988). In the setting of severe dopamine depletion, as seen in the dorsal striatum, dopaminergic therapy appears to have a positive effect. In areas relatively spared, however, such as the prefrontal cortex or ventral striatum as is seen in early Parkinson disease, dopamine replacement therapy can have deleterious effects. This is seen by performance on certain cognitive tasks (verbal fluency) improving in the "off" medication state and deteriorating in tasks of working memory (Gotham, Brown et al. 1988). A fundamental difference appears to exist between the impact of the various types of dopaminergic therapy used (Beaulieu, Gainetdinov 2011). This may be due, in part, to the post-synaptic receptor binding affinity of different medications. Levodopa binds to both D1 class receptors (D1, D5) and D2 class receptors (D2, D3, D4). The commonly used non-ergot dopamine agonists, ropinirole and pramipexole, both have a high affinity for the D2 receptor subtype, but pramipexole has additional affinity for the D3 receptor subtype (Beaulieu, Gainetdinov 2011). The use of pramipexole, but not other dopamine agonists, has been shown to have a negative effect on phonemic fluency performance (Domellof, Forsgren et al. 2013, Brusa, Bassi et al. 2003), short-term verbal memory and attentional-executive functions (Brusa, Bassi et al. 2003). Others have shown a U-shaped relationship between working memory and dopamine levels with both excessive and insufficient levels impairing performance on working memory (Cools, D'Esposito 2011).

Thus, while dopaminergic therapy has beneficial impact on some motor features of Parkinson disease, its effect on cognition is mixed. Although much of the impairment in executive dysfunction in Parkinson disease is attributable to the dopaminergic system effects in the frontal lobes, there is accumulating evidence of other circuits (brainstem nuclei, limbic structures and cerebral cortex) reflecting degeneration in the serotonergic, noradrenergic and cholinergic systems that are known to be involved in cognitive dysfunction in Parkinson disease (Sonnen, Postupna et al. 2010).

The Prefrontal Cortex

The prefrontal cortex has extensive cortical and subcortical connections associated with executive sub-processes to different regions and disruptions of these connections results in impaired executive function, working memory and attention (Alexander, DeLong et al. 1986, Burgess, Veitch et al. 2000, Rogers, Sahakian et al. 1998). Executive dysfunction in Parkinson disease is generally attributed to abnormal dorsolateral prefrontal-basal ganglia circuits as well as limbic pathways mediated by dopamine (Alexander, DeLong et al. 1986, Lewis, Dove et al. 2003). This disruption of prefrontal cognitive processing can occur anywhere in the cortico-striato-pallido-thalamo-cortical loop. The caudate has also been implicated in frontal cognitive functions, particularly procedural learning (Pascual-Leone, Grafman et al. 1995, Weiller, Ringelstein et al. 1990, Caplan, Schmahmann et al. 1990) which is impaired in Parkinson disease (Pascual-Leone, Grafman et al. 1995, Pascual-Leone, Grafman et al. 1993, Bruck, Portin et al. 2001) (Bruck, Portin et al. 2001).

Mild Cognitive Impairment

Mild cognitive impairment (MCI) is a risk factor for conversion to dementia and is now recognized as a common feature in non-demented individuals with Parkinson disease (Litvan, Goldman et al. 2012). Mild cognitive impairment may be qualitatively different from dementia which in Parkinson disease encompasses cognitive and behavioural impairment consistent with a "subcortical" pattern of dysfunction. Subcortical dementia is characterised by severely impaired performance on tasks assessing executive functions, working memory, procedural learning, visuospatial processing and memory encoding and retrieval, and by a greater prevalence of mood disorder and speech deficits (Cummings 1986, Glosser 2001, Jacobs, Marder et al. 1995, Piccirilli, D'Alessandro et al. 1997, Mahieux, Fenelon et al. 1998).

In non-demented individuals with Parkinson disease, the estimate of prevalence of MCI is ~25% (Litvan, Aarsland et al. 2011, Foltynie, Brayne et al. 2004, Janvin, Larsen et al. 2006, Aarsland, Bronnick et al. 2010, Mamikonyan, Moberg et al. 2009, Muslimovic, Post et al. 2009). The presence of Parkinson disease-MCI has been documented even in early, drug-naive patients (Aarsland, Bronnick et al. 2009, Muslimovic, Post et al. 2005, Yarnall, Breen et al. 2014). Although the time and onset of the continuum from less severe cognitive impairment to Parkinson disease-MCI and then to dementia is highly variable, in a population where up to 80% of individuals will eventually develop dementia (Hely, Reid et al. 2008, Aarsland, Andersen et al. 2003), it is important to understand who is most at risk for developing these disturbances. This will help to both guide treatment decisions and, perhaps equally importantly, to identify the underlying

neural substrates, in the hope of guiding future neuroprotective or therapeutic strategies to modulate further progression of impairment.

Mild cognitive impairment describes changes in cognitive abilities that do not significantly interfere with daily functional activities or meet the criteria for dementia but are changes, nonetheless, that exceed the expected decline for normal aging. Mild cognitive impairment usually presents with problems of memory, language, thinking and judgement. Until recently, there was no consensus for a definition of MCI in Parkinson disease, making it difficult to interpret or generalise results from research studies. An attempt to provide a more standard definition was made with the development of recent guidelines from the Movement Disorder Society Task Force (Litvan, Goldman et al. 2012). The guidelines characterise the earliest manifestations of cognitive impairment in Parkinson disease. These will help define the best predictors of conversion to dementia, provide a potential outcome measure for clinical trials, and present a common language to describe Parkinson disease-MCI for all stakeholders when describing Parkinson disease-MCI and its effects on daily function and quality of life. The guidelines suggest two different levels of criteria to determine Parkinson disease-MCI. Level I (abbreviated assessment) includes (i) Impairment on a scale of global cognitive abilities validated for use in Parkinson disease (Montreal Cognitive Assessment (Nasreddine, Phillips et al. 2005), Parkinson's Disease Cognitive Rating Scale (Pagonabarraga, Kulisevsky et al. 2008), Scales for Outcomes in Parkinson's Disease-Cognition [SCOPA-Cog] (Kulisevsky, Pagonabarraga 2009), Mattis Dementia Rating Scale [MDRS3] (Villeneuve, Rodrigues-Brazete et al. 2011)) or (ii) impairment on at least two tests, when a

limited battery of neuropsychological tests are performed. Level 2 criteria, considered the "gold standard", specifies how to make use of a more extensive neuropsychological test battery which includes two tests within each of the five cognitive domains (attention and working memory, executive, language, memory and visuospatial). From these tests, impairment (defined as ~1–2 SDs below appropriate norms or significant decline confirmed on serial cognitive testing) on ≥2 tests (either two impaired tests in one cognitive domain or one impaired test in two different cognitive domains) qualify for Parkinson disease-MCI. When available, the task force recommends incorporating information on decline from premorbid cognitive levels. Various sub-types of MCI have also been described: single-domain (abnormalities on two tests within a single cognitive domain, with other domains unimpaired), multiple-domains (abnormalities on at least one test in two or more cognitive domains), amnestic, non-amnestic (Litvan, Goldman et al. 2012) with the non-amnestic type most prevalent in Parkinson disease-MCI, with prominent executive, visual-spatial and attention dysfunction (Williams-Gray, Foltynie et al. 2007, Aarsland, Bronnick et al. 2010, Goldman, Weis et al. 2012, Levy, Jacobs et al. 2002, Woods, Troster 2003). Links between this multiple domain, non-amnestic cognitive impairment is associated with motor impairment suggesting a close interaction between the pathophysiology underlying motor and cognitive changes in Parkinson disease.

Neuroimaging, Cognitive Function and Parkinson Disease

The variety of neuroimaging techniques available to evaluate brain changes associated with cognitive changes seen in Parkinson disease is providing insights into structural and functional correlates of cognition disturbances in different

domains when combined with results from neuropsychological tests and the relationship of cognition to various motor impairments.

Structural MRI

Voxel-Based Morphometry

Voxel-based morphometry studies have been undertaken in untreated, early non-demented, MCI and demented patient groups. Studies assessing grey matter density and correlating changes to cognition have shown different results across the spectrum of cognitive impairment in Parkinson disease. In **untreated** early disease, pre-frontal atrophy was correlated with impaired sustained memory (Bruck, Kurki et al. 2004) and hippocampal atrophy was correlated with impaired vigilance and verbal memory (Bruck, Kurki et al. 2004, Beyer, Bronnick et al. 2013). In a drug-naive, early Parkinson disease cohort, a decrease in white matter volume in the anterior temporal lobe was observed, but no correlations to tests of cognition were found (Martin, Wieler et al. 2009). In non-demented Parkinson disease groups, studies have shown no regional atrophy and no correlation between grey matter loss and cognition (Dalaker, Zivadinov et al. 2010, Melzer, Watts et al. 2012, Weintraub, Doshi et al. 2011, Apostolova, Beyer et al. 2010, Hattori, Orimo et al. 2012). Other studies have found grey matter atrophy in the right parietal lobe associated with impaired visual memory tasks (Ellfolk, Joutsa et al. 2013) and left parietal lobe associated with reduced semantic fluency (Ellfolk, Joutsa et al. 2014). Atrophy in the right caudate was associated with impaired phonemic fluency (Ellfolk, Joutsa et al. 2014) and left putamen atrophy with poor memory tests of executive function (Camicioli, Gee et al. 2009). Significant grey matter reduction was seen in frontal and parieto-occipital regions with fine motor speed

and set-shifting associated with the occipital atrophy (Lee, Sen et al. 2013), and visuospatial and executive functions in the dorsolateral prefrontal cortex (Nagano-Saito, Washimi et al. 2005). Medial temporal lobe atrophy was seen but no correlation to cognitive test performance (Tam, Burton et al. 2005). Poor performance on memory tests was found to correlate with grey matter atrophy in the left and right temporal lobes (Camicioli, Gee et al. 2009). Hippocampal atrophy showed mixed results with no correlation to cognitive tests in one study (Tam, Burton et al. 2005) and an association with executive and visuospatial functions in another (Nagano-Saito, Washimi et al. 2005). One study demonstrated grey matter atrophy in the cerebellum to be associated with impaired executive function (Camicioli, Gee et al. 2009).

A number of studies have looked at brain changes in **non-demented** patients who were *pre-identified* as being non-demented. No formal neuropsychological testing was done during the study with which to correlate imaging changes. Findings of grey matter atrophy were found in the hippocampus (Junque, Ramirez-Ruiz et al. 2005, Burton, McKeith et al. 2004, Summerfield, Junque et al. 2005), frontal lobes (Burton, McKeith et al. 2004), amygdala (Junque, Ramirez-Ruiz et al. 2005, Burton, McKeith et al. 2004), left anterior cingulate gyrus (Summerfield, Junque et al. 2005), left superior temporal gyrus (Summerfield, Junque et al. 2005), occipital regions (Song, Lee et al. 2011) and striatum (Tinaz, Courtney et al. 2011).

The pattern of change observed in those with **MCI** shows involvement of more brain areas than those identified as non-demented but less than those with Parkinson disease dementia. Reductions in grey matter volumes in the left insular,

left superior frontal and left middle temporal areas were correlated with poor performance on tests of executive function, attention, memory and language as well as lower global cognition scores (Mak, Zhou et al. 2014). Global cognition was also correlated with gray matter atrophy in the temporal, parietal, frontal cortex, bilateral caudal hippocampus, amygdala and right putamen (Melzer, Watts et al. 2012). In a study of pre-identified patients with mild cognitive impairment, left frontal and both parietal lobes showed grey matter reduction (Beyer, Janvin et al. 2007).

Studies that correlated neuropsychological finding with grey matter changes in Parkinson disease **dementia** found visuospatial and executive functions were associated with reduced volumes in the dorsolateral prefrontal cortex and parahippocampal gyrus (Nagano-Saito, Washimi et al. 2005) and global cognitive scores with frontal and temporal cortex as well as hippocampus, posterior cingulate gyrus, primary visual cortex and caudate (Melzer, Watts et al. 2012, Camicioli, Moore et al. 2003). In *pre-identified* cohorts of Parkinson disease dementia, widespread grey matter atrophy has been observed in the parietal, pre-frontal, frontal and temporal areas (Melzer, Watts et al. 2012, Tam, Burton et al. 2005, Burton, McKeith et al. 2004, Song, Lee et al. 2011, Beyer, Janvin et al. 2007). Other studies have shown limbic area grey matter volume loss including hippocampus, thalamus, anterior cingulate insular and amygdala (Junque, Ramirez-Ruiz et al. 2005, Burton, McKeith et al. 2004, Summerfield, Junque et al. 2005). Caudate atrophy (Melzer, Watts et al. 2012, Apostolova, Beyer et al. 2010) was also observed.

Only one study has looked at the substantia innominata in the basal forebrain. Reduced volume of this cholinergic structure was significantly associated with global cognitive performance, attention and object naming (Choi, Jung et al. 2012), further implicating non-dopaminergic systems in cognition.

Diffusion-Weighted Tensor Imaging

The use of DTI to evaluate white matter has demonstrated significant changes in early Parkinson disease, Parkinson disease with MCI as well as in Parkinson disease dementia.

Decreased FA, representing loss of white matter integrity in anterior and posterior cingulate fibre tracts, was seen both in Parkinson disease with no identified cognitive impairment and Parkinson disease dementia, with lower FA values in the dementia group. These decreased FA values in the anterior cingulate correlated with a measure of global cognitive function in the dementia group (Kamagata, Motoi et al. 2012). In non-demented Parkinson disease, other authors have reported: no decrease in FA values for any tract (Hattori, Orimo et al. 2012); significant correlation between a measure of global cognitive function and decreased FA values in prefrontal white matter and corpus callosum (Kamagata, Motoi et al. 2013); no change in FA or MD values, but MD values within the corpus callosum that did correlate with a measure of global cognitive function (Wiltshire, Concha et al. 2010), and FA reductions in the left frontal/right temporal and bilateral anterior cingulate (Deng, Zhang et al. 2013). In those with MCI and those with Parkinson disease dementia, decreased FA values in many tracts (superior and inferior longitudinal fasciculus, inferior fronto-occipital fasciculus, uncinate fasiculus, cingulum, corpus callosum) were correlated to a measure of global cognitive

function (Hattori, Orimo et al. 2012). In a Parkinson disease dementia group, large FA reduction was seen in the anterior cingulate and corpus callosum (Deng, Zhang et al. 2013).

The abnormalities in white matter integrity shown with DTI in diffuse brain regions supports the contribution of white matter changes contributing to cognitive impairment in Parkinson disease that are different across the spectrum of disease stages.

Cortical Thickness Mapping

Cortical thickness changes in Parkinson disease are thought to be associated with meaningful functional differences and, with changes evolving over the course of the disease, are potentially useful in identifying Parkinson disease dementia. In **non-demented** early Parkinson disease, widespread cortical thinning is seen in the frontal, temporal, parietal, occipital, dorsolateral prefrontal, supplementary motor area and pre-supplementary motor area, posterior cingulate with correlation to tests of global cognition (Jubault, Gagnon et al. 2011, Biundo, Calabrese et al. 2013, Zarei, Ibarretxe-Bilbao et al. 2013, Tinaz, Courtney et al. 2011, Hanganu, Bedetti et al. 2013, Pereira, Svenningsson et al. 2014).

In cohorts of Parkinson disease with **MCI**, areas of atrophy were found in the right occipital-parietal region, middle frontal and occipital areas and cortical thickening in the right parietal-frontal and left temporal occipital areas. This thickening was thought to reflect compensatory neuroplasticity (Biundo, Calabrese et al. 2013, Hanganu, Bedetti et al. 2013). In another study, performance on visuospatial, recall, both semantic and phonemic fluency, working memory and attention tests were correlated to atrophy in temporal, parietal, frontal and occipital

regions, with tests of memory, executive function and visuospatial associated with temporal and frontal thinning (Pereira, Svenningsson et al. 2014).

Disease stage was associated with thinning of the medial frontal (premotor and supplementary motor cortex), posterior cingulate, precuneus, lateral occipital, temporal and dorsolateral prefrontal cortex (Zarei, Ibarretxe-Bilbao et al. 2013). In a recent longitudinal study, the rate of cortical thinning was higher in those with mild cognitive impairment, compared with healthy controls and Parkinson disease without mild cognitive impairment in a number of areas - temporal, occipital, parietal and supplementary motor area - which correlated to decline in a measure of global cognitive function. Also, a significant decrease in the volume of the amygdala and nucleus accumbens was observed in the group with mild cognitive impairment (Hanganu, Bedetti et al. 2014).

A pattern of changes in cortical thickness in Parkinson disease and an association with changes in measures of cognition is seen early in the disease and change together over time and with increasing cognitive impairment. It has been suggested that these changes may serve as a biomarker of conversion from mild cognitive impairment to dementia (Hanganu, Bedetti et al. 2014).

Functional Magnetic Resonance Imaging

Activation patterns with fMRI during cognitive tasks show differences between individuals with Parkinson disease compared to controls. Prefrontal cortex involvement in executive dysfunction in Parkinson disease is seen in decreased activation in the anterior and posterior frontal regions, primarily around the dorsolateral prefrontal cortex, striatum, anterior cingulate on various executive tasks of set-shifting and working memory (Sabatini, Boulanouar et al. 2000,

Dagher, Nagano-Saito 2007). The pattern of activation for a working memory and a set-shifting task in the prefrontal cortex of subjects with moderate Parkinson disease, on dopaminergic replacement therapy, with impairment in executive function was explored. Reduced activation in bilateral dorsolateral and ventrolateral prefrontal cortex and in bilateral caudate and right putamen was observed. These abnormalities were not seen in those without executive dysfunction (Lewis, Dove et al. 2003). These anomalies suggest that the nigrostriatal networks, particularly the caudate, play a significant role in cognitive functions. In contrast, the effects of dopamine on the cortical networks involved with working memory were explored with a working memory task. In a group of early stage individuals using dopaminergic replacement therapy with no cognitive impairment, increases in cortical activation (dorsolateral prefrontal cortex, anterior cingulate cortex and posterior parietal cortex) were seen in the "off" medication state compared to the "on" state, which correlated with a deterioration in cognitive performance (Mattay, Tessitore et al. 2002). These results suggest that the cognitive dysfunction seen in Parkinson disease may not be due to reductions in presynaptic dopaminergic functions of the nigrostriatal system, but rather result from depletion in dopaminergic input in the frontal lobes secondary to direct disruptions in the circuitry between the mesocortical dopaminergic system and the prefrontal cortex (Mattay, Tessitore et al. 2002). The differences in these results underscore the equivocal contribution of the caudate to cognitive dysfunction. In an fMRI paradigm testing visual attention and motor inhibition, fronto-striatal network and temporal-occipital cortex changes were observed, even in those with early Parkinson disease and with no evidence of cognitive impairment (Baglio, Blasi

et al. 2011). A comparable pattern of altered functional cerebral networks involved in a verbal memory task seen in a similar patient group was also reported (Ibarretxe-Bilbao, Zarei et al. 2011).

Resting State Functional Magnetic Resonance Imaging

Resting state networks are characterised by neuronal activity during rest, when there are no cognitive demands on the brain. In the default mode network, this activity is decreased or 'deactivated" when the brain is directed towards a task or goal (Raichle, MacLeod et al. 2001). In Parkinson disease, despite no cognitive impairment, decreased functional connectivity of the right medial temporal lobe and bilateral inferior parietal cortex were seen in the default mode network and this default mode network connectivity was significantly correlated with cognitive parameters (Tessitore, Amboni et al. 2012b). Dopaminergic therapy has been shown to have dose-dependent effect on the default mode network integrity in cognitively unimpaired Parkinson disease patients (Krajcovicova, Mikl et al. 2012).

Magnetic Resonance Spectroscopy

Magnetic resonance spectroscopy provides insights into the relationship between changes in cognition and a putative marker of neuronal integrity, NAA/Cr or NAA concentration. Studies of Parkinson disease and cognition have looked at various regions for evidence of decreased neuronal integrity. NAA concentrations in the dorsolateral prefrontal area correlating with frontal subcortical tasks were shown to be decreased in those with mild cognitive impairment and Parkinson disease compared to those identified as cognitively intact. In a group with

Parkinson disease dementia, the left hippocampus NAA concentrations correlated with a naming task (Pagonabarraga, Gomez-Anson et al. 2012).

In a cohort of those with Parkinson disease dementia compared to cognitively intact Parkinson disease and control groups, decreased NAA/Cr ratio observed in the occipital cortices were correlated with tasks of working memory and attention (Summerfield, Gomez-Anson et al. 2002). These results were also seen in temporoparietal regions (Hu, Taylor-Robinson et al. 1999).

Reduced NAA/Cr ratios were seen in the anterior cingulate cortex but not posterior cingulate in a group of Parkinson disease and this correlated with executive dysfunction, specifically attentional set-shifting and response inhibition. It was also correlated with the presences of hallucinations (Lewis, Shine et al. 2012). In contrast, in a group of Parkinson disease with mild to moderate disease, reduced NAA/Cr ratio in the posterior cingulate cortex correlated with a test of memory (Camicioli, Korzan et al. 2004).

In a group of non-demented individuals with Parkinson disease, NAA/Cr ratio in the pre-SMA was decreased, but did not show any correlation with measures of frontal function or global cognition (Camicioli, Hanstock et al. 2007). In a group of drug naive, early Parkinson disease subjects, this pre-supplementary motor area abnormality was not seen (Martin, Wieler et al. 2008b).

Positron Emission Tomography

Evaluation of the functional integrity of the nigrostriatal dopamine metabolic system with PET reveals a relationship to cognition in Parkinson disease. Cognitive deficits in early Parkinson disease may be due to dysfunction in the prefrontal cortex or striatum. A positive correlation was seen in the performance of executive

tasks in Parkinson disease subjects between caudate fluorodopa (F-DOPA) uptake and frontal dopamine activity (Bruck, Portin et al. 2001). In another study, in early, unmedicated Parkinson disease subjects, F-DOPA uptake was negatively correlated in the right caudate on a test of attention but no significant correlation with the putamen or caudate on other tests of executive function were found (Bruck, Portin et al. 2001). In other studies, a significant reduction in F-DOPA uptake correlated with tests of working memory in the anterior putamen (Cheesman, Barker et al. 2005, Cropley, Fujita et al. 2008), and executive function in the caudate (Bruck, Portin et al. 2001, Cheesman, Barker et al. 2005, Cropley, Fujita et al. 2008, Niethammer, Tang et al. 2013, Jokinen, Karrasch et al. 2013). These results point to dopamine depletion within the caudate nucleus playing a central role in impaired cognitive task performance in Parkinson disease. Another mechanism contributing to cognitive impairment includes alterations in cholinergic pathways in the posterior brain regions which are implicated in the emergence of dementia (Hirano, Shinotoh et al. 2012, Bohnen, Kaufer et al. 2006, Bohnen, Kaufer et al. 2003).

This association between cognitive impairment, particularly executive function and memory, in Parkinson disease and brain metabolic activity has been further shown using positron emission tomography and a variety of ligands to evaluate glucose utilization and DAT binding in the caudate and putamen. The reduced metabolism observed in frontal and parietal association areas suggests disturbance in these brain regions in Parkinson disease is associated with impaired cognition (Huang, Tang et al. 2007, Edison, Ahmed et al. 2013). This demonstration of deterioration of various parts of the dopaminergic and cholinergic

metabolic pathways in Parkinson disease underscores the complexity of understanding the pathophysiology underpinning cognitive impairment.

Other Markers of Impaired Cognition

A novel approach to understanding the pathophysiology of cognitive impairment in Parkinson disease looked at cerebral spinal fluid (CSF) markers of Alzheimer pathology, tau and amyloid β, and grey matter volume measured by VBM. A correlation between gray matter in frontal and parietal structures and these CSF markers was found; the CSF markers significantly correlated with memory, naming and visuospatial scores, as well as semantic and phonetic verbal fluencies and the areas of decreased grey matter volume correlated with the CSF markers that correlated with abnormal cognitive functions (Compta, Ibarretxe-Bilbao et al. 2012). This suggests that these CSF markers may serve as a biomarker of cognitive impairment and dementia in Parkinson disease.

Summary

When reviewing the literature on cognitive impairment and Parkinson disease, direct comparisons between studies can be difficult. This includes the heterogeneity of study cohorts with respect to disease duration, age and cognitive status; use of different neuropsychological tests with varying cut-off scores; inconsistent definition of Parkinson disease-MCI and Parkinson disease dementia; and variation in image analysis related variation in delineation of anatomic boundaries, segmentation techniques and statistical thresholds. Nevertheless, cognitive dysfunction in Parkinson disease is evident across the continuum from early to late disease. Dysfunction in multiple domains is seen with contributions

from striatal and cortical dopamine loss, cortical neuronal loss, dysfunction of other neurotransmitter systems, (particularly the cholinergic system) as well as alteration in structural and functional connectivity in multiple regions. The contributions of cortical and subcortical dysfunction to cognitive impairment may be related not only to the stage of the disease but also to medication effects. Nigrostriatal pathways are clearly more affected early in the disease course than mesocortical networks. Later disease may involve functional impairment in the basal ganglia as well as in the frontal cortex. Understanding the emergence, progression and underlying neural substrates of this debilitating feature of Parkinson disease will help direct further research supporting the treatment and management of this feature and ease the burden of those affected by Parkinson disease.

Cognition and Gait

Cognition and gait are no longer thought of as structurally and functionally distinct processes. While the task of walking does encompass aspects of automaticity, there is increasing evidence suggesting a close relationship with more conscious engagement. Attention to internal and external cues and judgement of the environment, in addition to coordinated motor programs, are requirements of successful gait. Degeneration of the structural and/or functional neural substrates in one has deleterious effects on the other. This is exemplified in Parkinson disease where both gait disturbance and cognitive dysfunction "walk" together. Parkinson disease is characterised by a loss of a normal, automatic gait pattern (Morris, Iansek et al. 1996, Hausdorff 2009, Hausdorff, Schaafsma et al. 2003b). This altered gait is accompanied by cognitive impairments, particularly in the domains of attention and executive function, deficits which may be seen even in drug-naive

individuals with Parkinson disease and in early disease (Schaafsma, Giladi et al. 2003, Dubois, Pillon 1997, Rochester, Hetherington et al. 2004, Baltadjieva, Giladi et al. 2006b). When the system is taxed by the demands of two tasks that require attention, dual-task prioritisation must take place; this process is impaired in those with Parkinson disease and FOG (Vandenbossche, Deroost et al. 2012a).

Postural Instability and Gait Disturbance

Several studies have shown that those with more postural instability and axial symptoms have greater cognitive impairment (Domellof, Forsgren et al. 2013, Verbaan, Marinus et al. 2007) and a faster rate of cognitive decline (Burn, Rowan et al. 2006, Alves, Larsen et al. 2006). Postural instability, axial deficits and gait disturbance respond poorly to dopamine replacement therapy which is critical to the normal function of fronto-striatal circuits; denervation of cholinergic neurons in the pedunculopontine nucleus has been implicated in these features (Bohnen, Muller et al. 2009, Rochester, Yarnall et al. 2012, Yarnall, Rochester et al. 2011). Postural instability and gait disturbance have also been correlated to poor performance on attentional and executive function tests (Bohnen, Kaufer et al. 2006). This suggests that gait disturbance, including FOG, and cognitive impairment may not be directly related to dopaminergic mechanisms, but may rather be a result of disruption in multiple neurotransmitter systems.

Neural Substrates Underlying Impaired Cognition and Freezing of Gait

Attempts to understand the neural substrates of the debilitating gait phenomenon, freezing of gait, have included its link to cognitive deficits and it has been hypothesised that cognitive dysfunction itself may be a mechanism associated

with the emergence of FOG (Nutt, Bloem et al. 2011). The evidence supporting this connection is growing, with many studies showing a correlation between impairment on neuropsychological tests of frontal lobe function, particularly executive function and attention, and the emergence, frequency and severity of FOG episodes (Naismith, Shine et al. 2010, Amboni, Cozzolino et al. 2008, Amboni, Barone et al. 2010). It has been shown that focused attention and cues can help to overcome FOG (Spildooren, Vercruysse et al. 2010, Giladi, Hausdorff 2006a, Nieuwboer 2008, Baker, Rochester et al. 2008). Increased cognitive loading with a task that divides attention (dual- task) (Yogev-Seligmann, Hausdorff et al. 2008, Almeida 2009), or tasks with higher attentional demands (Giladi, McMahon et al. 1992) increases the frequency and severity of FOG episodes.

The inability to successfully engage various executive domains to compensate for or assist with alterations in gait has been suggested as a basis for FOG (Plotnik, Giladi et al. 2009, Maruyama, Yanagisawa 2006). Disruption in the cortico-striatal system secondary to striatal dopamine depletion has been implicated in the emergence of FOG. Another hypothesis has been put forth suggesting that a disruption in the tight regulation of parallel neural networks (controlling motor, cognitive and limbic functions) that pass through the basal ganglia is implicated in FOG. The loss of striatal dopamine, coupled with the loss of the ability to appropriately process competing but complementary cognitive, motor or limbic demands, allows for "cross-talk" between the networks which may result in sudden, transient inhibition of the thalamus and pedunculopontine nucleus resulting in a freezing episode (Lewis, Barker 2009a).

The heterogeneous nature of Parkinson disease, cognitive impairment and gait disturbance has lead to investigations as to a subtype of presentation that might predispose an individual to the emergence of FOG. The presence of FOG has been associated with faster progression of executive dysfunction (Amboni, Barone et al. 2010), and affective symptoms, including depression, apathy and anxiety, have been associated with the presence of FOG (Naismith, Shine et al. 2010, Amboni, Cozzolino et al. 2008, Lieberman 2006).

Imaging Cognition and Freezing of Gait

Several neuroimaging studies have investigated the underlying neural substrates associated with impaired cognition and FOG. Structural imaging studies, using VBM, have shown posterior grey matter atrophy (including the posterior cingulate) in individuals with FOG which correlated with poor performance on tests of frontal lobe function and more severe motor impairment (Tessitore, Amboni et al. 2012a). Freezing of gait in Parkinson disease is thought to be a result of abnormal interactions between fronto-parietal cortical regions and subcortical structures, such as the striatum. In activation studies using fMRI, during a simultaneous cognitive and motor task, those with FOG were less able to appropriately recruit cortical (pre-supplementary motor area) and subcortical (striatum and anterior insula) regions within the cognitive control network (Shine, Matar et al. 2013b). Supporting the hypothesis that FOG is a result of disrupted communication between complementary but competing networks, (Lewis, Barker 2009a) an imagined gait task during functional MRI study, showed functional decoupling between the basal ganglia network and the cognitive control network in each hemisphere associated with sudden halting of movement in those with FOG

(Shine, Matar et al. 2013c). Results from a resting state fMRI study show FOG to be significantly correlated with impaired visual and executive-attention networks (with poor performance on tests of frontal function, particularly tests of executive function) (Tessitore, Amboni et al. 2012b).

The close relationship between cognitive deficits and FOG and between fMRI aspects of cortical function and FOG points to the complex pathogenesis of these features which implicates involvement of multiple levels including cortical, subcortical and brainstem regions. Our understanding of the exact nature of this relationship is evolving and remains to be fully elucidated.

Current Studies

The overall goal of this research is to evaluate longitudinal changes in motor function and cognition in Parkinson disease which can be quantified clinically and with brain imaging (magnetic resonance imaging and/or spectroscopy). We suggest that imaging markers of abnormal brain structure and function will correlate with these clinical changes and serve as biomarkers of disease progression. Loss of dopaminergic neurons in the substantia nigra of the basal ganglia is the prominent pathology associated with many motor features that characterise Parkinson disease.

Increased levels of iron in the substantia nigra are seen in Parkinson disease and can be estimated from measured R_2* values. Iron, per se, is not the focus of interest but rather it is used as a marker of neuronal structural integrity. Changes in cognition contribute to disability seen in Parkinson disease. Loss of neuronal integrity, as measured by NAA/Cr ratio, in an area implicated in both cognitive decline and motor dysfunction in Parkinson disease, the pre-supplementary motor

area, may be related to clinical presentation and progression. There is a tight integration between decline in gait and decline in cognition. In order to investigate these features, we evaluated the evolving relationship between clinical manifestations of Parkinson disease, reflected in changes of gait and cognition, and *in vivo* structural and functional changes as measured by high field magnetic resonance imaging and spectroscopy in early, untreated individuals with Parkinson disease over 36 months.

In the first study (Chapter 2) we evaluated changes in midbrain iron content as a marker of neuronal loss in a group of early untreated Parkinson disease subjects compared to a control group and correlated evolving motor dysfunction in with these changes.

In the second study (Chapter 3) we investigated changes in this same iron-rich midbrain region to determine if changes were associated differently between a cohort of early, untreated Parkinson disease subjects who developed FOG over three years compared to one who did not.

In the third study (Chapter 4) we examined the relationship of cognitive changes and clinical motor features, including FOG, with a magnetic resonance spectroscopy maker of regional neuronal loss, NAA/Cr ratio, in the pre-supplementary motor area. We compared a group of early, untreated Parkinson disease to controls, and then divided the patients into subgroups of those who developed FOG compared to those who did not, over 36 months.

General Methodology

Subjects

Individuals, sequentially seen in the University of Alberta Movement Disorders Program, who, within 2 years preceding the study, had met the standard criteria for clinically definite Parkinson disease and, at the time of entry into the study, had not started symptomatic treatment were eligible for participation. Those with significant medical, neurologic (other than Parkinson disease), orthopedic or psychiatric conditions were excluded. All eligible subjects were approached for participation. Of those who expressed an interest in the study, approximately 5 declined upon further explanation of the study requirements. A total of 29 Parkinson disease subjects agreed to participate in this study. One subject died from a cause unrelated to Parkinson disease prior to the completion of the study and one subject declined participation after the first visit, expressing concern about exposure to a strong magnetic field.

A control group of healthy age- and sex-matched individuals were recruited from the spouses, family and friends of various patients seen in the University of Alberta Movement Disorders Program, as well as from the general university community. Individuals with significant medical, neurological, orthopedic or psychiatric conditions or a family history of Parkinson disease were excluded. Control subjects were included to establish the degree of normal variation and longitudinal age-related changes that may be present in the magnetic resonance studies, and tests of cognition and motor function. A total of 16 control subjects agreed to participate in this study. One subject was excluded following the

incidental finding of a benign brain tumor during baseline MRI scan. This participant was referred to a neurosurgeon for subsequent follow up.

This pool of participants was used for all three studies presented, although specific subject differed for each paper. Adequate data was not available for analysis for all subjects, mainly due to poor quality of imaging data. Those subjects whose data were not available for inclusion in the analysis did not differ with respect to clinical measures of the included cohort.

Dopaminergic Therapy

The introduction of dopaminergic therapy to address Parkinson disease symptoms over the course of this 36 month study was expected. At month 36, if study participants had commenced treatment, medications were converted to levodopa equivalent dose (LED) using the following recommended conversion factors (Tomlinson, Stowe et al. 2010) and summed together to obtain a total LED. (See Table 1.3)

Table 1.3: Levodopa equivalent dose

▪ immediate release levodopa dose × 1 ▪ controlled release levodopa dose × 0.75 ▪ pramipexole dose × 100 ▪ ropinirole dose × 2	▪ rotigotine x 30 ▪ rasagiline x 100 ▪ amantadine x 1
▪ entacapone or Stalevo - levodopa x 0.33 (irrespective of entacapone dose, levodopa dose is multiplied by 0.33 to give LED subtotal for entacapone which is then added to the levodopa dose and other LEDs to give total LED)	

Clinical Assessments

A battery of standardised clinical and neuropsychological assessments was administered at baseline, 18 months and 36 months. These included:

Disease Burden

The **Unified Parkinson Disease Rating Scale** [UPDRS] (Fahn, Elton et al. 1987) is a widely-utilised standard rating tool used to longitudinally monitor features of Parkinson disease. The UPDRS consists of subscales evaluating cognitive and emotional status (UPDRS I – mentation, behavior and mood; range 0-16); daily functioning (UPDRS II – activities of daily living; range 0-52); motor symptoms (UPDRS III – motor examination; range 0-108); and side effects of therapy (UPDRS IV – complications therapy; range 0-23). A higher overall score of sections I - III, as well as a score for each subscale, indicates greater burden of disease.

Motor Assessments

The motor assessments chosen have been shown to be valid and reliable in Parkinson disease testing basic performance of gait and balance.

Timed Up and Go

Timed Up and Go (TUG) (Podsiadlo, Richardson 1991) is a test of basic functional mobility in which an individual is asked to stand up from a chair, walk forward at a comfortable and safe pace for 3 metres, turn around and return to sit on the chair. The number of seconds taken to complete the task is recorded. A cut-off score of >11.5 seconds indicates a higher risk for falls in Parkinson disease (Nocera, Stegemoller et al. 2013).

Berg Balance Scale

The **Berg Balance Scale** (Berg, Wood-Dauphinee et al. 1992, Qutubuddin, Pegg et al. 2005) is a measure of static and dynamic balance. This scale assesses

the performance of 14 functional tasks of increasing challenge to balance, scored from 0-4, where a maximum score of 56 indicates no fall risk and a score of <45 indicating a risk for falling.

Timed 14 m walk

The **Timed 14 m walk** asks the subject to walk 7 metres; turn and walk back 7 metres. The time and number of steps are recorded.

Self-Reported Health Status and Emotional Functioning

Parkinson's Disease Questionnaire-39

The **Parkinson's Disease Questionnaire-39** (PDQ-39) (Peto, Jenkinson et al. 1998) is a 39-item, self-report questionnaire, assessing Parkinson disease-specific health-related quality over the previous month in eight domains of function: mobility, activities of daily living, emotional well-being, stigma, social support, cognition, communication and bodily discomfort. Scores on the PDQ-39 range from 0 to 100, with a higher score indicating poorer reported quality of life.

Beck Depression Inventory–II

The **Beck Depression Inventory–II** (BDI–II) (Beck, Steer, Brown, 1996) is a self-report measure of subjective symptoms of depression that assesses psychological functioning (total score range 0-63) with higher total scores indicating greater depression. In Parkinson disease, a cut-off score of 14 is recommended to identify mild depression (Visser, Leentjens et al. 2006).

Neuropsychological Assessments

The comprehensive neuropsychological test battery used in these studies was compiled in collaboration with Dr. Nancy Fisher, a registered neuropsychologist, and

Dr. Richard Camicioli, a behavioral neurologist experienced in movement disorders. Cognitive evaluation was conducted by Satwinder Sran, a research assistant, trained by Dr. Fisher to administer the tests. Measures were chosen to quantify cognition in the following domains: attention, memory, language, executive function, and visuo-spatial function. The measures were chosen for their validity and ease of applicability for patients with motor impairment. These measures were administered at baseline, 18 and 36 months, while patients were in the clinically defined "off" state. Alternate forms, where available, were used at different time points to avoid learning effects.

Mini-Mental State Exam

The **Mini-Mental State Exam** (MMSE) (Folstein, Folstein et al. 1975) is a structured screening tool of global cognitive function assessing orientation, verbal memory, language, attention/calculation, and visuoconstructive abilities. A score of <24 out of 30 is considered abnormal for those with college education (<23/30 for those with high school education).

Frontal Assessment Battery

The **Frontal Assessment Battery** (FAB) (Dubois, Slachevsky et al. 2000) is a screening battery evaluating several frontal function domains, with a lower score indicating more executive domain dysfunction.

Trail Making Test

The **Trail Making Test** (Spreen, Strauss 1998) is composed of two conditions, Trail Making Test A and Trail Making Test B. Trail Making Test A, a speeded visual task of attention, requires subjects to connect consecutively

numbered dots as quickly as possible. Trail Making Test B requires the participant to alternate between consecutive numbers and letters in a speeded task of executive functioning.

Verbal Fluency-letters

The **Verbal Fluency-letters** (Controlled Oral Word Association Test) (Benton, Hamsher et al. 1994) is a test of phonemic fluency where participants generate a list of words based upon a phonemic cue.

Wechsler Memory Scale–III Digit Span

The **Wechsler Memory Scale–III Digit Span** (Wechsler 1997) is a task that requires the participant to hold and reproduce a string of numbers in two conditions: forward and backward. Digit Span is considered a task of attention and working memory.

California Verbal Learning Test-Second Edition

The **California Verbal Learning Test-Second Edition** (CVLT-II) (Delis, Kramer et al. 2000) evaluates verbal episodic memory including acquisition trials, immediate free recall (IR), short delayed free recall (SDR), long delayed free recall (LDR), delayed recognition (DR), semantic clustering (SC) where higher scores represent better performance.

Judgment of Line Orientation

Judgment of Line Orientation (JOL) (Benton, Hamsher et al. 1983) is a test of visuospatial functions in which subjects are asked to match the angle and orientation of lines of space.

Chapter 2: Longitudinal Midbrain Changes in Early Parkinson's Disease: Iron Content Estimated from R_2*/MRI

This paper has been submitted for publication:

Wieler M, Gee M, Martin WRW. Longitudinal Midbrain Changes in Early Parkinson's Disease: Iron Content Estimated from R_2*/MRI

Abstract

Objective: To determine whether, in patients with early Parkinson's disease, longitudinal changes in midbrain iron content are associated with declining motor function over a period of three years.

Methods: Nineteen untreated subjects with early Parkinson disease and 13 age- and sex-matched control subjects were followed clinically for 36 months. MRI with a 3 tesla magnet was performed at baseline, 18 months and 36 months with a multiple gradient echo sequence designed for rapid single-scan mapping of the proton transverse relaxation rate R_2*. R_2* was calculated for midbrain and forebrain basal ganglia regions.

Results: A difference in measured R_2* values between patients and controls was observed at baseline ($p = 0.035$) but not at 18 or 36 months in the lateral substantia nigra pars compacta (SNc). Linear regression indicated significant correlations between the change in R_2* values in the lateral SNc and the change score in UPDRS III ($p = 0.008$) and the PDQ-39 -mobility sub-score ($p = 0.03$) from baseline to 36 months.

Conclusions: High field strength MRI demonstrates lateral SNc abnormalities that progress over a period of 3 years in early Parkinson disease consistent with increased iron content, corresponding to the known distribution of neuronal loss occurring in this disorder, and correlating with motor symptomatology. Larger and longer investigations with more precise mapping of iron-containing midbrain structures are needed to fully evaluate the potential of R_2* as a biomarker of disease progression in Parkinson disease.

Introduction

Parkinson's disease is a slowly progressive neurologic disorder characterized clinically by varying combinations of rest tremor, rigidity, bradykinesia, postural instability and gait disorder. The primary change underlying many of the motor features of Parkinson disease is a gradual loss of dopaminergic neurons projecting from the substantia nigra pars compacta (SNc) to the striatum, resulting in decreased striatal dopamine content. Lateral SNc neuronal loss has been reported to be particularly correlated with the clinical features of Parkinson disease (Greffard, Verny et al. 2006, Lee, Schulzer et al. 1994). Evidence from post-mortem studies suggests increased iron content in the substantia nigra in Parkinson disease (Sofic, Riederer et al. 1988, Dexter, Wells et al. 1989), with the changes most marked in severe disease (Youdim, Ben-Shachar et al. 1990), suggesting that measurement of regional iron content in the SNc may provide an indication of the pathological severity of the disease.

While brain structures with high non-heme iron deposition, including the substantia nigra, can be visualized readily on conventional clinical MR images, attempts to use these images to differentiate individuals with classic Parkinson disease from healthy controls have generally been unsuccessful. In contrast, T_2* -based methods using gradient echo MRI sequences, are better suited to capture the influence of magnetic field inhomogeneities produced by the presence of paramagnetic ions in tissue thereby more accurately reflecting midbrain iron content (Ye, Allen et al. 1996).

We previously reported a cross-sectional study of early, untreated Parkinson disease subjects with MRI changes consistent with increased iron content in the

lateral SNc suggesting that the degree of increase correlated with the severity of motor symptomatology (Martin, Wieler et al. 2008a). The objective of the present longitudinal study was to explore the potential progressive changes in midbrain iron content associated with declining motor function over a period of three years in this cohort of early, untreated Parkinson disease subjects. We used a high-field MRI (3 tesla) method designed to minimize magnetic field inhomogeneities arising from tissue-tissue and air-tissue interfaces thereby improving the specificity of transverse relaxation measurements for changes in iron content (Wild, Martin et al. 2002, Ye, Martin et al. 1996).

Methods

Subjects

In this longitudinal case controlled study, 19 sequential subjects with early untreated Parkinson disease were recruited from the Movement Disorders Program located at the Glenrose Rehabilitation Hospital, matched for age with 13 control subjects and followed for 36 months. Control subjects were healthy, free of neurological and psychiatric disease and recruited from the community as well as from patients' relatives and friends. All patients fulfilled standard criteria for a clinical diagnosis of Parkinson disease (Hughes, Daniel et al. 1992) as assessed by a movement disorders neurologist (WM). All Parkinson disease subjects participated in a previously published study utilizing dihydrotetrabenazine/PET (Martin, Wieler et al. 2008c) and displayed striatal PET abnormalities that were consistent with a diagnosis of Parkinson disease. At the start of the study, none of the Parkinson disease participants required pharmacologic treatment with levodopa or other dopaminergic medications to address Parkinson disease symptoms. In

order to minimize the potential impact of medication on the clinical measures in subsequent evaluations, if medications had been initiated to treat Parkinson disease symptoms, participants were examined in the clinically defined "off" state, a minimum of 12 hours after the withdrawal of all dopaminergic medications. Clinical features were rated with the Unified Parkinson's Disease Rating Scale (UPDRS) part III (Fahn, Elton et al. 1987) by one of two raters (MW or WM) who had a high inter-rater reliability (intra-class correlation coefficient for the total motor UPDRS = 0.99, determined in a separate group of patients with Parkinson disease). All follow-up assessments for subjects were rated by the same rater (MW or WM). Other clinical evaluations included the mini-mental state exam (MMSE) (Folstein, Folstein et al. 1975), Timed Up and Go (Podsiadlo, Richardson 1991), 14 metre walk (with turn), reflecting our interest in mobility changes in early Parkinson disease, the Berg Balance Test and the Parkinson's Disease Questionnaire (Peto, Jenkinson et al. 1998) (PDQ-39). The PDQ-39 is a self-report quality of life tool with eight sub-domains (mobility, activities of daily living, emotional well-being, stigma, social support, communication cognitions, communications and bodily discomfort) that can be used to calculate a single index score or reported as sub-domains. These were all administered by one investigator (MW) at each time point. Participants were followed for 36 months with clinical evaluations and scans completed at baseline, 18 and 36 months. The study was approved by the Health Research Ethics Board of the University of Alberta and all subjects gave written informed consent.

Data Acquisition

MRI data were acquired using a Magnex 3 tesla research magnet (Magnex Scientific Ltd., Abingdon, U.K.) with actively shielded gradients, controlled with a Surrey Medical Imaging Systems console (Surrey Medical Imaging Systems, Ltd., Guildford, U.K). A multiple gradient echo sequence designed for rapid and optimal single-scan mapping of the proton transverse relaxation rate, R_2^* ($R_2^* = 1/T_2^*$), developed by our group, was used.(Wild, Martin et al. 2002) A series of gradient echo images for each slice was acquired at echo times (TE) ranging from 5 ms to 55 ms in 10 ms intervals for a total of six images and a repetition time of 428 ms. With the aid of sagittal gradient echo scout images to guide slice placement, two sets of images were acquired. The first set was comprised of four 5 mm thick slices on a plane at 45° from a plane containing the anterior commissure (AC) and the posterior commissure (PC), as illustrated in Figure 2.1, with the PC placed in the center of the slab of slices. These images provided data for the red nucleus and substantia nigra. The second set of images consisted of five 5 mm thick slices with the slice plane placed 10º from the plane containing both the AC and PC (see Figure 2.1) with the PC placed approximately in the center of the slice slab. This second set of images provided data for the globus pallidus (GP) and putamen (Pu). The field of view for both sets of images was 300 x 300 mm^2 with 128 x 128 data points acquired and with the matrix subsequently zero-filled to 256 x 256 points for both sets of slices. The total scan time for each participant was approximately 30 minutes. In order to optimise the reproducibility of slice placement on follow-up MR scans, the axial images and orthogonal scout images for each set of slices for each subject on follow-up scans was compared directly to the baseline images to ensure

similar axial slice placement and anatomical localization of the 3-dimensional volume.

Image Processing and Analysis

Image processing and analysis was performed using custom software written in MATLAB (The MathWorks, Inc., Natick, MA). From the series of echo images, R_2* was determined using a voxel-wise least-squares fit to:

$$\ln(M) = (-R_2^*)(TE) + b$$

where M is the signal intensity for a particular voxel in the echo image acquired at TE and b is the intercept. In principle, $b = 0$ in this relationship; however it is included to account for experimental variability and improve the fit. Region specific R_2* values were measured by averaging the values obtained from the selected voxels. To avoid bias, the individual responsible for region-of-interest (ROI) analysis (MG) was blinded to subject group allocation. As described in our previous publication (Martin, Wieler et al. 2008a), ROIs were drawn on the substantia nigra pars reticulata (SNr) and SNc. The SNr and SNc were then subdivided into medial and lateral components. The SNr was identified as the band of low signal in the ventrolateral midbrain, while the region between the SNr and the red nucleus was considered to represent the SNc. In the forebrain, ROIs were drawn on the GP and Pu by reference to a standard neuroanatomical atlas (Talairach J 1988). ROI placement on follow-up scans was made visually in direct comparison with baseline scans to ensure similar anatomical localization.

Statistical Methods

Demographic data for patients and controls were compared using t-tests and Fisher exact tests. The R_2* values were compared between the controls and Parkinson disease subjects using a longitudinal repeated measures analysis of variance (rANOVA) where the within-subject factor was time point, with three levels (baseline, 18 months, and 36 months) and the between-subject factor was group, with two levels (controls and Parkinson disease). For each subject, we obtained a measure of R_2* change over time by performing a linear regression of R_2* value versus scan time (baseline, 18, and 36 month) to obtain R_2* slope (s^{-1}/month). The R_2* slope values were then compared to the change clinical measures (month 36 value - baseline value) using linear regression; the Pearson correlation coefficient (r^2) and associated p-value are reported. The clinical measures analysed consist of the UPDRS III, Timed Up and Go, 14 metre walk and PDQ39 mobility sub-score. The sequentially rejective Bonferroni correction for multiple comparisons was applied to the p-values. Individual results for Parkinson disease participants' R_2* slope and UPDRS III were analysed with a one way ANOVA followed by pairwise multiple comparisons using the Holm-Sidak method to examine relationship between these parameters. Statistical analysis was performed using IBM SPSS Statistics Version 21.0 for Windows (IBM Corp., Armonk, NY).

Results

At baseline, the 19 Parkinson disease and 13 control subjects did not differ with respect to gender, age, MMSE score or measures of gait and balance ability as measured by Timed Up and Go, the 14 metre walk test, and the Berg Balance Test. There was a significant difference in education with the control group having a

higher level of education. The demographic information and clinical status of the cohort are summarized in Table 2.1. At baseline, none of the group of early, mildly affected Parkinson disease participants required symptomatic treatment. At month 36, all but 5 of the 19 Parkinson disease subjects had begun dopaminergic therapy. Two subjects were taking a dopamine agonist alone, 10 subjects were taking levodopa/carbidopa and 2 were taking a combination of levodopa/carbidopa and entacapone.

At baseline, a difference in measured R_2* values was observed in the lateral SNc between control and Parkinson disease subjects ($p = 0.035$) using an independent t-test. Other brain regions studied did not show baseline differences in R_2* values. This baseline significant difference between groups in the measured R_2* values in the lateral SNc was no longer evident at month 36. When looking at the lateral SNc R_2* values for the individual subjects over time, we see that each group has a large variance at each time point and that at 36 months, the variance in the Parkinson disease group (mean 26.3 ± 5.0) is larger than that in the controls (mean 25.9 ± 3.9) (Figure 2.2).

In the Parkinson disease cohort, there were significant correlations between the measured R_2* change in the lateral SNc and the change score in some of the clinical measures from baseline to 36 months (Table 2.2). The correlations were significant for the change in UPDRS III score ($p = 0.008$; Figure 2.3), and the PDQ-39 mobility sub-score ($p = 0.03$; figure 2.4). Subjects with lower UPDRS III scores at baseline tended to show a decrease in measured R_2* with time; those with a higher score showed a significant increase in R_2* ($p = 0.015$) (Table 2.3). Other

change scores in clinical measures of disease severity showed no correlation with measured R_2* changes.

Discussion

This longitudinal study followed control subjects and patients with early, mild Parkinson disease, who did not require symptomatic treatment at baseline, for 36 months utilizing high field MR imaging to quantify R_2* as an estimate of midbrain iron content. In the Parkinson disease group we correlated changes in measured R_2* values with changes in clinical measures of various aspects of disease severity, focussing on measures relating to mobility.

We previously published baseline data for this cohort and demonstrated increased R_2* values in the lateral SNc in the Parkinson disease group, consistent with a local increase in regional iron content (Martin, Wieler et al. 2008a). The close relationship between this MR measure and direct quantitation of regional iron content (Martin, Wieler et al. 2008a) correlates with the extant neuropathological evidence of selective degeneration of neurons in this region in Parkinson disease (Fearnley, Lees 1991). In contrast to the baseline findings, however, our longitudinal data do not show a significant difference in measured R_2* in the lateral SNc between the Parkinson disease and control groups at month 36. The data do show a large variance in measured R_2* values in both groups at both baseline and at 36 months. This could be a reflection of the natural variation in regional iron content (Hallgren B 1958) and may account for our inability to demonstrate a significant change at month 36 between the Parkinson disease and control groups in this relatively small group of subjects.

The variance between the groups is dissimilar with a larger variance in the Parkinson disease group than in the control group (Figure 2.2). This suggests that the Parkinson disease group may not be homogeneous, despite relatively similar disease duration amongst included patients. The range of baseline UPDRS III scores, 7 - 26, suggests heterogeneity in terms of clinical disease severity. One would expect a corresponding degree of heterogeneity in the underlying neuropathology. The R_2* heterogeneity that we have observed may be a direct reflection of the underlying variability in neuropathology. An alternate explanation for the lack of a significant difference between controls and patients at 36 months is the possibility of a floor effect due to the marked loss of nigral cells associated with disease progression in the Parkinson disease group or to a ceiling effect related to striatal iron accumulation.

Additional support for the presence of heterogeneity in this patient group comes from the R_2* slope values. Patients with less severe disease at 36 months, on the basis of their UPDRS III scores, tended to have a negative R_2* slope, whereas more severely affected participants had positive slopes. This suggests a difference in midbrain pathology between these subjects with increased nigral iron content being closely associated with increased severity of motor features of the disease.

Iron is only one contributor to the R_2* signal; it may be that other processes are occurring in the 36 months between scans affecting the R_2* values. For example, disease-related tissue changes including neuronal loss may result in an increase in free water, thereby altering R_2* values (Grabill, Silva et al. 2003).

The precise location of the substantia nigra and the anatomical boundaries separating the SNc and the SNr are not easily distinguished on MRI (Oikawa, Sasaki et al. 2002, Duguid, De La Paz et al. 1986, Stern, Braffman et al. 1989), particularly in subjects with Parkinson disease where there is progressive neurodegeneration, particularly in SNc. This imprecise localisation may account for inconsistencies in the literature with respect to iron content and measured R_2* in the substantia nigra in Parkinson disease. In this longitudinal study, we felt it was important to retain the same localisation of substantia nigra and placement of ROIs as in our previously published cross-sectional data and took care to place ROIs on follow-up studies in precisely the same location as in baseline scans. If imprecision in ROI placement was introduced in our original methodology, it will be reproduced in the longitudinal data. Perhaps with refined techniques or higher field strength systems providing better spatial resolution, more accurate delineation of these midbrain structures will be possible in the future.

Most previous data regarding midbrain iron content in Parkinson disease have come from cross-sectional studies; few longitudinal studies have been reported. A recent publication looking at R_2* with a 1.5 tesla MRI system (Ulla, Bonny et al. 2013) reported a significant increase in measured R_2* in the substantia nigra, caudate and putamen that correlated with clinical markers of disease progression over 3 years. The authors concluded that R_2* was a promising tool for monitoring disease progression and treatment effects related to various interventions. Patients in this study, however, presumably had more advanced Parkinson disease than did our patients since they were receiving treatment at the time of the initial evaluation. Furthermore, this report does not include valid clinical

evidence of disease severity since motor evaluations were done in the "on" state and were therefore subject to the effects of symptomatic medication. In contrast, our data indicate that there are limitations to the potential for R_2* for monitoring disease progression and treatment since.

A more recent paper (Rossi, Ruottinen et al. 2014) described a 2 year follow-up of Parkinson disease-related changes in iron content as measured by R_2*. These investigators were unable to confirm a correlation between MRI parameters and either clinical measures or a change in clinical measures. The study methodology, particularly with respect to patient characteristics and evaluation, however, make comparisons to our cohort difficult.

Although the absolute measures of R_2* in our data do not show an increase in the SNc with time, we did observe a significant change over 36 months in the Parkinson disease group that correlates to measures of disease severity that reflect decline in motor function. These results support the notion that nigral iron content does indeed correlate with the progression of motor symptomatology in Parkinson disease. However, the underlying conundrum that faces us is how iron is involved in the neurodegeneration of nigral cells in Parkinson disease: does increased iron content point to an underlying cause or is it merely a marker of dying neurons? This question cannot be answered from our data alone.

Our data indicate heterogeneity of the Parkinson disease group. The development of a marker of disease progression that can account for the heterogeneity seen in most patient groups is important to evaluate trials both of symptomatic treatment and of potential neuroprotective compounds in Parkinson disease. This heterogeneity may explain, in part, the lack of efficacy in large

clinical trials that fail to support promises seen in animal work. Measures of R_2* reflecting midbrain iron content, given our observations, may not provide a sufficiently sensitive measure to evaluate disease progression in small patient groups but, if measured at baseline in the setting of a clinical trial, could help decrease the heterogeneity of the study group. Larger and longer investigations with more precise mapping of iron-containing midbrain structures are needed to parse out the potential value of R_2* as a biomarker of disease progression in Parkinson disease.

Acknowledgements

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Tables

Table 2.1: Demographic and baseline characteristics of participants

	Control Subjects	Parkinson disease Subjects	p-value
Subjects (female, male)	13 (5, 8)	19 (5, 14)	n.s.
Baseline age, years (mean ± SD)[range]	56.0 ± 6.9 [46.1 - 66.5]	59.8 ± 7.3 [45.0 - 72.1]	n.s.
Time between baseline and month 36 scan, years (mean ± SD) [range]	3.1 ± 0.2 [2.6 – 3.3]	3.0 ± 0.2 [2.4 – 3.3]	n.s
Education, years (mean ± SD)[range]	18.0 ± 2.8 [14 - 23]	13.7 ± 2.6 [11 - 20]	0.0001
MMSE (mean ± SD)[range]	29.8 ± 0.4 [29 - 30]	28.7 ± 1.9 [24 - 30]	n.s
Disease duration at baseline, years (mean ± SD) [range]	-	1.8 ± 1.3 [0.0 - 5.6]	-
TUG at baseline (mean ± SD) [range]	7.8 ± 1.5 [6.0 - 11.7]	8.6 ± 1.3 [7.0 – 12.4]	n.s
14 m walk (mean ± SD) [range]	11.4 ± 2.0 [7.8 – 14.8]	11.7 ± 1.5 [9.5 – 15.5]	n.s
UPDRS III at baseline (mean ± SD) [range]	-	14.3 ± 5.1 [7 - 26]	-
Berg Balance Score (mean ± SD) [range]	-	55.2 ± 1.5 [49 - 56]	-
PDQ-39 Score (mean ± SD) [range]	-	20.2 ± 13.0 [4 - 62]	-

Abbreviations:
MMSE = Mini-Mental State Exam; **TUG** = Timed Up and Go; **UPDRS** = Unified Parkinson's Disease Rating Scale; **PDQ-39** = Parkinson's Disease Questionnaire-39

Table 2.2: Substantia nigra pars compacta R_2* change correlations with change in clinical scores

Region	UPDRS III score change[1]		TUG time change[1]		14m speed change[1]		BBS total change[1]		PDQ-39 mobility subscore change[1]	
	r^2	p-value	r^2	p-value	r^2	p-value	r^2	p-value	r^2	p-value
medial SNc	0.1	0.1	0.0	0.9	0.08	0.3	0.04	0.4	0.3	0.01
lateral SNc	0.4	0.008*	0.04	0.4	0.0	1.0	0.04	0.4	0.3	0.03*

[1]change = Month 36 value - Baseline value
*significant after correction for multiple comparisons using sequentially rejective Bonferroni method

Abbreviations:
r^2 = Pearson product-moment correlation squared; **UPDRS III** = Unified Parkinson's Disease Rating Scale, Part III; **TUG** = Timed Up and Go; **BBS** = Berg Balance Scale; **PDQ-39** = Parkinson's Disease Questionnaire-39; **SNc** = substantia nigra pars compacta

Table 2.3: Longitudinal Individual Results of UPDRS III and R_2* Slope

UPDRS III			R_2^* Slope		
Baseline	36 Months	Change	Baseline	36 Months	Slope
9	***11***	***2***	***23.76***	***18.31***	***-0.151***
11	17	6	28.37	23.68	-0.130
13	***28***	***15***	***24.93***	***20.66***	***-0.119***
15	14	-1	26.96	22.91	-0.112
12	17	5	27.14	24.16	-0.083
8	22	14	24.23	21.39	-0.079
12	***23***	***11***	***28.04***	***26.04***	***-0.065***
18	29	11	32.28	29.95	-0.065
10	***16***	***6***	***24.25***	***22.39***	***-0.052***
7	17	10	23.57	21.95	-0.045
11.5[1,3]	**19.4**[1,4]	**7.9**[5]	**26.35**[6]	**23.14**[7]	
26	40	14	25.57	25.88	0.009
16	37	21	26.67	27.31	0.018
15	31	16	24.94	26.81	0.052
21	34	13	25.12	26.98	0.052
22	30	8	23.16	25.08	0.053
10	26	16	33.67	36.08	0.067
16	39	23	23.70	28.89	0.144
12	***29***	***17***	***26.20***	***33.72***	***0.209***
19	36	17	25.83	36.66	0.301
17.4[2,3]	**33.6**[2,4]	**16.1**[5]	**26.10**[6]	**29.71**[7]	

Participants in bolded italics remained untreated for the duration of the study.

Green bands denote mean values for the column above.

[1] Negative R2* slope: baseline vs 36 month UPDRS III: p = 0.002
[2] Positive R2* slope: baseline vs 36 month UPDRS III: p < 0.001
[3] Baseline UPDRS III: negative vs positive R2* slope: p = 0.046
[4] 36 month UPDRS III: negative vs positive R2* slope: p < 0.001
[5] UPDRS III change: negative vs positive R2* slope: p = 0.002
[6] Baseline R2*: negative vs positive R2* slope: p = 0.897
[7] 36 month R2*: negative vs positive R2* slope: p = 0.032

Figure Legend

Figure 2.1: Schematic showing slice placement.

For the substantia nigra, four slices were placed parallel to the line labeled "a" and for the putamen and globus pallidus, five slices were placed parallel to the line labeled "b."

AC = anterior commissure; **PC** = posterior commissure

Figure 2.2: Lateral substantia nigra pars compacta R_2^* value vs time for each subject.

Circles depict individual R_2^* values for subjects with Parkinson's disease at baseline, 18 months and 36 months; Squares depict individual values for control subjects at the same time points.

Figure 2.3: Lateral substantia nigra pars compacta (SNc) R_2* change vs change in UPDRS III score.

The regression line was determined with the least squares method (solid line); the 95% CI is indicated. R_2* change indicates the slope of the change in R_2* over baseline, 18 month and 36 month scans (see text). Unified Parkinson's Disease Rating Scale motor (UPDRS III) score change was calculated as the 36 month score - baseline score.

Figure 2.4: Lateral substantia nigra pars compacta (SNc) R_2* change vs change in PDQ-39 mobility subscore.

The regression line was determined with the least squares method (solid line); the 95% CI is indicated. R_2* change indicates the slope of the change in R_2* over baseline, 18 month and 36 month scans (see text). PDQ-39 mobility subscore change was calculated as the 36 month score - baseline score.

Figures

Figure 2.1: Schematic showing slice placement

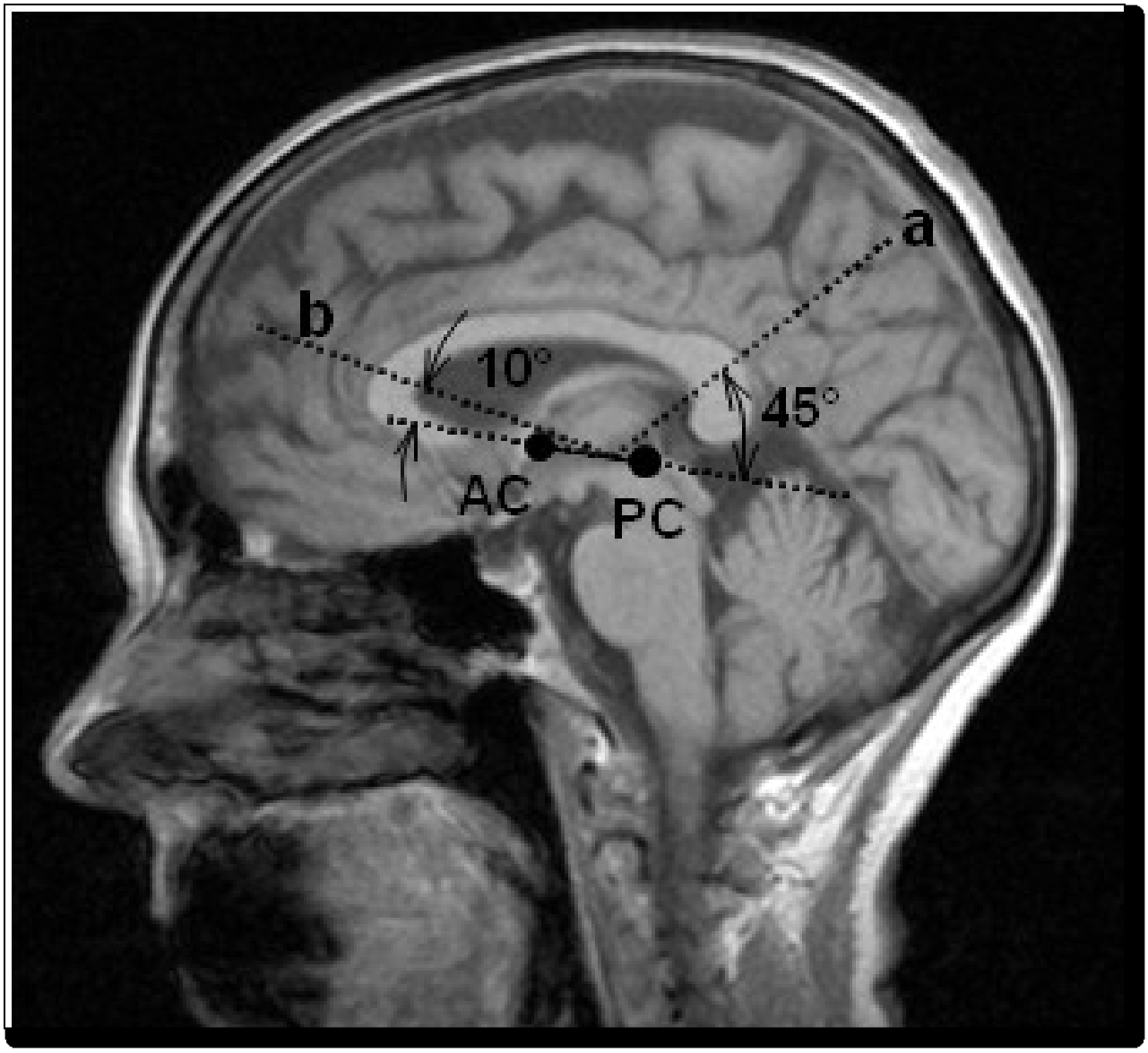


Figure 2.2: Lateral substantia nigra pars compacta R_2^* value vs time for each subject

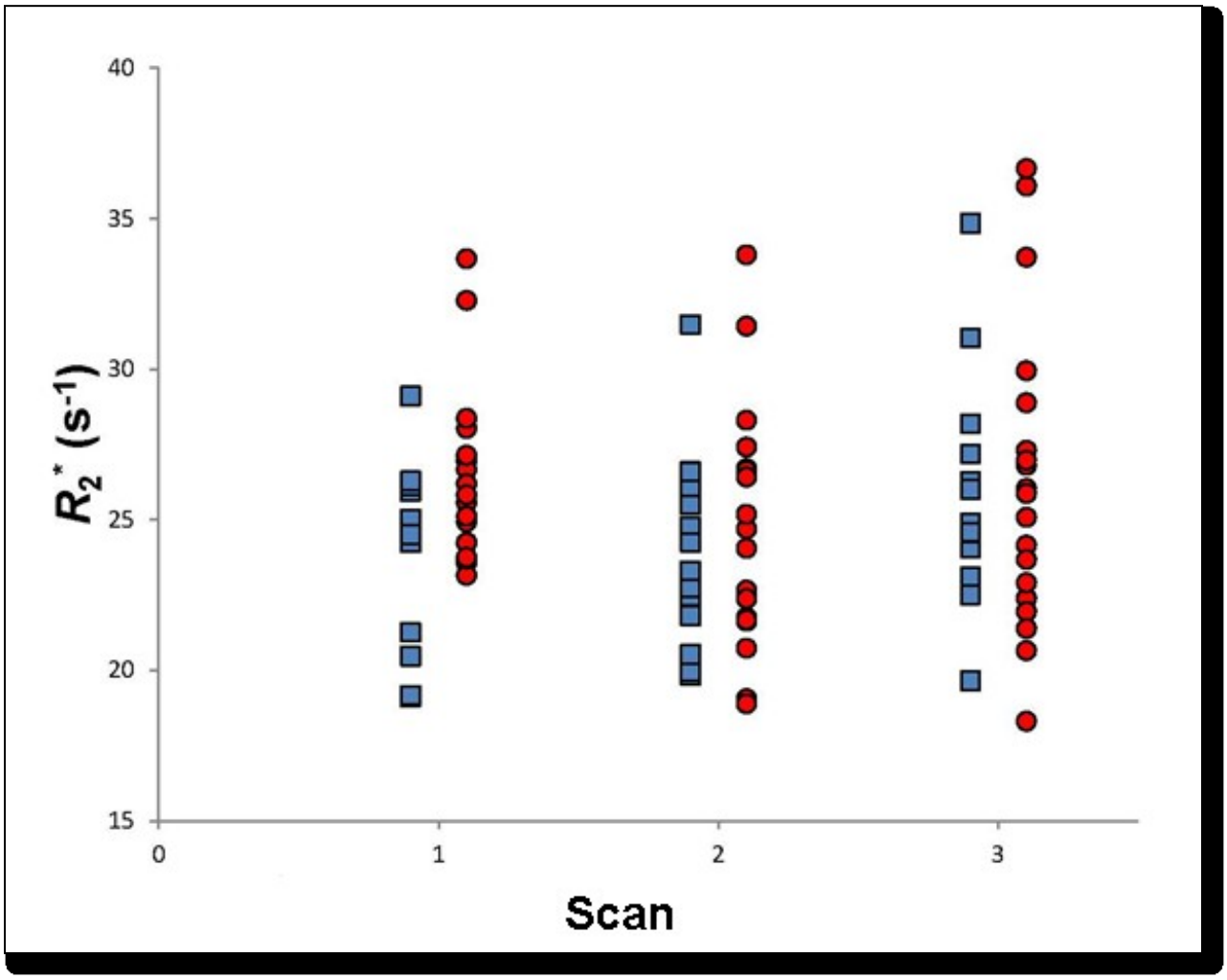


Figure 2.3: Lateral substantia nigra pars compacta (SNc) R_2* change vs change in UPDRS III score.

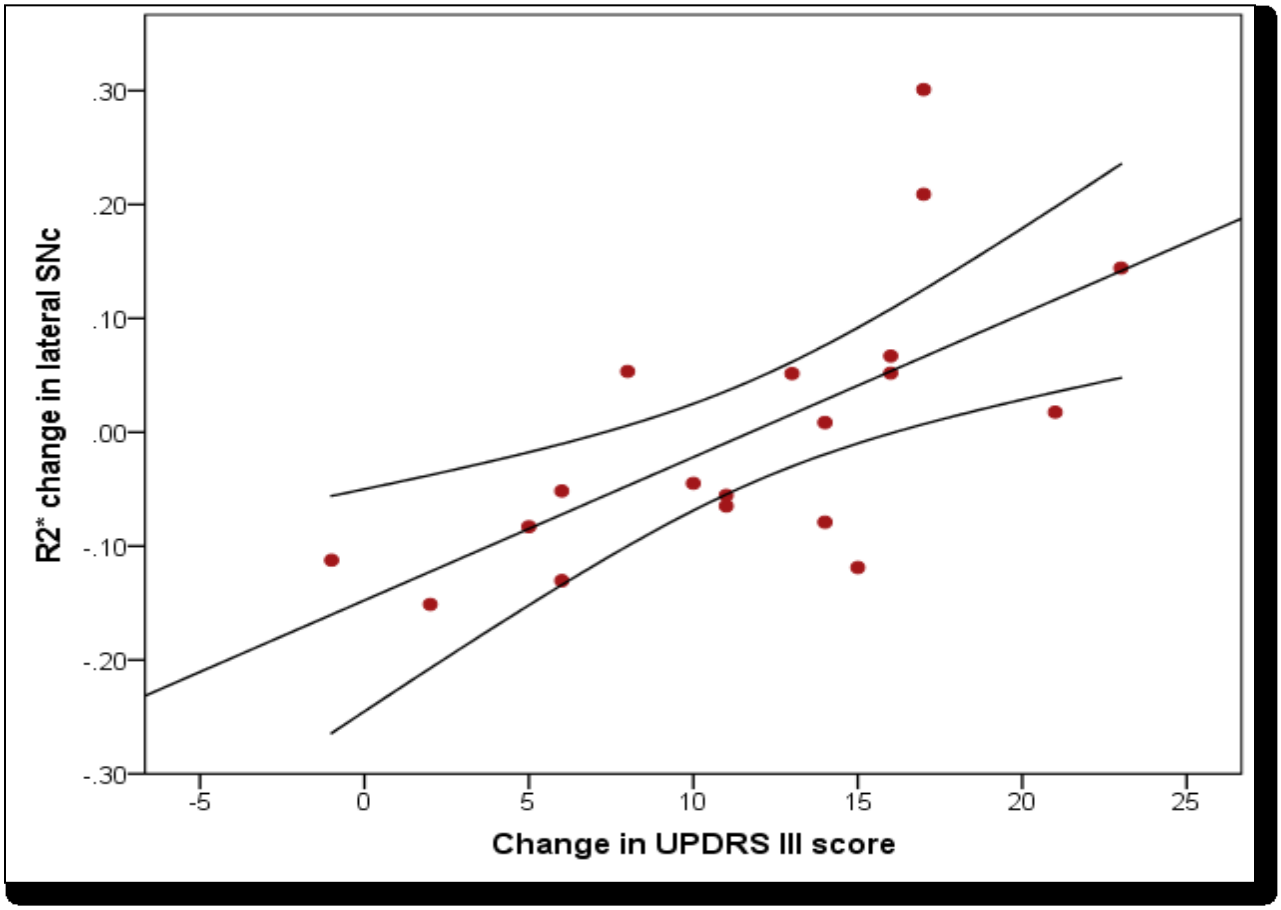
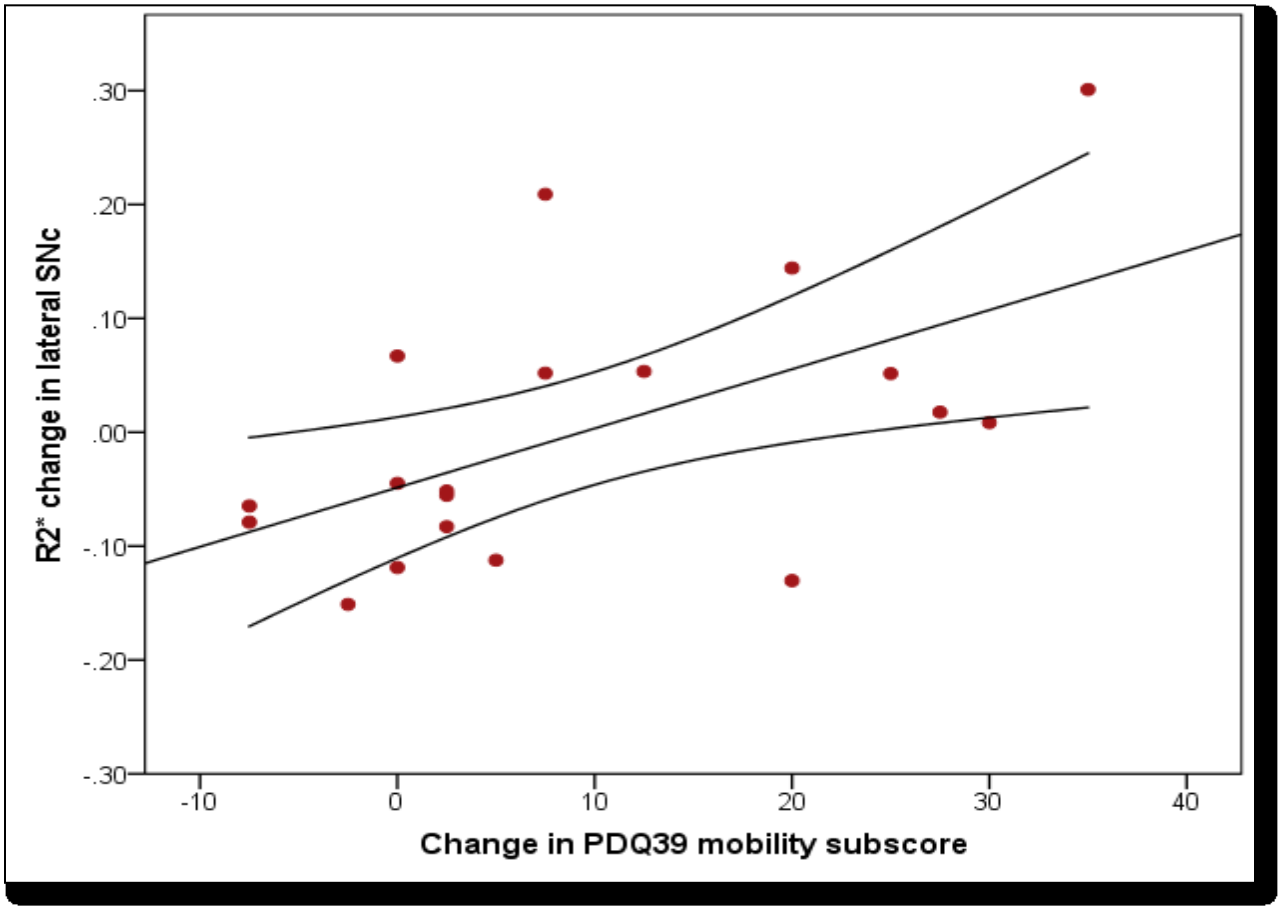


Figure 2.4: Lateral substantia nigra pars compacta (SNc) R_2* change vs change in PDQ-39 mobility subscore.



Chapter 3: Freezing of Gait in Early Parkinson's Disease: Nigral Iron Content Estimated from MRI

This chapter is in preparation for submission:

Wieler M, Gee M, Camicioli R, Martin WRW. Freezing of Gait in Early Parkinson's Disease: Nigral Iron Content Estimated from MRI

Abstract

Objective: To determine whether evolving changes in nigral iron content and their association with declining motor function in early Parkinson's disease differentiated subjects who developed freezing of gait (FOG) over three years of follow-up from those who did not.

Methods: A cohort of previously untreated individuals with early Parkinson disease (n = 19) was followed clinically for 36 months with clinical evaluations and MRI scans performed at baseline, 18 and 36 months. A multiple gradient echo sequence designed for rapid single-scan mapping of proton transverse relaxation rate R_2* provided an index of local iron content in the basal ganglia. The cohort was divided into two groups based on the development of FOG during 36 months of follow-up.

Results: There were significant baseline differences between those who developed FOG (n=7) and those who did not (nFOG; n=12) in Unified Parkinson's Disease Rating Scale (UPDRS) III scores, time to complete a 14 m walk and Timed Up and Go. There was a significant correlation between measured R_2* change values in the lateral substantia nigra pars compacta (SNc) and the change in UPDRS III score ($p = 0.002$) from baseline to 36 months. Those in the FOG group showed a greater change in UPDRS III score and lateral SNc iron content than those in the nFOG group.

Conclusions: Individuals destined to develop FOG early in Parkinson disease have more motor impairment at baseline and more rapid deterioration in motor function and SNc changes suggestive of increased iron content in comparison to those who

do not develop FOG suggesting a difference in midbrain pathology between these groups.

Introduction

Parkinson disease is a common neurodegenerative disorder. It is both a motor disorder characterized by varying combinations of rest tremor, rigidity, bradykinesia, and gait/balance impairment and a non-motor disorder with features including disturbances of speech, autonomic function, sleep, cognition and mood (Hely, Morris et al. 1999).

The primary change underlying many of the motor features of Parkinson disease is a gradual loss of dopaminergic neurons projecting from substantia nigra compacta (SNc) to striatum, resulting in decreased striatal dopamine content. A decrease of ~80% of striatal dopamine precedes the emergence of clinical features of Parkinson disease; motor symptoms then gradually worsen with further neuronal loss (Marsden 1992). Lateral SNc neuronal loss has been reported to be particularly correlated with the clinical motor features of Parkinson disease (Greffard, Verny et al. 2006, Lee, Schulzer et al. 1994). We have previously shown a significant correlation in early Parkinson disease between increasing iron content in the lateral SNc (estimated from MRI) and measures of disease severity that reflect a longitudinal decline in motor function over three years (Wieler, Gee, Martin, 2014).

An important source of disability in Parkinson disease that emerges and worsens with disease progression is gait impairment, including freezing of gait (FOG) (Giladi, McMahon et al. 1992). Freezing of gait refers to a transient reduction or absence of forward progression when there is an intention to walk (Giladi, McMahon et al. 1992). It manifests as start hesitation, turning hesitation, target hesitation and open-area hesitation. The unpredictable nature of freezing

episodes is associated with increased falls, negatively impacting health-related quality of life. The response of FOG to dopaminergic replacement therapy is variable and complicated. In some individuals, FOG episodes will improve with levodopa ("off" freezing) while other episodes ("on" freezing) may be resistant to or even exacerbated by the addition of levodopa. Little is known about the pathological substrates underlying this debilitating feature of Parkinson disease.

While many reports of early Parkinson disease rely on disease duration to define a group as "early", there is often little attention paid to potential clinical heterogeneity within a cohort with relatively similar disease duration. The primary objective of this study was to determine if changes in nigral iron content were associated differently with declining motor function in a cohort of previously untreated Parkinson disease subjects divided into two subgroups: one who developed FOG over three years of follow-up compared to one who did not. We used a high-field MRI [3 tesla] method designed to minimize magnetic field inhomogeneities arising from tissue-tissue and air-tissue interfaces thereby improving the specificity of transverse relaxation measurements, R_2*, for changes in iron content (Wild, Martin et al. 2002, Ye, Martin et al. 1996).

Subjects

The Parkinson disease participants presented in this study are part of a larger longitudinal project, other parts of which have been previously published (Martin, Wieler et al. 2008a, Martin, Wieler et al. 2009, Martin, Wieler et al. 2008b). We recruited 19 subjects with early, untreated Parkinson disease from the University of Alberta Movement Disorders Program in Edmonton, Alberta, Canada. All fulfilled standard criteria for a clinical diagnosis of Parkinson disease (Hughes, Daniel et al.

1992) as assessed by a movement disorders neurologist (WM). The subjects had participated in a previous study examining presynaptic dopaminergic function measured with dihydrotetrabenazine/positron emission tomography (PET) (Martin, Wieler et al. 2008c), and all exhibited striatal PET abnormalities that were consistent with a diagnosis of Parkinson disease. These mildly affected participants did not require symptomatic treatment with levodopa or other dopaminergic medications at the start of the study. If dopaminergic therapy was initiated for treatment of Parkinson disease symptoms after baseline assessments, subjects were subsequently examined in the clinically defined "off" state, a minimum of 12 hours after the last ingestion of dopaminergic medication at subsequent time points in order to obviate the effects of medication on underlying disease state. Clinical symptoms in the Parkinson disease cohort were rated with the Unified Parkinson's Disease Rating Scale (UPDRS) (Fahn, Elton et al. 1987) by one of two raters (MW and WM) who had a high inter-rater reliability (intra-class correlation coefficient for the total motor UPDRS = 0.99, determined in a separate group of patients with Parkinson disease). All follow-up assessments for subjects were rated by the same rater (MW or WM). All other clinical assessments were administered by one investigator (MW). A variety of tools were used to assess subjects including: Unified Parkinson's Disease Rating Scale (UPDRS) III (Fahn, Elton et al. 1987) mini-mental state exam (Folstein, Folstein et al. 1975) (MMSE), Frontal Assessment Battery (Dubois, Slachevsky et al. 2000) (FAB), Timed Up and Go (Podsiadlo, Richardson 1991) (TUG), 14 metre walk (with turn), Berg Balance Scale (Berg, Wood-Dauphinee et al. 1992) (BBS) and the Parkinson's Disease Questionnaire-39 (Peto, Jenkinson et al. 1998) (PDQ-39). The self-reported PDQ-39, assessing eight

areas of health and daily activities affecting quality of life, was used to calculate a single index score or individual sub-domains to give an indication of health-related quality of life. Participants were followed for 36 months with clinical evaluations and MRI scans completed at baseline, 18 and 36 months. The study was approved by the Human Research Ethics Board of the University of Alberta and all subjects gave written informed consent.

MRI Data Acquisition

MRI data were acquired using a Magnex 3 tesla research magnet (Magnex Scientific Ltd., Abingdon, U.K.) with actively shielded gradients, controlled with a Surrey Medical Imaging Systems console (Surrey Medical Imaging Systems, Ltd., Guildford, U.K). We used a multiple gradient echo sequence designed for rapid and optimal single-scan mapping of the proton transverse relaxation rate, R_2^* ($R_2^* = 1/T_2^*$), developed by our group (Wild, Martin et al. 2002). A series of gradient echo images for each slice was acquired at echo times (TE) ranging from 5 ms to 55 ms in 10 ms intervals for a total of six images and a repetition time of 428 ms. With the aid of sagittal gradient echo scout images to guide slice placement, two sets of images were acquired. The first set was comprised of four 5 mm thick slices on a plane at 45° from a plane containing the anterior commissure (AC) and the posterior commissure (PC), as illustrated in Figure 3.1, with the PC placed in the center of the slab of slices. These images provided data for the red nucleus and substantia nigra. The second set of images consisted of five 5 mm thick slices with the slice plane placed 10º from the plane containing both the AC and PC (see Figure 3.1) with the PC placed approximately in the center of the slice slab. This second set of images provided data for the globus pallidus (GP) and putamen (Pu). The

field of view for both sets of images was 300 x 300 mm^2 with 128 x 128 data points acquired and with the matrix subsequently zero-filled to 256 x 256 points for both sets of slices. The total scan time for each participant was approximately 30 minutes. In order to optimise the reproducibility of slice placement on follow-up MR scans, the set of scout images for each subject on follow-up scans was compared directly to those obtained at baseline to ensure identical slice placement on longitudinal studies.

Image Processing and Analysis

Image processing and analysis was performed using custom software written in MATLAB (The MathWorks, Inc., Natick, MA). From the series of echo images, R_2* was determined using a voxel-wise least-squares fit to:

$$\ln (M) = (-R_2^*) (TE) + b$$

where M is the signal intensity for a particular voxel in the echo image acquired at TE and b is the intercept. In principle, b = 0 in this relationship; however, it is included to account for experimental variability and improve the fit. Averaged values from acquired from selected voxels gave a measure of region specific R_2* values. As previously described (Martin, Wieler et al. 2008b), region of interest (ROI)s were drawn on the substantia nigra pars reticulata (SNr), and SNc which were then subdivided into medial and lateral components. The SNr was identified as the band of low signal in the ventrolateral midbrain, while the region between the SNr and the RN was considered to represent the SNc. In the forebrain, ROIs were drawn on the GP and Pu by reference to a standard neuroanatomical atlas. ROI placement on follow-up studies was optimized by direct visual comparison to

those placed on baseline images. The individual responsible for region-of-interest (ROI) analysis (MG) was unaware of which subjects developed FOG to avoid potential bias.

Statistical Methods

Demographic data were compared using t-tests and Fisher exact tests. The R_2* values were compared between the subjects with Parkinson disease nFOG and Parkinson disease FOG using a longitudinal repeated measures analysis of variance (rANOVA) where the within-subject factor was the time point, with three levels (baseline, 18 months, and 36 months,) and the between-subject factor was group with two levels. For each subject, we obtained a measure of R_2* change over time by performing a linear regression of R_2* value versus scan time (baseline, 18, and 36 month) to obtain R_2* slope (s^{-1}/scan). The R_2* slope values were then compared to the change clinical measures (month 36 value - baseline value) using linear regression; the Pearson correlation coefficient, r^2, and associated p-value are reported. The clinical measures reported include the UPDRS III, Timed Up and Go, FAB, BBS, 14 metre walk and PDQ-39 mobility sub-score. The sequentially rejective Bonferroni correction for multiple comparisons was applied to the p-values. Statistical analysis was performed using IBM SPSS Statistics Version 21.0 for Windows (IBM Corp., Armonk, NY).

Results

Baseline demographics and clinical status of the cohort are summarized in Table 3.1. Longitudinal clinical results are reported in Table 3.2. The development of FOG is based on UPDRS II #14 >1 (rare freezing when walking, may have start

hesitation). The two groups, i.e. those who developed FOG (n=7) during 36 months of follow-up and those who did not (nFOG; n=12), did not differ at baseline with respect to age, disease duration, education or cognitive status (as reflected by the MMSE score or the PDQ39. There were, however, significant baseline differences between the groups in UPDRS III scores, BBS, time to complete a 14 m walk with turn and TUG.

By month 36, only 6/12 nFOG subjects had initiated dopaminergic therapy while all 7 FOG participants had initiated dopaminergic therapy. The FOG group had a significantly higher levodopa equivalent dose (Tomlinson, Stowe et al. 2010) (FOG 501 mg ± 178; nFOG 231 mg ± 236, p = 0.02; Table 3.3). All participants reported a UPDRS II #14 of 0 (no freezing) at baseline. By month 36, 2 subjects in the FOG group reported UPDRS II #14 score of 1 (rare freezing when walking, may have start hesitation), 3 a score of 2 (occasional freezing when walking), and 2 a score of 3 (frequent freezing; occasionally falls because of freezing). Two FOG subjects and 3 nFOG subjects had a baseline score of 1 on UPDRS III #29 (walks slowly, may shuffle with short steps but no festination or propulsion). By month 36, 5 nFOG subjects had a score of 1 and 5 in the FOG group had a score of 1 and 2 had a score of 2 (walks with difficulty but requires little or no assistance; may have some festination, short steps or propulsion) on UPDRS III #29. No subject reported any falls (UPDRS II #13 = 0) at baseline; 1 subject in each group reported a UPDRS II #13 score of 1 (rare falling) at 36 months.

At baseline, an independent t-test showed a difference in measured R_2* values in the lateral SNr (p = 0.004) between the FOG and nFOG groups. Other

brain areas (lateral or medial SNc, GP or Pu) showed no difference in measured R_2* values between the groups.

With the application of a longitudinal model, there were significant differences between the 2 groups in the lateral SNr (p=0.006), the lateral SNc (p=0.04), and the medial SNc (p=0.04). There were no differences found in the medial SNr, GP or Pu. There was a significant scan by group interaction in the lateral SNc (p = 0.03). (See Tables 3.4)

After correction for multiple comparisons, there was a significant correlation between the measured R_2* change values (month 36 - baseline) in the lateral SNc and the change in the UPDRS III score (p = 0.002) from baseline to 36 months (See Figure 3.2). Those in the FOG group showed a greater change in UPDRS III score than those in the nFOG group. Other change scores in clinical measures of disease severity showed no correlation with measured R_2* change values in any other region of the substantia nigra.

Discussion

This longitudinal study followed subjects with early, mild Parkinson disease, not requiring symptomatic treatment at baseline, for 36 months. The group was divided into two cohorts: those who developed FOG and those who did not. All subjects were examined in the clinically defined "off" condition to decrease symptomatic effects of medication on evaluation of the underlying disease process, though it is recognized that lingering effects on symptoms may remain after such a short time of medication withholding (Hauser, Holford 2002).

Utilizing high field MR imaging to quantify R_2* as an estimate of nigral iron content and a battery of clinical assessments to evaluate disease severity, we

evaluated the changes occurring over time in these two groups to determine if the two groups evolved differently with respect to these measures.

In keeping with our previously published data (Wieler, Gee, Martin, 2014), the entire Parkinson disease cohort demonstrated increasing R_2* values in the lateral SNc with time, consistent with a local increase in iron content. Interestingly, while the R_2* values of both groups changed over time, the measured change was different between those who reported FOG at 36 months and those who did not, suggesting a pathological process that differed in the two groups. Other regions in the substantia nigra, including the lateral SNr and the medial SNc, also showed differences in measured R_2* between the groups.

The increase in measured R_2* for the lateral SNr, although different between the groups, was not associated with disease progression. Previous studies using transcranial sonography (TCS) have been reported to show an increase in echogenicity of the substantia nigra in more than 90% of Parkinson disease patients (Berg, Siefker et al. 2001). This hyperechogenic signal is thought to reflect abnormal iron accumulation in the substantia nigra, based on evidence from both animal and post mortem studies (Berg, Becker et al. 1999). In a five-year longitudinal study, the area of substantia nigra hyperechogenicity detected with TCS was shown not to change as Parkinson disease symptoms progressed (Berg, Merz et al. 2005). Although the technique of TCS does not allow for differentiation of the substantia nigra into the SNc and SNr portions, the R_2* values we report in the lateral SNr that remain unchanged over 36 months may compliment the notion that an increase in iron content of the undifferentiated substantia nigra seen with TCS is a trait of Parkinson disease that is unrelated to progression of the disease.

When looking at the lateral SNc, we show that the two groups evolved differently over time with respect to disease progression as evidenced by the correlation of measured R_2* and measures of disease severity reflected in UPDRS III scores. The FOG group progressed more rapidly on this clinical measure, suggesting a difference in midbrain pathology in these subjects, with increased nigral iron content being closely associated with increased severity of motor features of the disease.

The determination of FOG can be difficult as it often does not appear during a clinic visit despite being reported by the individual as significantly disabling. Report by history remains the primary method of assessing the presence of FOG, aided by questionnaires (some with video to show what is meant by FOG) (Nieuwboer, Rochester et al. 2009, Giladi, Shabtaia et al. 2000) to help quantify frequency and severity. Our study relied on a single question on the UPDRS to divide the participants into FOG or nFOG cohorts, thereby potentially underestimating its true prevalence. We also did not differentiate between "on" freezing and "off" freezing introducing a potential limitation to the interpretation of this data. However, all participants were followed by a movement disorders specialist and were receiving medical treatment that was optimised by that physician. This would suggest that FOG was unlikely to be due to under-treatment of Parkinson disease symptoms.

There remains debate within the literature as to the precise location of the substantia nigra and how best to separate the SNc and SNr on MRI scans (Oikawa, Sasaki et al. 2002, Lotfipour, Wharton et al. 2012, Manova, Habib et al. 2009). The inexorable progression of the disease further complicates the identification of a structure with evolving pathological changes. This imprecision in defining midbrain

structures may have contributed to the heterogeneity of results in studies looking at iron content and measured R_2* values. The localisation of substantia nigra and placement of ROIs in the present study was identical to our previous reports of midbrain iron content (Martin, Wieler et al. 2008a) in order to maintain consistency within our studies. This does not, of course, preclude imprecision in our localisation nor differences compared to other reports in the literature.

The emergence of FOG is a complex and troublesome feature for individuals living with Parkinson disease affecting quality of life (Moore, Peretz et al. 2007) and contributing to risk of falling (Lim, van Wegen et al. 2008). Freezing can manifest in both the "off" and "on" states and is often refractory to standard medical treatment. It is a poorly understood phenomenon with the underlying pathophysiology yet to be fully elucidated. No one in this *de novo* group reported FOG at baseline yet, despite similar disease duration and baseline MMSE scores, 37% of subjects had developed FOG by 36 months.

This is the first longitudinal study, to our knowledge, to identify differences in measured R_2* values as a reflection of midbrain iron content to discriminate between subjects who developed FOG and those who did not. A marker that would help discriminate, early in the disease course, those destined to develop FOG in three years may be a helpful addition when designing clinical trials. This would have the effect of reducing the heterogeneity of trial cohorts and may accurately distinguish those who might benefit from a specific drug intervention or regime. Ours was a fairly small cohort. Larger cohorts followed for a longer time are needed to confirm increased SNc iron content, reflected by increased measured R_2* values, as a potential biomarker for the development of FOG in Parkinson disease.

Acknowledgements

The authors thank the subjects for their willing cooperation and Richard Camicioli for his critical comments. The authors have no competing interests. The study was funded by the Canadian Institutes of Health Research (CIHR).

Tables

Table 3.1: Demographic and baseline characteristics of cohort

	Parkinson disease no FOG	Parkinson disease FOG	p-value
Subjects (female, male)	12 (5, 8)	7 (1, 6)	n.s.
Baseline age (years) (mean ± SD)[range]	60.6 ± 8.5 [45.0 - 72.1]	58.5 ± 4.9 [51.6 - 63.7]	n.s.
Disease duration at baseline (years) (mean ± SD)[range]	1.8 ± 1.4 [0.5 - 5.6]	1.9 ± 1.1 [0.0 - 3.3]	n.s.
Time between baseline and Month 36 scan (years) (mean ± SD) [range]	3.1 ± 0.1 [2.9 – 3.3]	3.1 ± 0.1 [2.9 – 3.2]	n.s.
MMSE (mean ± SD)[range]	28.4 ± 1.9 [24 - 30]	28.3 ± 2.3 [25 - 30]	n.s.
Education, years (mean ± SD)[range]	13.7 ± 2.6 [11 - 20]	13.9 ± 2.7 [11 - 19]	n.s.
TUG (seconds) (mean ± SD) [range]	8.3 ± 0.7 [7.3 - 10]	9.8 ± 1.5 [9.0 – 12.4]	0.008
14 m walk (seconds) (mean ± SD) [range]	11.9 ± 0.9 [9.8 – 13]	13.3 ± 1.9 [9.5 – 15.5]	0.005
UPDRS III (mean ± SD) [range]	12.2 ± 4.0 [7 - 22]	18.0 ± 5.0 [10 - 26]	0.01
Berg Balance Score (mean ± SD) [range]	55.8 ± 0.4 [55 - 56]	54.0 ± 2.3 [49 - 56]	0.01
PDQ-39 Score (mean ± SD) [range]	17.8 ± 15.4 [4 - 62]	20.3 ± 13.3 [7 - 44]	n.s.

Abbreviations:
MMSE = Mini-mental state exam; **TUG** = Timed Up and Go; **UPDRS** = Unified Parkinson's Disease Rating Scale; **PDQ-39** = Parkinson Disease Questionnaire-39

Table 3.2: Longitudinal Clinical Results

Assessment		Parkinson disease nFOG	Parkinson disease FOG
UPDRS III (mean ± SD)	baseline	12.17 ± 4.0	18.0 ± 4.9
	month 18	18.7 ± 7.0	28.4 ± 8.0
	month 36	21.3 ± 6.9	34.4 ± 5.1
TUG (seconds) (mean ± SD)	baseline	8.3 ± 0.72	9.8 ± 1.5
	month 18	10.0 ± 1.4	10.6 ± 1.5
	month 36	10.5 ± 1.6	13.6 ± 4.2
14 m walk (seconds) (mean ± SD)	baseline	11.2 ± 0.9	13.3 ± 1.9
	month 18	13.1 ± 1.8	15.8 ± 3.4
	month 36	14.5 ± 3.5	16.7 ± 4.1
Berg Balance Score (mean ± SD)	baseline	55.8 ± 0.4	54.0 ± 2.3
	month 36	55.5 ± 0.5	53.4 ± 1.7
	month 36	54.3 ± 0.8	51.7 ± 1.7
PDQ-39 Score (mean ± SD)	baseline	17.8 ± 15.4	20.3 ± 13.3
	month 18	26.36 ± 18.9	22.7 ± 7.1
	month 36	26.36 ± 18.1	37.0 ± 8.5

Abbreviations:
UPDRS = Unified Parkinson's Disease Rating Scale; **TUG** = Timed Up and Go; **PDQ-39** = Parkinson Disease Questionnaire-39

Table 3.3: Levodopa equivalent dose at 36 Months

	Parkinson disease no FOG (n=12)	Parkinson disease no FOG LED mg	Parkinson disease FOG (n=7)	Parkinson disease FOG LED mg
no antiparkinsonian medication	5	0	0	0
Levodopa	4	475 ± 126*	3	450 ± 150
dopamine agonist (pramipexole or ropinirole)	1	120	1	400
levodopa plus dopamine agonist (pramipexole or ropinirole)	0	0	3	587 ± 260
levodopa and **rasagiline**	1	550	0	0
amantadine	1	200	0	0
LED Total[1]		**231 ± 236[#]**		**501 ± 178[#]**

* mean ± standard deviation
[1] Levodopa equivalent dose
[#] significance p = 0.02

Table 3.4: Longitudinal Results R_2* (s^{-1}) ± standard deviation

Region of Interest		Controls	Parkinson disease	Parkinson disease nFOG	Parkinson disease FOG
SNc-medial	baseline	26.1 ± 4.7	28.2 ± 6.4	26.8 ± 5.1	30.7 ± 8.0
	month 18	26.2 ± 6.3	30.8 ± 6.4	28.5 ± 3.2	34.9 ± 8.5
	month 36	29.2 ± 7.6	29.3 ± 5.2	27.3 ± 4.1	32.8 ± 5.1
SNc-lateral	baseline	23.9 ± 3.0	26.2 ± 2.8	25.5 ± 1.8	27.6 ± 3.8
	month 18	23.8 ± 3.2	24.5 ± 4.0	23.6 ± 2.9	26.2 ± 5.1
	month 36	25.9 ± 3.9	26.3 ± 5.0	23.9 ± 3.9	30.2 ± 4.4
SNr-medial	baseline	38.4 ± 5.8	40.1 ± 6.4	38.8 ± 6.4	42.4 ±6.3
	month 18	34.5 ± 6.7	39.2 ± 4.1	37.8 ± 3.3	41.6 ± 4.5
	month 36	39.8 ± 6.3	38.8 ± 7.3	36.3 ± 6.4	43.2 ± 6.8
SNr-lateral	baseline	37.5 ± 4.6	35.0 ± 5.5	32.7 ± 5.7	38.8 ± 1.8
	month 36	34.2 ± 5.6	34.3 ± 5.9	31.7 ± 4.9	38.8 ± 4.6
	month 36	34.6 ± 4.1	37.3 ± 5.0	35.3 ± 4.8	40.8 ± 3.1
GP-whole	baseline	39.5 ± 4.3	37.0 ± 5.6	35.8 ± 5.1	39.2 ± 6.2
	month 18	38.0 ± 4.8	36.6 ± 5.3	35.3 ± 4.8	39.1 ± 5.7
	month 36	37.7 ± 4.3	37.3 ± 5.5	36.1 ± 4.9	39.5 ± 6.4
Pu-whole	baseline	30.9 ± 2.6	31.6 ± 5.0	30.3 ± 5.2	33.9± 3.7
	month 18	31.5 ± 3.8	32.3 ± 5.0	31.6 ± 4.9	33.5 ± 5.3
	month 36	32.0 ± 4.2	32.8 ± 5.0	31.7 ± 4.6	34.8 ± 5.4

Abbreviations:
SNc = Substantia nigra pars compacta; **SNr** = Substantia nigra pars reticulata; **GP** = Globus pallidus; **Pu** = Putamen

Figure Legend

Figure 3.1: Schematic showing slice placement

For the substantia nigra, four slices were placed parallel to the line labeled "a". For the putamen and globus pallidus, five slices were placed parallel to the line labeled "b."

AC = anterior commissure; **PC** = posterior commissure

Figure 3.2: Lateral substantia nigra pars compacta (SNc) R_2* change vs change in UPDRS III score

Figures

Figure 3.1: Schematic Showing Slice Placement

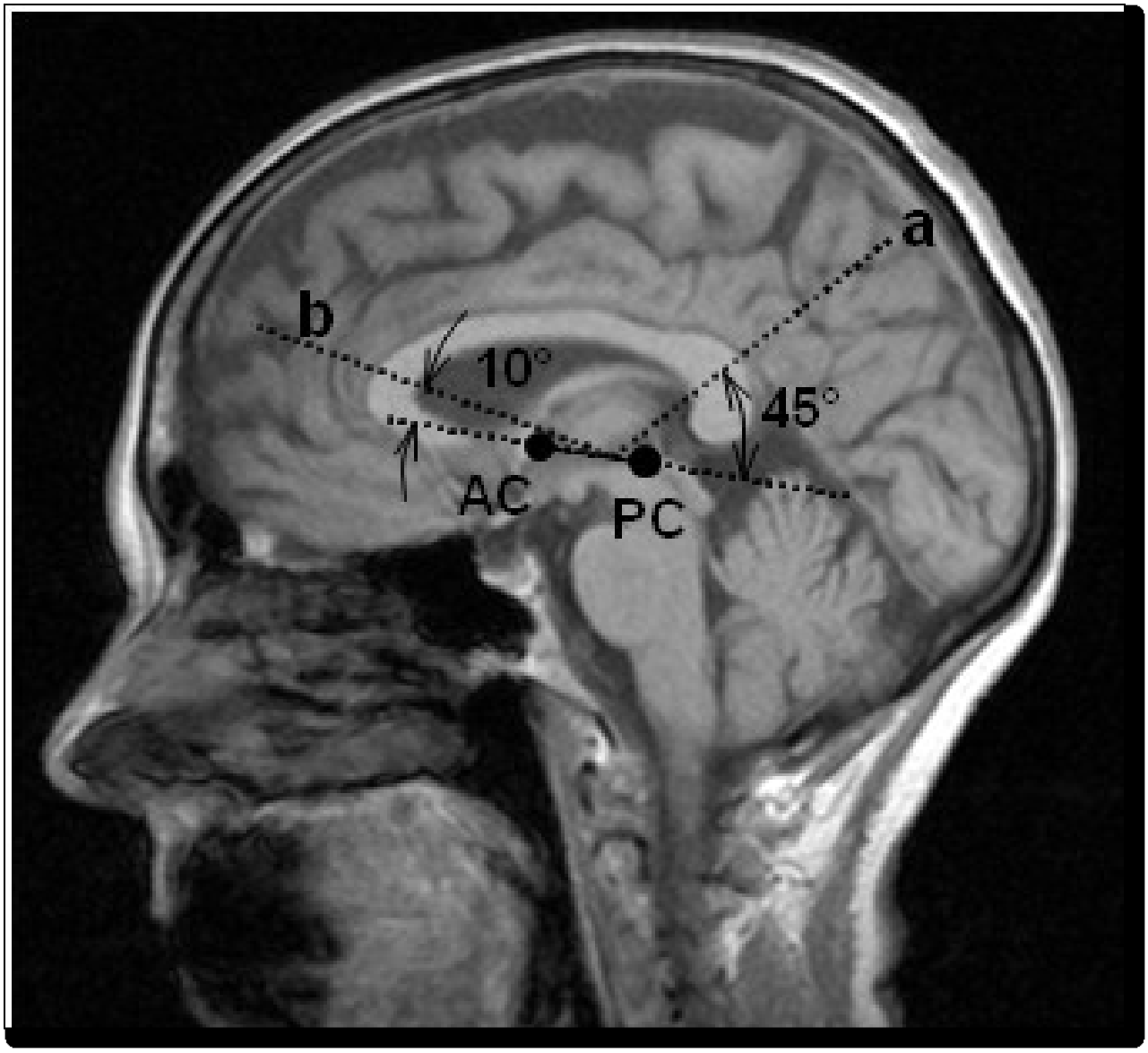
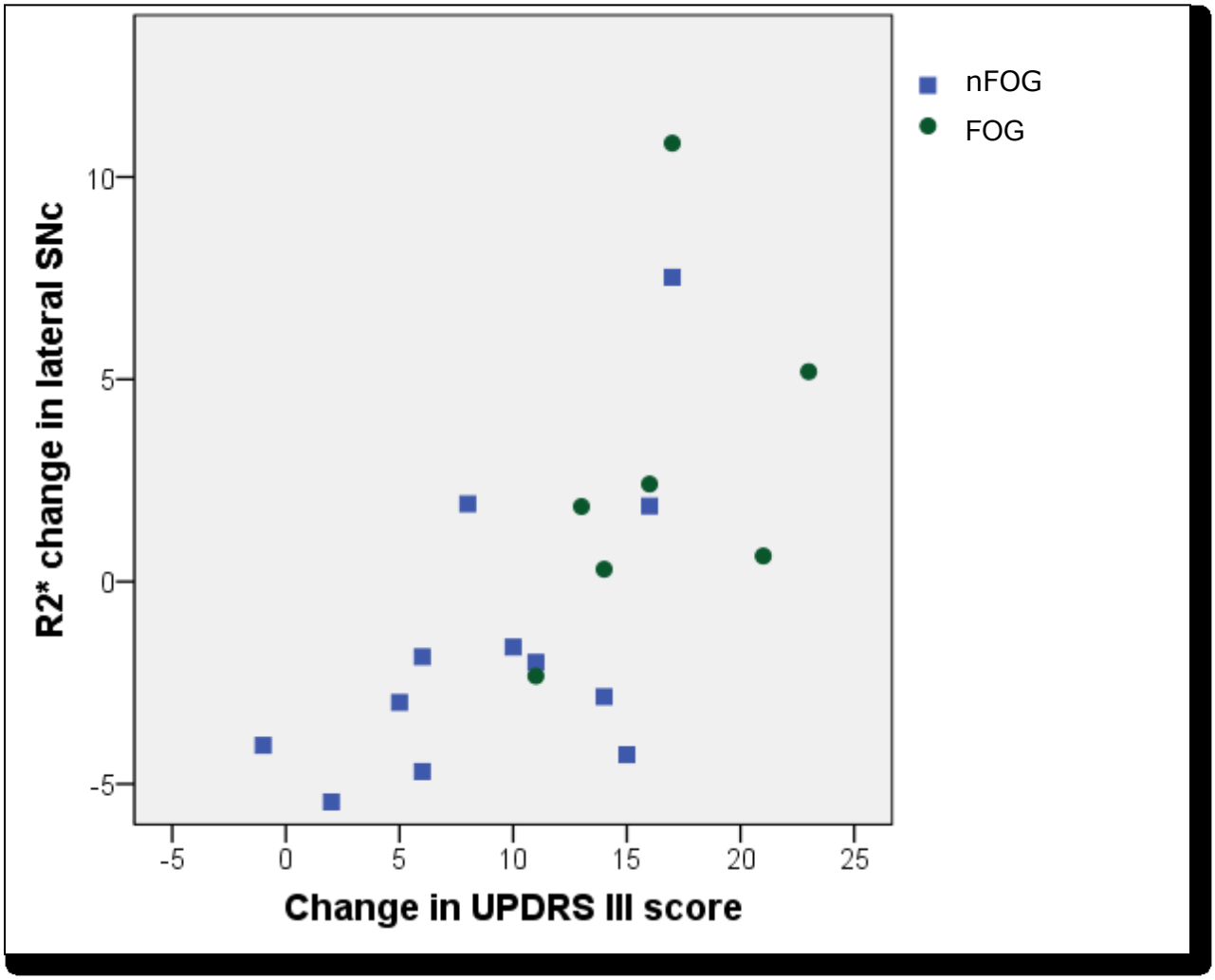


Figure 3.2: Lateral substantia nigra pars compacta (SNc) R_2* change vs change in UPDRS III score



Chapter 4: Emergence of Freezing of Gait in Parkinson disease: Are there predictors?

This chapter is in preparation for submission:

Wieler M, Gee M, Hanstock C, Camicioli R, Martin WRW. Emergence of Freezing of Gait in Parkinson disease: Are there clinical or imaging predictors?

Abstract

Objective: To determine whether individuals with Parkinson's disease who develop freezing of gait (FOG) early in the course of the disease have cognitive impairment, motor dysfunction and/or neuronal loss from the pre-supplementary motor area predictive of the development of FOG.

Methods: A cohort of previously untreated individuals with early Parkinson disease (n = 26) was followed for 36 months with clinical evaluations and single voxel magnetic resonance spectroscopy (MRS) at baseline, 18 and 36 months. Metabolite ratios were determined in the pre-supplementary motor area with MRS. The cohort was divided into two groups based on the development of FOG during 36 months of follow-up. A control group of 15 age-matched individuals underwent the same evaluations.

Results: There were significant baseline differences between Parkinson disease and controls on multiple cognitive tests but no difference in pre-supplementary motor area MRS measures. Those who developed FOG (n=10) had worse motor function at baseline than those who did not (nFOG; n=16) on the Timed Up and Go test and the 14 m walk test; these features as well as Unified Parkinson's Disease Rating Scale motor scores progressed more rapidly in the FOG group than in the nFOG group.

Conclusions: Significant cognitive deficits associated with frontal lobe dysfunction are present in patients with early, untreated Parkinson disease but impaired cognition was not predictive of the development of FOG. In contrast, individuals who developed FOG had worse motor function at baseline and more rapid decline in

motor scores over 36 months indicating an association between disease burden and the development of FOG.

Introduction

Parkinson disease is a progressive neurodegenerative disorder heterogeneous in both clinical presentation and disease progression. While the etiology and pathogenesis of Parkinson disease remain unclear, Parkinson disease was traditionally considered a disorder of motor function with motor manifestations characterised clinically by varying combinations of rest tremor, rigidity, bradykinesia, and gait/balance impairment, including freezing of gait (FOG). The primary change underlying many of these motor features is a gradual loss of dopaminergic neurons projecting from substantia nigra compacta (SNc) to striatum, resulting in decreased striatal dopamine content (Marsden 1992). There is also evidence of progressive neuronal loss from cortical areas and basal ganglia (Halliday, Hely et al. 2008). It has become increasingly clear, however, that non-motor features including cognitive impairment (particularly executive function), dementia, mood disorders, autonomic dysfunction and sleep disorders play a critical role in the disability associated with disease progression. Many of these features are not thought to be responsive to dopamine. The structural/functional neural substrates of cognitive and gait dysfunction, including freezing of gait (FOG), are not well understood. These features are unresponsive or poorly responsive to dopaminergic therapy. Despite evidence of a relationship between gait disorders and cognition, such as dual-tasking (Spildooren, Vercruysse et al. 2010, Camicioli, Oken et al. 1998, Rochester, Hetherington et al. 2004, Vercruysse, Devos et al. 2012), it remains unclear if these features evolve together and/or if they share a common pathophysiology.

Cognitive impairment in Parkinson disease, ranging from subtle deficits to mild cognitive impairment (MCI) to dementia is seen in up to 80% of individuals with Parkinson disease (Hely, Reid et al. 2008, Aarsland, Andersen et al. 2003). The evolution, degree and pattern of impairment is highly variable but studies point to dysfunction in frontal/executive, verbal memory and visuospatial/visuoperceptual domains (Levy, Jacobs et al. 2002, Woods, Troster 2003). Recent data suggests that impaired verbal memory and attention are associated with conversion from MCI to dementia (Svenningsson, Westman et al. 2012). Neuropsychological tests administered to individuals with Parkinson disease with FOG (PD FOG) and without FOG (PD nFOG) have identified some cognitive domains that may be more affected in those with FOG, particularly executive function (Naismith, Shine et al. 2010, Amboni, Cozzolino et al. 2008, Amboni, Barone et al. 2010, Vandenbossche, Deroost et al. 2013, Nantel, McDonald et al. 2012, Vandenbossche, Deroost et al. 2012b). There is also evidence suggesting that individuals with Parkinson disease and MCI may have gait impairment (Goldman, Weis et al. 2012, Poletti, Frosini et al. 2012, Amboni, Barone et al. 2012). Given that MCI can occur even in early, drug naive patients, it is possible that it shares common neural substrates or may contribute to gait impairment in Parkinson disease.

People with Parkinson disease and gait impairment commonly have stooped posture, reduced step length and width, reduced or absent arm swing and impaired balance. In some individuals, FOG, a troublesome feature seen in Parkinson disease distinct from bradykinesia and rigidity, emerges (Bartels, Balash et al. 2003). Freezing of gait has been defined as a "brief, episodic absence or marked reduction of forward progression of the feet despite having the intention to walk"

(Nutt, Bloem et al. 2011), where individuals state that their feet feel "glued to the floor". This unpredictable, episodic phenomenon can be one of the most debilitating features of Parkinson disease, responsible for falls and reduced mobility, and significantly impacting quality of life (Moore, Peretz et al. 2007).

Numerous studies have examined physical situations that provoke FOG (Spildooren, Vercruysse et al. 2010, Spildooren, Vercruysse et al. 2010, Schaafsma, Balash et al. 2003, Nutt, Bloem et al. 2011, Cowie, Limousin et al. 2012, Almeida, Lebold 2010, Snijders, Haaxma et al. 2012), including confined spaces (doorways, narrow halls), turns, initiating gait, and approaching an object. There is also a body of literature that supports the notion that dual-tasking or divided attention increases the frequency and/or severity of FOG episodes (Bloem, Hausdorff et al. 2004, Spildooren, Vercruysse et al. 2010, Camicioli, Oken et al. 1998, Giladi, Hausdorff 2006b). The literature also shows cueing strategies and specific attention to be helpful in overcoming some situations of freezing (Willems, Nieuwboer et al. 2006, Nieuwboer, Kwakkel et al. 2007, Snijders, Nijkrake et al. 2008).

Similar to findings of MCI impairment in untreated Parkinson disease (19%) (Aarsland, Bronnick et al. 2009), there is evidence of FOG in *de novo* Parkinson disease (Bloem, Hausdorff et al. 2004, Giladi, McDermott et al. 2001, Garcia-Ruiz 2011) and in approximately 25% of patients in the early stages of Parkinson disease (Giladi, McDermott et al. 2001, Tan, McGinley et al. 2011). This despite FOG being thought of as primarily a phenomenon of longer disease duration with approximately 80% of individuals with more advanced Parkinson disease reporting this troubling feature (Hely, Reid et al. 2008), primarily in the "off" medication

state. Freezing in the "off" state is often responsive to levodopa (Schaafsma, Balash et al. 2003) implicating decreased striatal dopamine in its pathological process but it can also be exacerbated with increased doses of levodopa (Ambani, Van Woert 1973). Thus the underlying pathophysiology associated with FOG remains unclear (Nutt, Bloem et al. 2011).

There is evidence of a link between decreased functional connectivity in executive-attention networks and visual networks measured with functional magnetic resonance imaging (fMRI) in FOG in Parkinson disease as well as impaired performance on tests of frontal lobe function (Tessitore, Amboni et al. 2012b). In an fMRI study of imagined gait with Parkinson disease subjects, FOG was associated with both a decrease in activity in the frontal and posterior parietal regions and an increase in activity in the midbrain mesencephalic locomotor region, a region putatively intimately involved in locomotion (Snijders, Leunissen et al. 2011a). We demonstrated (Ba, Wieler et al. 2014), in a pilot study looking at structural connectivity of the midbrain pedunculopontine nucleus, a significant deficit between the pedunculopontine nucleus region and pre-supplementary motor area in Parkinson disease subjects compared to control and in PD FOG compared with PD nFOG. These finding were consistent with a larger study (Fling, Cohen et al. 2013) showing reduced connectivity only in the right hemisphere between the pedunculopontine nucleus and the cerebellum, thalamus and many frontal regions in PD FOG. They also reported poorer performance on some cognitive tasks, specifically in those with FOG in those with more left hemisphere lateralized pedunculopontine nucleus tract volume. Taken together, these data suggest a

complex interaction of cortical and subcortical structures in the pathophysiology underlying cognitive and gait abnormalities seen in Parkinson disease.

This co-occurrence of FOG and cognitive impairment has led to models suggesting multisystem involvement in the pathogenesis of this phenomenon (Giladi, Hausdorff 2006a, Lewis, Barker 2009a). A recent model suggests dysfunction of normal neurophysiological and neurochemical processes across multiple domains of the central nervous system, including cortical, subcortial and brainstem regions, resulting in the inability of motor and cognitive fronto-striatal circuits to concurrently carry out their task with resulting FOG episodes (Lewis, Barker 2009b, Shine, Naismith et al. 2011, Shine, Matar et al. 2013a).

Evidence for cortical dysfunction in Parkinson disease comes in part from cognitive impairments that occur commonly, but also from direct measurements of cortical structure and function made with MRI and MR spectroscopy. Brain imaging in a higher-level gait disorder (gait ignition failure) points to lesions in the supplementary motor area underlying an inability to initiate gait and to make turns (Nadeau 2007). Magnetic resonance spectroscopy (MRS) is a non-invasive technique that is used to provide a measurement of N-acetyl aspartate (NAA), a putative marker of neuronal integrity, where a reduction in NAA correlates with neuronal loss or decreased neuronal function (Adalsteinsson, Sullivan et al. 2000). There is some evidence of a reduction of this marker in the pre-supplementary motor area in Parkinson disease that discriminates Parkinson disease from healthy controls (Camicioli, Hanstock et al. 2007) and that reduction of this marker in the anterior cingulate is correlated with executive dysfunction in Parkinson disease (Lewis, Shine et al. 2012).

Evidence in the literature supports the discrimination of **healthy control subjects** from **Parkinson disease** on the basis of cognitive and motor function. We investigated these two groups on the basis of neuropsychological and motor tests to determine if in this early *de novo* Parkinson disease cohort there were differences between the groups at baseline and evaluated change over 36 months. We hypothesized that this group of early, untreated Parkinson disease patients would show cognitive impairment and gait changes compared to controls at baseline with differential change over time. Moreover, we hypothesized that pre-supplementary motor area neuronal dysfunction, as measured by MRS, would show a similar pattern.

Mounting evidence points to early impaired gait, falls and freezing as a predictor for significant cognitive impairment (Anang, Gagnon et al. 2014). Given that individuals with Parkinson disease and FOG are proposed to have cortical dysfunction which manifests as cognitive impairment (primarily executive dysfunction) and the tight integration of movement control, falls (Bloem, Grimbergen et al. 2006) and executive function, we wanted to investigate how a cohort of *de* novo Parkinson disease subjects, followed for 36 months would change with respect to cognition and evaluate the development of FOG. We hypothesized that those participants who developed FOG would have greater impairment on cognitive test performance, particularly on measures of executive function, have greater difficulty with motor tasks and have evidence of more pre-supplementary motor area neuronal loss as measured by MRS than those who did not report FOG.

Subjects

In this longitudinal case controlled study, 26 subjects with early untreated Parkinson disease were recruited from the University of Alberta Movement Disorders Program located in Edmonton, Alberta. These participants were free of other neurologic disease and had an MMSE score ≥ 24. Fifteen individuals, matched for age and healthy and free of neurological and psychiatric disease, were recruited from patients' spouses and friends and from the general university community to act as the control group. Both groups were followed for 36 months and evaluated at baseline, 18 months and 36 months.

All Parkinson disease participants fulfilled the standard criteria for a clinical diagnosis of Parkinson disease (Hughes, Daniel et al. 1992) as assessed by a movement disorders neurologist (WM). All individuals in the Parkinson disease cohort displayed striatal dihydrotetrabenazine/positron emission tomography (PET) abnormalities consistent with a diagnosis of idiopathic Parkinson disease, supporting the clinical diagnosis (Martin, Wieler et al. 2008c).

When the study commenced, none of the Parkinson disease participants were judged to need treatment with levodopa or other dopaminergic medications to address Parkinson disease symptoms. During the ensuing 36 months, all Parkinson disease participants continued to be followed by a movement disorders neurologist who optimized anti-parkinsonian medications, as required. If, at subsequent evaluations, anti-parkinsonian medication had been started to address Parkinson disease symptoms, all clinical and cognitive assessments were done in the clinically defined "off" state, a minimum of 12 hours after the withdrawal of all dopaminergic medications. Imaging studies were done in the "on" state to ensure participant

comfort. The emergence of FOG was based on a UPDRS II #14 >1 (rare freezing when walking, may have start hesitation). The study was approved by the Human Research Ethics Board of the University of Alberta and all subjects gave written informed consent.

Clinical Assessments

Clinical symptoms in the Parkinson disease cohort were rated with the Unified Parkinson's Disease Rating Scale (UPDRS) (Fahn, Elton et al. 1987) by one of two raters (MW and WM) who had a high inter-rater reliability (intra-class correlation coefficient for the total motor UPDRS = 0.99, determined in a separate group of patients with Parkinson disease). All follow-up assessments for subjects were rated by the same rater (MW or WM). Additional evaluations of the Parkinson disease group included the Berg Balance Scale (Berg, Wood-Dauphinee et al. 1992) (BBS) and a self-report quality of life tool, Parkinson's Disease Questionnaire (Peto, Jenkinson et al. 1998) (PDQ39). Other clinical evaluations administered to both groups included Timed Up and Go (Podsiadlo, Richardson 1991) (TUG), 14 metre walk (with turn) and the (self-administered) Beck Depression Inventory II (Beck, Steer, Brown 1996). These clinical evaluations were administered by the same rater on all occasions (MW).

Cognitive Battery

Neuropsychological tests were performed to evaluate various cognitive domains. (See Table 4.1) The tests examined global function, executive function, memory, language, visuospatial function and attention, domains in which abnormalities were already documented as affected in early Parkinson disease.

Tests scores were adjusted for age, education and sex, as appropriate, using normative data (Heaton, Miller et al. 2004, Ruff, Light et al. 1996). Standardized scores (T-scores or z-scores) were used, with a higher score indicating better performance for Verbal Fluency, Digit Span forward and Digit Span backwards, Trail Making Test-A, Trail Making Test-B. Cognitive assessments were administered in a quiet, well-lit room by a trained research assistant, with breaks provided as needed to maintain participant engagement.

Magnetic Resonance Imaging

All MR images were acquired on a 1.5 T Siemens Sonata scanner at the Peter S. Allen MR Research Centre, University of Alberta. To determine slice placement for subsequent imaging, a set of orthogonal localizer images was used. Axially oriented T_1-weighted images, parallel to the anterior commissure (AC)-posterior commissure (PC) line, were acquired using a magnetization prepared rapid gradient echo (MPRAGE) sequence with an echo time (TE) of 3.9 ms and a repetition time (TR) of 2120 ms with two acquisitions. The in-plane field of view was 230 x 201 mm^2, with a matrix of 256 x 224, and 176 slices (1 mm slice thickness) were acquired. The data were zero-filled to a matrix of 512 x 448 yielding a final voxel size of 0.45 x 0.45 x 1 mm^3.

Magnetic Resonance Spectroscopy

Single voxel MRS data were acquired following registration to MR images in sagittal, coronal and axial planes. Imaging included a sagittal gradient-echo sequence (repetition time (TR) 199 ms, echo time (TE) 4.6 ms, slice thickness 5 mm), a coronal T_2-eighted sequence (TR 6330 ms, TE 83 ms, slice thickness 3

mm), and a T_1-weighted axial 3D magnetization prepared rapid acquisition gradient echo (MPRAGE) sequence (TR 2120 ms, TE 3.9 ms, TI (inversion time) 1100 ms with 176 slices, each 1 mm thick). A line was placed at the AC, perpendicular to the AC- PC line on a sagittal image. The pre-supplementary motor area voxel measuring 2x2x2 cm^3 was then situated such that its posterior edge abutted the perpendicular line. The voxel was then rotated counter clockwise to place its superior border parallel to the cortical surface. (See Figure 4.1). Axial and coronal images were used to confirm voxel placement in the midline.

Water suppressed spectra were obtained using a Point Resolved Spectroscopy (PRESS) sequence (TR 5 1600 ms, TE 5 80 ms, 64 averages, 1024 data size) and peak areas calculated with LC model (Provencher 1993). The metabolite basis set spectra used for the analysis were generated by numerical simulation. From the peak areas for N-acetyl aspartate (NAA), choline (Cho), and creatine/phosphocreatine (Cr), the metabolite ratios NAA/Cr and Cho/Cr were calculated. A typical spectrum from a control, a PD nFOG and a PD FOG subject is illustrated in Figure 4.2.

The voxels selected for MR spectroscopic study were segmented into their gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF) components using Magnetization Prepared RApid Gradient Echo (MPRAGE) images using an algorithm developed in SPM2 (http://www.fil.ion.ucl.ac.uk/spm/) in Matlab 7.0 (The Mathworks, Natick, MA). This required the spectroscopy voxel to be registered with each of the GM, WM or CSF component segments using an in-house program to transform the coordinates of the voxel into the frame of reference of the segmented images.

Reproducibility of MR spectroscopy PRESS volume placement on follow-up MR scans was achieved using a set of images illustrating the prior location for each subject. Following the standard registration procedure described above, fine adjustments to match the exact location on follow-up scans were performed to ensure a co-registered anatomical location between scans.

Statistical Methods

Baseline demographic data were compared using independent sample t-tests and Fisher exact tests. A repeated measures ANOVA was conducted to examine the effects of the group (control vs PD and PD nFOG vs PD FOG) and time (scans at baseline, 18 months, 36 months) on measures of cognition and motor performance. Multivariate test Wilks' lambda is reported. If Box's M test of equality of covariance matrices was violated for multivariate tests, Pillai's Trace was reported (Field 2005). Bonferroni post hoc tests were performed in case of significant scan differences. The relationships between the change (month 36 value - baseline value) in cognitive and clinical measures and changes in pre-supplementary motor area NAA/Cr and Cho/Cr in the groups were analysed using a linear regression. The Pearson correlation coefficient, r^2, and associated p-value are reported. Data are presented as means ± SD unless otherwise indicated. Statistical analysis was performed using IBM SPSS Statistics Version 212.0 for Windows (IBM Corp., Armonk, NY).

Results

Main Results Controls vs. Parkinson disease Group

Baseline

Demographics

Differences were detected between the groups. (See Table 4.2.) The two groups did not differ with respect to age, or female/male distribution, but did differ significantly with respect to education ($p = 0.004$). The Parkinson disease cohort showed more depressive symptoms as measured by the Beck Depression Inventory II than the control group (mean score 7.4 ± 5.8 vs 3.2 ± 2.8, $p = 0.012$) but did not meet the current cut off criteria for depression (BDI-II score=13/14) (Litvan, Goldman et al. 2012). Although the MMSE showed a statistically significant difference between the groups (28.7 ± 1.9 vs 29.9 ± 0.4, $p=0.003$), this small difference was not considered to be clinically significant. There was no statistically significant difference between the groups with respect to the TUG or 14 m walk times.

Neuropsychological Testing

There were significant baseline differences on multiple cognitive tests between the two groups. The score on the FAB, was worse in the Parkinson disease group (15.6 ± 2.0 vs 16.9 ± 1.2, $p=0.021$). The scores on two CVLT-II subtests (immediate recall [IR] and semantic categories [SC]) were significantly different with the Parkinson disease performance worse than controls (CVLT-II IR 49.3 ± 9.6 vs 58.7 ± 9.3, $p=0.004$; CVLT-II SC -0.12 ± 1.2 vs 1.2 ± 1.9, $p=0.024$) indicating impairment in memory. The Parkinson disease group had impaired visuomotor speed reflected in Trail Making-A (40.4 ± 12.8 vs 48.8 ± 6.6, $p=0.022$). In Trail

Making Test-B, a measure of set-shifting, the Parkinson disease group showed impairment (45.5 ± 11.4 vs 52.9+ ± 11.0, p=0.049). Visual-spatial processing as measured by JOL, also showed the Parkinson disease group had worse scores (26.8 ± 3.1 vs 30.3 ± 2.0, p<0.000).

Motor Assessments

There were no significant differences between the groups with respect to the TUG, 14 m walk or 14 m steps times.

MRS Spectroscopy

There were no significant differences for any of the MRS measures of NAA/Cr, Cho/Cr between the controls and Parkinson disease.

Longitudinal Results

Neuropsychological Testing

A significant interaction was observed between the groups and time indicating that spontaneous word generation, working memory, attention and set-shifting was significantly different for the two groups over time, (Verbal Fluency [Wilks' lambda =0.988, F(2,38)=4.005, p=0.026], Trail Making Test-B [Wilks' lambda =0.783, F(2,38)=3.210, p=0.050], and Digit Span backwards [Wilks' lambda =0.7.425 F(2,38)=6.262, p=0.002]).

A main effect for time was observed only in the CVLT-II LDR with Parkinson disease group getting slightly worse and control groups showing an improvement in this test of memory between baseline and month18 only [Wilks' lambda =0.827 F(2,38)=3.965, p=0.027].

A significant main effect for comparing the groups (control vs Parkinson disease) in the scores of most of the cognitive tests was observed suggesting that the Parkinson disease group had more impairment on cognitive function compared to the control group in measures of memory, set shifting, visuospatial function and executive function (CVLT-II IR: $F(1,39)=11.356$, $p=0.002$; CVLT-II short delay recall: $F(1,39)=7.880$, $p=0.008$); CVLT-II long delay recall: $F(1,39)=16.886$), $p=0.012$; CVLT-II SC: $F(1,39)=13.610$, $p=0.001$; Digit Span Backwards: $F(1,39)=7.100$, $p=0.011$; FAB: $F(1,39)= 5.971$, $p=0.019$; JOL:$F(1,39)=13.518$,$p=0.001$; Trail Making Test-A: $F(1,39)=11.462$, $p=0.002$; Trail Making Test-B: $F(1,39)=11.336$, $p=0.002$).

There was a significant interaction with time and group on the BDI-II indicating that depression was different for the two groups over time with the Parkinson disease group reporting higher scores over time [Wilks' lambda $=0.972$, $F=(2,38)=3.478$, $p=0.041$]. (See Table 4.3)

Motor Assessments

There was a significant interaction between time and 14 m walk speed [Wilks' lambda $=0.837$, $F(2,37)=3.606$, $p=0.037$] as well as the number of steps needed to walk 14 m [Wilks' lambda $=0.847$, $F(2,36)=3.243$, $p=0.050$] with post hoc testing indicating that speed and step rate was significantly different between the two group between baseline and 36 months and baseline and 18 months but not between 18 months and 36 months, with the Parkinson disease group walking more slowly and taking more steps.

The main effect for time TUG for was significant [Wilks' lambda=$F(2,37)=19.184$, $p<0.000$]. Post hoc analysis revealed that the TUG times

were significantly different between all time points with both groups walking more slowly. There was also a significant main effect for the number of steps needed to walk 14 m [Wilks' lambda=F(2,36)=4.830, p=0.014]

The main effect for group differences in TUG time and 14 m walk speed were both significant indicating that the Parkinson disease group were slower than the control (F(1,36)=10.614, p=0.002 and F(1,36)=5.859, p=0.021). (See Table 4.4)

MRS Spectroscopy

There were no significant main effects for MRS measures of NAA/Cr, Cho/Cr. (See Table 4.5.) There were no significant correlations between baseline to 36 month change of the metabolic ratios and change in any clinical or neuropsychological test.

Main Results PD nFOG vs. PD FOG

The data from the 16 Parkinson disease participants who did not report FOG (PD nFOG) and 10 Parkinson disease participants who did report FOG (PD FOG) and who had cognitive testing at all three time points were compared.

Baseline

Demographics

The two groups did not differ at baseline with respect any demographic measures. (See Table 4.2)

Neuropsychological Testing

There were no measured differences between the groups on any cognitive measure.

Motor Assessments

There was no significant difference between the groups with respect to UPDRS total score, UPDRS III score, Berg Balance scale or PDQ-39 score. There was a significant difference between the groups with respect to the TUG (8.0 ± 0.8 vs 9.5 ± 1.4, p=0.003) and 14 m walk times (11.1 ± 0.9 vs 12.7 ± 1.8) and 14 m steps (10.93 ± 1.05 vs 12.1 ± 1.37. p=0.021) with the FOG group being slower than the nFOG group and taking more steps to walk 14 m.

MRS Spectroscopy

There were no statistically significant differences for any of the MRS measures of NAA/Cr, Cho/Cr. (See Table 4.5.)

Longitudinal Results

The levodopa equivalent dose (LED) did not differ between the two groups at month 36 when all the subjects were considered (nFOG = 295.0 ± 231.5 mg , range 0 - 1000, FOG = 418.5 ± 231.5, range 0-840). Of the seven subjects who had not commenced dopaminergic therapy at month 36, six were in the nFOG group. When looking only at the participants (nFOG n=10, FOG n=9) who were on dopaminergic therapy at 36 months, the non-significant difference in LED remained (nFOG 472 ± 243.0, range 120-1000, FOG 465.0 ± 189.6, range 225-840).

Neuropsychological Testing

There was a significant interaction between the groups and time observed for CVLT-II IR indicating that the FOG group worsened over time while the nFOG group improved slightly between baseline and month 36 [Wilks' lambda= 0.647, F(2,23)=6.267, p=0.007].

No significant main effect for group was seen in any of the cognitive tests indicating that the presence of FOG was not accompanied with cognitive impairment that differentiated it from those without FOG. (See Table 4.3)

Motor Assessments

There was a significant group x time interaction for UPDRS III scores [Wilks' lambda =0.757, F(2,23)=3.693, p=0.041], the TUG times [Wilks' lambda =0.757, F(2,23)=6.482, p=0.006], 14 m steps [Wilks' lambda =0.724, F(2,23)=4.389, p=0.024]and BBS scores (Pillai's Trace F(2,23)=3.562, p=0.045) with post hoc testing revealing the UPDRS III scores, the TUG times, 14 m steps and BBS scores were significantly different between all time points.

The main effect for time on these measures of motor function was also significant: 14 m walk speed [Wilks' lambda =F(2,23)=15.348, p<0.000]. Post hoc analysis revealed that the 14 m walk was only different between baseline and month 18 and baseline and month 36 but not between month 18 and month 36.

The main effect for group differences was seen in all measures of motor function including the 14 m walk speed (F(1,24)=13.947, p=0.001) indicating that the FOG group had a higher disease burden and were slower than the nFOG group.

There was a significant group x time interactions for PDQ 39 scores indicating that quality of life, as measured by the PDQ39, was different between the groups and the FOG group reported lower quality of life at 36 months than the nFOG group [Wilks' lambda =0.598, F(2,23)=7.746, p=0.003]). (See Table 4.4.)

MRS Spectroscopy

There were no statistically significant differences for any of the metabolite ratios, NAA/Cr, Cho/Cr between PD nFOG and PD FOG. There were no significant correlations between baseline to 36 month change of the metabolic ratios and change in any clinical or neuropsychological test. (See Table 4.5)

Discussion

This study examined two separate but related questions by evaluating cortical function in two distinct ways. Firstly, did cognition and motor function change differently over 36 months between a cohort of 26 early, untreated Parkinson disease subjects compared to 10 controls as well as between the 10 Parkinson disease subjects who reported FOG at 36 months compared to the 16 Parkinson disease subjects who did not? Secondly, did the neuronal viability marker, NAA/Cr, in the pre-SMA differ between these same groups, providing a possible neural correlate of Parkinson disease and/or FOG in Parkinson disease?

The baseline differences, on standardized neuropsychological tests, between control and Parkinson disease subjects across a number of cognitive domains associated with frontal lobe function is consistent with the literature (Owen, James et al. 1992, Muslimovic, Post et al. 2009, Lees, Smith 1983, Downes, Roberts et al. 1989, Dujardin, Degreef et al. 1999). This confirms that even in *de novo* Parkinson disease, impairment in cognition is present, specifically with respect to set-shifting, verbal episodic memory and visuospatial function. Over time, the control and Parkinson disease groups changed differently with respect to measures of working memory, spontaneous word production and set-shifting consistent with evolving cognitive decline previously reported in Parkinson disease (Hely, Reid et al. 2008,

Aarsland, Andersen et al. 2003) and differences in performance observed at baseline between the groups were maintained.

In the literature, both attention and executive function are associated with Parkinson disease gait speed and variability (Yogev, Giladi et al. 2005, Lord, Baker et al. 2011, Lord, Rochester et al. 2010). When examining this Parkinson disease cohort, we also found that cognitive impairment was associated with slower speed and increased steps taken to walk 14 m.

It has been postulated that cognitive dysfunction in Parkinson disease is one of the mechanisms associated with the development of the troublesome phenomenon of FOG (Nutt, Bloem et al. 2011). There is increasing evidence that FOG is correlated with both cognitive dysfunction and faster progression of cognitive dysfunction, particularly in the executive domain (Naismith, Shine et al. 2010, Amboni, Cozzolino et al. 2008, Amboni, Barone et al. 2010, Giladi, McMahon et al. 1992). However, although we hypothesized that there would be baseline differences on tests of cognition between the nFOG and FOG groups, none were found. Similarly, the hypothesis that the groups would both change and change differently over 36 months was generally not supported by our data. The only significant difference on the cognitive measures between the groups over time was CVLT-II immediate recall, with the FOG group declining while the nFOG group improved slightly. This isolated difference does not lend itself to generalization of significant cognitive impairment that is specific to the FOG group though the development of memory impairment may signal a transition toward developing more significant future cognitive impairment.

A number of measures of motor function (TUG, 14m walk and 14 m steps) discriminated between the PD nFOG and PD FOG group at baseline. While both groups were walking more slowly by 36 months, the PD FOG group was walking significantly slower, taking more steps and progressed at a faster rate to walking more slowly than the nFOG group. In addition, although the UPDRS III was not different between the groups at baseline, the FOG group had more disease burden, as indicated by higher UPDRS III scores, and more rapid disease progression over 36 months.

Supporting previous reports in the literature in which impaired cognition and FOG in Parkinson disease are associated with poorer quality of life (Moore, Peretz et al. 2007, Lawson, Yarnall et al. 2014), our data suggest that although quality of life, as reported on the PDQ 39, was no different between the groups at baseline, the FOG group developed significantly poorer quality of life by month 36.

The pre-supplementary motor area, with its extensive cortical and subcortical connectivity (Tessitore, Amboni et al. 2012b, Wu, Long et al. 2011, Catani, Dell'acqua et al. 2012), is intimately involved with both motor and cognitive functions, including the cognitive control of actions that require rapid updating, inhibition, or switching (Nachev, Wydell et al. 2007). The pre-supplementary motor area has been shown to be differentially active over the course of Parkinson disease with increased activity in early disease (Eckert, Peschel et al. 2006) and decreased activation with longer disease duration (Sabatini, Boulanouar et al. 2000, Fukuda, Mentis et al. 2001, Cunnington, Egan et al. 2001, Thobois, Dominey et al. 2000). There is also evidence from post mortem studies of a selective loss of pre-supplementary motor area cortico-cortical projecting pyramidal neurons

(MacDonald, Halliday 2002). In the present study, there were no differences in pre-supplementary motor area NAA/Cr comparing the Parkinson disease cohort to controls suggesting that in this relatively early Parkinson disease group, significant neuronal dysfunction or loss in this brain area thought to be important in both cognitive function and motor control, has not yet developed. This supports the notion that early cognitive and motor dysfunction is related instead to striatal dysfunction and resulting cortical changes attributable to reduced striatal dopamine (Alexander, DeLong et al. 1986, Taylor, Saint-Cyr et al. 1986, Dubois, Pillon 1997).

An imaging marker that would assist in the identification of those with Parkinson disease who are destined to develop FOG would be useful for prognostication and perhaps ultimately for treatment. Unfortunately, the present study does not support pre-supplementary motor area NAA/Cr as a biomarker to distinguish between PD nFOG and PD FOG in a relatively early disease state (4.7 years $\pm$ 1.3 yr from diagnosis). Future studies should evaluate the potential for structural imaging tools such as voxel-based morphometry and/or cortical thickness mapping at demonstrating pre-supplementary motor area pathology. We relied on the UPDRS II, question 14 to identify the emergence of FOG. At the time this study was commenced, validated tools to assess FOG were just emerging and not yet in widespread usage (Nieuwboer, Rochester et al. 2009, Giladi, Shabtaia et al. 2000). Our study would have been enhanced with a more comprehensive picture of the nature of the FOG rather than merely its presence or absence. Future studies should include the New Freezing of Gait Questionnaire (Nieuwboer, Rochester et al. 2009) which assesses both frequency and severity of freezing episodes. As with

many studies in the literature examining FOG, we did not differentiate between "on" freezing and "off" freezing.

We followed participants for only 36 months which may not be long enough to see the evolution of changes in the pre-supplementary motor area neuronal integrity associated with disease progression. Given that a previous study in subjects with longer disease duration did show a difference in this marker compared to controls (Camicioli, Hanstock et al. 2007), future studies involving longer follow up may ultimately support this as a biomarker of disease progression and a risk for the development of FOG in Parkinson disease as dysfunction in the pre-supplementary motor area may contribute to the development of dopamine non-responsive features associated with disease progression.

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Tables

Table 4.1: Neuropsychological tests

Neuropsychiatric Test	Cognitive Domain	Cognitive Function
Mini-Mental State Exam		▪ global cognitive function
Frontal Assessment Battery	executive functions	▪ screening task
California Verbal Learning Test-II	memory	▪ verbal learning ▪ organization ▪ memory
Verbal Fluency - letters (COWAT)*	language	▪ spontaneous word generation
Trail Making Test A	executive functions	▪ Attention ▪ processing speed
Trail Making Test B	executive functions	▪ set-shifting
Digit Span	executive functions	▪ working memory ▪ attention
Judgment of Line Orientation	visuospatial functions	▪ orientation judgement

*Controlled Oral Word Association Test

Table 4.2: Demographic and baseline characteristics of cohort

	Control	PD	p-value	Parkinson disease no FOG	Parkinson disease FOG	p-value
Subjects (female, male)	15 (7, 8)	26 (8, 18)	n.s	16 (6, 10)	10 (2, 8)	n.s
Baseline age in years	56.8 ± 7.5*	60.4 ± 7.4	n.s.	60.2 ± 7.5	60.73 ± 7.6	n.s.
Disease duration at baseline (years)	-	1.7 ± 1.3	-	1.7 ± 1.3	1.9 ± 1.3	n.s.
Baseline to Month 36 scan (years)	3.0 ± 0.2	3.0 ± 0.2	n.s.	3.1 ± 0.2	3.0 ± 0.1	n.s.
Education (years)	17.5 ± 3.10	14.5 ± 3.0	0.004	14.6 ± 3.1	14.4 ± 2.8	n.s.
BECK	3.2 ± 2.8	7.4 ± 5.8	0.003	7.7 ± 6.1	7.0 ± 5.5	n.s.
MMSE	29.9 ± 0.4	28.7 ± 1.9	0.003	28.8 ± 1.8	28.5 ± 2.1	n.s.
UPDRS III	-	14.33 ± 6.2	-	12.8 ± 6.6	16.8 ± 4.8	n.s.
UPDRS Total		20.21 ± 8.2		19.06 ± 8.9	22.05 ± 6.9	n.s
TUG (seconds)	7.8 ± 1.5	8.6 ± 1.3	n.s.	8.0 ± 0.8	9.5 ± 1.4	0.003
14 m walk (seconds)	11.38 ± 2.0	11.7 ± 1.5	n.s.	11.1 ± 0.9	12.7 ± 1.8	0.018
14 m walk (steps)	11.46 ± 1.15	11.38 ± 1.30	n.s.	10.93 ± 1.05	12.1 ± 1.37	0.021
Berg Balance Score	-	755.2 ± 1.5	-	55.7 ± 0.6	54.3 ± 2.1	n.s
PDQ-39 Score	-	19.2 ± 13.3	-	19.3 ± 14.3	19.1 ± 12.4	n.s.
FAB	16.9 ± 1.2	15.6 ± 2.0	0.021	15.5 ± 2.3	15.7 ± 1.4	n.s.
CVLT-II Immediate Recall	58.7 ± 9.3	49.3 ± 9.6	0.004	49.3 ± 9.2	49.5 ± 10.7	n.s.
CVLT-II Short Delay Recall	0.83 ± 1.0	0.06 ± 1.5	n.s.	0.28 ± 1.4 [4 - 62]	-0.30 ± 1.2 [4 - 62]	n.s.
CVLT-II Long Delay Recall	0.70 ± 0.84	-0.04 ± 1.5	n.s.	0.10 ± 1.6	-0.25 ± 1.3	n.s.
CVLT-II Semantic Categories	1.2 ± 1.9	-0.12 ± 1.2	0.024	-0.13 ± 0.53	-0.10 ± 1.9	n.s.
CVLT-II Delayed Recognition	0.13 ± 0.85	0.52 ± 3.1	n.s.	-0.12 ± 0.9	1.6 ± 4.8	n.s.

	Control	PD	p-value	Parkinson disease no FOG	Parkinson disease FOG	p-value
Digit Span Forward	41.1 ± 8.8	40.1 ± 10.7	n.s.	39.0 ± 10.9	417 ± 10.5	n.s.
Digit Span Backward	36.9 ± 6.8	34.6 ± 10.0	n.s.	33.63± 10.5	36.2 ± 9.4	n.s.
Trail Making Test A	48.8 ± 6.6	40.4 ± 12.8	0.022	41.4 ± 9.8	38.6 ± 16.9	n.s.
Trail Making Test B	52.9+ ± 11.0	45.5 ± 11.4	0.049	47.19 ± 6.1	42.7 ± 168	n.s.
Judgment of Line	30.3 ± 2.0	26.8 ± 3.1	0.00	27.3 ± 3.1	26.0 ± 3.0	n.s.
Verbal Fluency	51.2 ± 9.2	48.6 ± 12.3	n.s.	48.9 ± 13.4	48.3 ± 11.1	n.s.

*mean ± standard deviation

Abbreviations:
FOG = freezing of gait; **MMSE** = Mini-mental state exam; **TUG** = Timed Up and Go; **UPDRS** = Unified Parkinson's Disease Rating Scale; **PDQ-39** = Parkinson's Disease Questionnaire–39; **FAB** = Frontal Assessment Battery; **CVLT-II** = California Verbal Learning Test - Second Edition

Table 4.3: Neuropsychological test - longitudinal data

Assessment		Controls	Parkinson disease	Parkinson disease no FOG	Parkinson disease FOG
CVLT-II Immediate Recall	baseline	58.67 ± 9.34	49.35 ± 9.56	49.25 ± 9.18	49.50 ± 10.66
	month 18	55.40 ± 5.14	47.62 ± 11.90	47.94 ± 12.30	47.10 ± 11.86
	month 36	59.80 ± 5.95	48.38 ± 12.43	51.93 ± 11.19	42.70 ± 12.74
CVLT-II Short Delay Recall	baseline	0.83 ± 1.01	0.06 ± 1.37	0.28 ± 1.45	-0.30 ± 1.21
	month 18	0.57 ± 1.37	-0.29 ± 1.18	-0.28 ± 1.21	-0.30 ± 1.21
	month 36	0.93 ± 0.65	0.08 ± 1.16	0.16 ± 1.14	-0.05 ± 1.26
CVLT-II Long Delay Recall	baseline	0.70 ± 0.84	-0.04 ± 1.50	0.09 ± 1.63	-0.25 ± 1.32
	month 18	0.30 ± 0.86	-0.40 ± 1.10	-0.38 ± 1.07	-0.45 ± 1.21
	month 36	0.83 ± 0.62	-0.12 ± 1.24	0.09 ± 0.99	-0.45 ± 1.57
CVLT-II Semantic Cluster	baseline	1.23 ± 1.94	-0.12 ± 1.19	-0.13 ± 0.53	-0.10 ± 1.85
	month 18	1.53 ± 1.58	-0.27 ± 1.35	-0.25 ± 1.10	-0.30 ± 1.75
	month 36	1.23 ± 1.94	-0.23 ± 1.03	-0.19 ± 0.81	-0.30 ± 1.35
CVLT-II Delayed Recognition	baseline	0.13 ± 0.85	0.52 ± 3.10	-0.13 ± 0.92	1.55 ± 4.84
	month 18	0.43 ± 0.84	0.23 ± 1.21	0.22 ± 1.17	0.25 ± 1.34
	month 36	0.73 ± 0.68	0.00 ± 1.35	0.16 ± 1.14	-0.25 ± 1.67
Digit Span Forward	baseline	41.07 ± 8.82	40.08 ± 10.65	39.06 ± 10.94	41.70 ± 10.54
	month 18	44.00 ± 6.78	42.35 ± 9.04	42.63 ± 9.10	41.90 ± 9.41
	month 36	45.40 ± 8.43	39.85 ± 10.47	39.44 ± 11.09	40.50 ± 9.95
Digit Span Backwards	baseline	36.93 ± 6.80	34.62 ± 9.96	33.63 ± 10.47	36.20 ± 9.39
	month 18	42.13 ± 6.49	33.19 ± 8.97	32.69 ± 10.29	34.00 ± 6.78
	month 36	41.67 ± 6.10	33.35 ± 9.00	33.13 ± 9.44	33.70 ± 8.64

Assessment		Controls	Parkinson disease	Parkinson disease no FOG	Parkinson disease FOG
Verbal Fluency	baseline	51.19 ± 9.20	48.64 ± 12.32	48.90 ± 13.40	48.30 ± 11.10
	month 18	52.52 ± 6.45	46.43 ± 13.13	46.80 ± 14.60	45.90 ± 11.04
	month 36	49.24 ± 6.50	48.82 ± 12.95	49.20 ± 13.83	48.28 ± 12.11
Judgement of Line Orientation	baseline	27.31 ± 3.09	26.00 ± 3.02	30.27 ± 1.98	26.81 ± 3.07
	month 18	27.88 ± 2.53	28.40 ± 3.20	30.20 ± 3.59	28.08 ± 2.76
	month 36	27.69 ± 3.50	28.20 ± 2.57	30.80 ± 2.15	27.88 ± 3.13
Trail Making Test A	baseline	48.80 ± 6.56	40.35 ± 12.78	41.44 ± 9.83	38.60 ± 16.94
	month 18	49.00 ± 8.39	42.04 ± 15.25	42.13 ± 18.15	41.90 ± 9.83
	month 36	50.20 ± 5.77	39.12 ± 11.33	39.34 ± 9.04	37.80 ± 14.73
Trail Making B	baseline	52.87 ± 10.99	45.46 ± 11.36	47.19 ± 6.15	42.70 ± 16.79
	month 18	54.27 ± 9.33	42.77 ± 10.70	43.13 ± 10.88	42.20 ± 10.96
	month 36	58.07 ± 10.19	46.08 ± 11.20	48.25 ± 5.52	42.60 ± 16.62
MMSE	baseline	29.87 ± 0.35	28.65 ± 1.85	29.75 ± 1.77	28.50 ± 2.07
	month 18	29.80 ± 0.41	28.19 ± 1.72	28.19 ± 1.83	28.20 ± 1.62
	month 36	29.73 ± 0.59	28.54 ± 1.59	28.94 ± 1.53	27.90 ± 2.73
FAB	baseline	16.93 ± 1.16	15.58 ± 1.98	15.50 ± 2.31	15.70 ± 1.42
	month 18	16.47 ± 1.50	15.50 ± 1.92	15.38 ± 2.09	15.70 ± 1.70
	month 36	17.20 ± 1.32	15.88 ± 2.25	15.69 ± 2.39	16.20 ± 2.10
BDI-II	baseline	3.20 ± 2.83	7.42 ± 5.81	7.69 ± 6.15	7.00 ± 5.52
	month 18	2.80 ± 3.88	8.85 ± 5.92	9.50 ± 7.32	7.80 ± 2.49
	month 36	2.00 ± 3.00	10.08 ± 7.65	9.81 ± 8.03	10.50 ± 7.53

Table 4.4: Longitudinal motor measures

Assessment		Controls	Parkinson disease	Parkinson disease no FOG	Parkinson disease FOG
UPDRS III	baseline	N/A	14.3 ± 6.2	12.8 ± 6.6	16.8 ± 4.8
	month 18	N/A	21.5 ± 8.4	18.4 ± 7.7	26.4 ± 7.4
	month 36	N/A	24.5 ± 8.6	20.1 ± 6.5	31.6 ± 6.8
BBS	baseline	N/A	55.2 ± 1.5	55.7 ± 0.6	54.3 ± 2.1
	month 18	N/A	54.4 ± 1.7	55.3 ± 0.8	53.0 ± 1.8
	month 36	N/A	53.0 ± 2.0	54.2 ± 0.8	51.2 ± 1.9
TUG	baseline	7.8 ± 1.5	8.6 ± 1.3	8.0 ± 0.8	9.5 ± 1.5
	month 18	8.5 ± 0.8	10.0 ± 1.3	9.8 ± 1.3	10.3 ±1.3
	month 36	9.3 ± 0.9	11.8 ± 3.4	10.4 ± 1.6	14.0 ± 4.4
14 m walk	baseline	11.4 ± 2.0	11.7 ± 1.5	11.1 ± 0.9	12.7 ± 1.8
	month 18	12.3 ± 2.3	14.0 ± 3.0	13.0 ± 1.7	15.7 ± 3.8
	month 36	12.2 ± 1.0	15.2 ± 3.8	14.0 ± 3.2	17.1 ± 4.0
14 m steps	baseline	11.4 ± 1.2	11.4 ± 1.3	10.9 ± 1.1	12.1 ± 1.4
	month 18	11.6 ± 2.3	12.2 ± 1.5	11.8 ± 1.2	12.9 ± 1.8
	month 36	11.56 ± 1.3	13.3 ± 2.7	12.1 ± 1.2	15.3 ± 3.2

*mean ± standard deviation

Abbreviations:
FOG = freezing of gait; **UPDRS III** = Unified Parkinson's Disease Rating Scale, Part III; **BBS** = Berg Balance Scale; **TUG** = Timed Up and Go

Table 4.5: Spectroscopy data

		Controls	Parkinson disease	Parkinson disease no FOG	Parkinson disease FOG
NAA/Cr ratio - pre-supplementary motor area[#]	baseline	1.52 ± 0.15*	1.47 ± 0.17	1.45 ± 0.14	1.50 ± 0.20
	month 18	1.44 ± 0.14	1.43 ± 0.12	1.45 ± 0.12	1.41 ± 0.12
	month 36	1.48 ± 0.10	1.49 ± 0.19	1.47 ± 0.20	1.52 ± 0.18
Cho/Cr ratio - pre-supplementary motor area[#]	baseline	0.69 ± 0.01*	0.66 ± 0.12	0.64 ± 0.14	0.69 ± 0.11
	month 18	0.66 ± 0.11	0.72 ± 0.18	0.64 ± 0.07	0.80 ± 0.23
	month 36	0.67 ± 0.08	0.74 ± 0.15	0.69 ± 0.15	0.78 ± 0.20

* mean ± standard deviation
[#] multivariate tests showed no significant differences

Abbreviations:
FOG = freezing of gait; **NAA =** *N*- acetylaspartate; **CR** = creatine

Figure Legend

Figure 4.1: Schematic Showing Voxel Placement

Sagittal image showing pre-supplementary motor area voxel placement.

AC = anterior commissure; **PC** = posterior commissure

Figure 4.2: Representative Spectra from Cohort

Proton spectrum from a 2 x 2 x 2 cm^3 voxel located on the midline regions of the pre-supplementary motor area. The data shown highlight the metabolic peaks from *N*-acetylaspartate (NAA), Creatine plus phosphocreatine (Cr/PCr), and choline-containing compounds (Cho) and illustrate both the experimental data (black) and the LCModel fit (gray) for a control subject, a Parkinson disease subject with no freezing of gait and a Parkinson disease subject with freezing of gait.

Figure 4.1: Voxel Placement

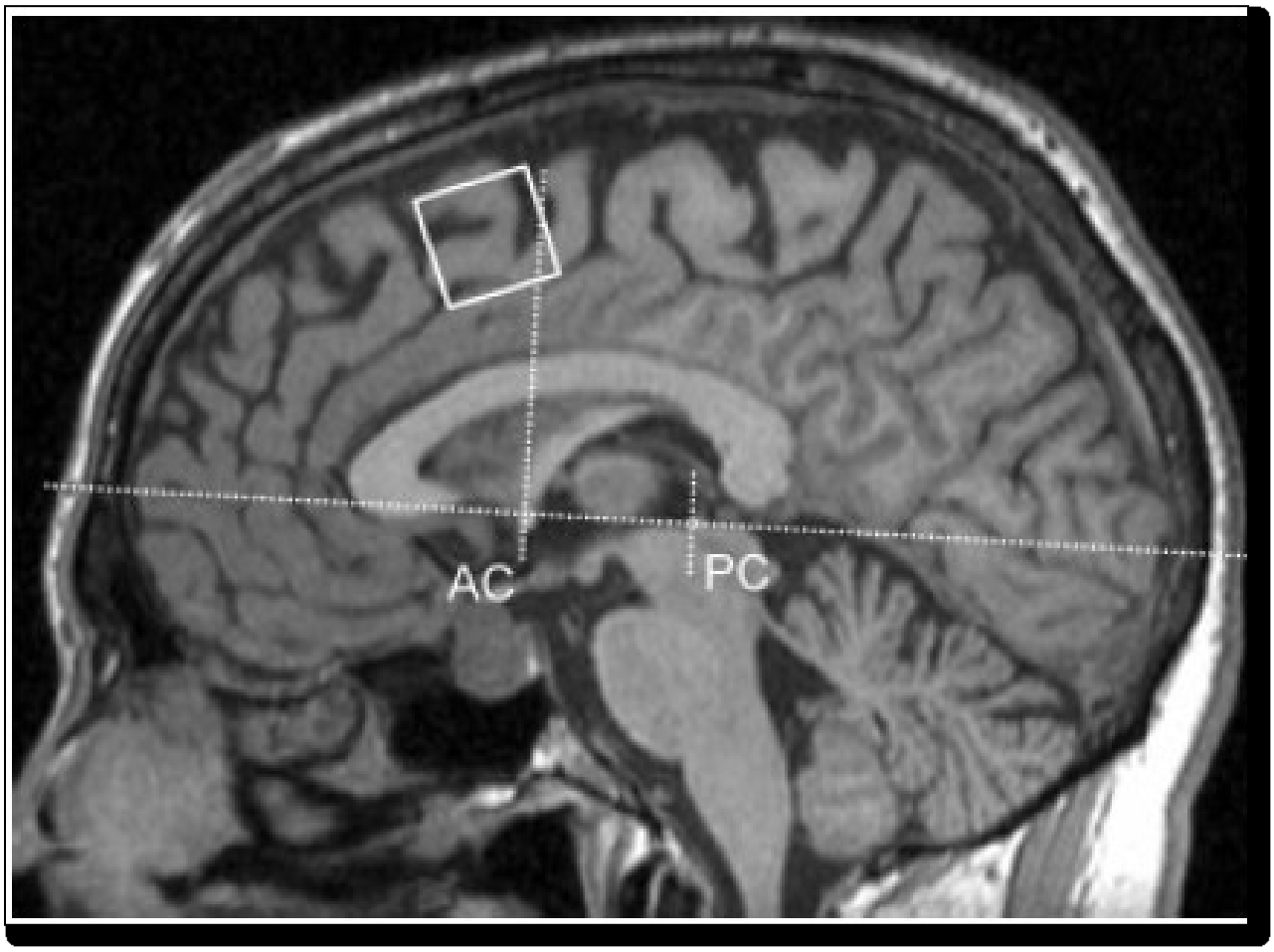
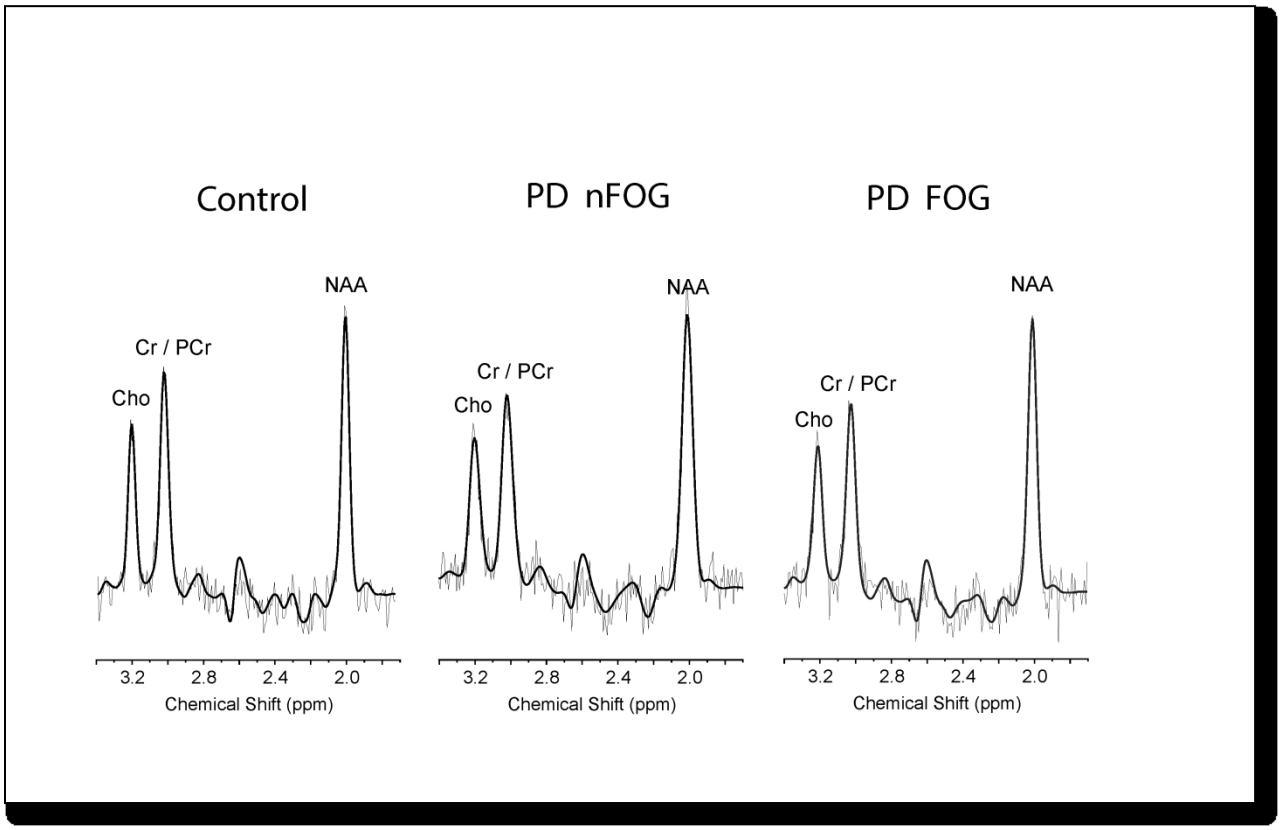


Figure 4.2: Representative Spectra from Cohort



Summary of Major Findings

The goal of this dissertation was to better understand the longitudinal changes in Parkinson disease progression with respect to motor function and cognition and to quantify these clinical changes along with changes in brain structure and function. The objective was to show that imaging markers of abnormal brain structure and function would correlate with these clinical changes and serve as biomarkers of disease progression. To this end, we investigated: 1) how iron accumulation in midbrain structures was associated with declining motor function in a group of individuals with early, untreated Parkinson disease compared to a control group, 2) the association between freezing of gait, declining motor function and iron accumulation in midbrain structures in a group of individuals with early, untreated Parkinson disease who developed freezing of gait by 36 months after baseline compared to those who did not, and 3) how changes in cognition and motor function, and measures of regional neuronal loss from the pre-supplementary motor area in a group of individuals with early, untreated Parkinson disease compared to those in a control group and the association between these changes and the development of freezing of gait.

Chapter 2

Deposition of nigral iron is evident in healthy individuals with increasing accumulation seen with age, though the implications of this increase remain unclear (Zecca et al. 2004, Martin, Ye & Allen 1998). In Parkinson disease, there is evidence of further increase in nigral iron content beyond that expected from simple aging (Dexter et al. 1989). Studies using MRI show proton relaxation rate R_2* correlates with iron concentrations in brain tissue (Ye, Martin & Allen 1996) and

increased measures of R_2* in substantia nigra of individuals with Parkinson disease (Gorell et al. 1995). There are data suggestive of a role for iron in the degenerative process of dopaminergic neurons, but it is not clear if iron is a cause of cell death or simply occurs in parallel - an epiphenomenon.

In an earlier cross-sectional study, we demonstrated increased iron in the SNc in early Parkinson disease that was correlated to severity of motor disability and differentiated the Parkinson disease group from healthy controls (Martin, Wieler & Gee 2008). In the current longitudinal study, this discrimination was no longer evident between the groups at three years. The change, however, in iron content as estimated by R_2* did correlate with change in motor symptoms over three years in the Parkinson disease group. This is consistent with our hypothesis that SNc iron content is correlated with the progression of motor symptoms in Parkinson disease and may have utility as an imaging marker for Parkinson disease progression. The heterogeneity of the Parkinson disease group, however, may have accounted for the failure of this measure to allow differentiation of the Parkinson disease group from controls at three years.

Chapter 3

The emergence of gait disturbance, particularly FOG, is a disabling feature of Parkinson disease of unclear pathogenesis. To explore the relationship between changes in midbrain iron content and the development of this disabling feature, we evaluated two subgroups of an early, untreated Parkinson disease cohort, followed over 36 months. At baseline, those who went on to develop FOG were more severely impaired from a motor perspective with slower gait and higher UPDRS III scores. The measured R_2* values of both groups changed over time. The

measured change in lateral SNc was different between those who reported FOG at 36 months and those who did not develop FOG. The FOG group progressed more rapidly with respect to disease severity, as reflected by UPDRS III scores, suggesting a more aggressive pathological process in the midbrain in this group. Other regions in the substantia nigra, including the lateral SNr and the medial SNc, also showed differences in measured R_2* between the groups. Increased nigral iron content appears to be closely associated with severity of motor features in Parkinson disease. This is, to our knowledge, the first longitudinal study to observe differences in midbrain iron content, as measured R_2* values, to differentiate subjects in whom FOG emerged from those who did not.

Chapter 4

Cognitive impairment and motor dysfunction coexist in Parkinson disease, (Yogev et al. 2005, Lord et al. 2010, Lord et al. 2011) both with variable response to dopaminergic replacement therapy. Freezing of gait in Parkinson disease, a frequent troublesome phenomenon, is often unresponsive to dopaminergic treatment It is unclear if there are common underlying neural substrates for these two disabling features of Parkinson disease, impaired cognition and FOG. This study is consistent with other reports (Poletti et al. 2012, Arnaldi et al. 2012) that showed subtle cognitive differences in early, untreated Parkinson disease, when compared to healthy controls, at baseline. By 36 months, multiple spheres of cognition were impaired in the Parkinson disease cohort compared to controls. Not surprisingly, the Parkinson disease group also showed increasing impairment in measures of motor function. Abnormal activation of the pre-supplementary motor area has been described in tests of both motor (D'Ostilio et al. 2013, Eckert et al.

2006) and cognitive (Obeso et al. 2013, Hikosaka et al. 1996) function in individuals with Parkinson disease and there are reports of lower pre-supplementary motor area NAA/Cr ratios in a group of older individuals with Parkinson disease. In this study, this marker of pre-supplementary motor area neuronal integrity did not discriminate between the two groups.

The emergence of FOG has been proposed to be associated with cognitive dysfunction (Nutt et al. 2011). Evidence supporting the correlation of FOG and cognitive impairment, as well as faster progression of cognitive decline, is emerging (Amboni et al. 2008, Amboni et al. 2010, Naismith, Shine & Lewis 2010, Giladi et al. 1992). In our study, performance of tests of cognition in early disease did not discriminate those who went on to develop FOG. Similarly, the hypothesis that the groups would change and would change differently over 36 months was generally not supported by our data. This study did confirm more initial impairment and a more rapid decline in measures of motor disease burden in the FOG group. The FOG cohort, supporting previous findings, reported worsening in reported quality of life over three years. There were no baseline or 36 month differences detected between freezers and non-freezers on measures of pre-supplementary motor area NAA/Cr ratio.

In summary, NAA/Cr ratios, as a marker of neuronal integrity in pre-supplementary motor area, did not discriminate between Parkinson disease and controls and Parkinson disease FOG vs nFOG at baseline or a differential change in the groups over time. We were unable to show evidence of neuronal loss using this methodology in this cohort of Parkinson disease subjects.

Significance and Clinical Implications

There is compelling motivation to identify a biomarker of disease progression in Parkinson disease. The search for a neuroprotective or disease modifying therapy continues to be hampered by the lack of adequate outcome measures and measures of disease progression. Current clinical outcome measures suffer from inter-rater differences and insensitivity to subtle, but perhaps clinically meaningful, changes. A biomarker that reproducibly and accurately quantifies pathologic change and is sensitive to change over time and unaffected by symptomatic treatment would be extremely beneficial in the setting of a clinical trial. This biomarker would need to correlate with clinical deterioration, be readily accessible, cost effective and, importantly, acceptable to patients. Non-invasive neuroimaging is a good candidate for such a biomarker. An objective imaging marker would be useful to discriminate between subtypes of Parkinson disease to possibly allow recruitment of an enriched, more homogeneous clinical trial cohort.

One explanation for the lack of success in human studies of compounds that showed success in treating animal models of Parkinson disease is the diversity of clinical profile and underlying pathology between Parkinson disease subtypes. Our midbrain iron data suggest that there is a great deal of heterogeneity in this population, but that measures of iron in the substantia nigra correlate with the development of FOG. Perhaps by using this measure early, one could increase homogeneity in the setting of a prospective clinical trial. The marker of neuronal loss in the pre-supplementary motor area failed to correlate both with disease status and disease progression in this cohort of early Parkinson disease. These results conflict with data from an older cohort with longer disease duration

(Camicioli et al. 2007). This suggests that NAA/Cr ratios may be useful biomarker in a more advanced patient population.

Rigorous systematic validation of a potential biomarker must be done prior to its use in neuroprotective or therapeutic clinical trials. High nigral iron levels, increasing with age, are evident in healthy individuals (Zecca et al. 2004). These levels increase even further in Parkinson disease (Dexter et al. 1989). This increase may been seen in some studies of early Parkinson disease (Martin, Wieler & Gee 2008) and change in SNc iron accumulation is correlated with worsening motor (Martin, Wieler & Gee 2008) and cognitive (Rossi et al. 2014, Pagonabarraga et al. 2012) function but not in other studies.(Jin et al. 2012) Increases in nigral iron have been implicated in the neurodegeneration seen in Parkinson disease, but it is not clear to what extent iron plays a role in the degenerative process.

The possibility that removing iron might prevent further damage or reverse existing damage holds appeal. A recent Phase 2 trial, (Devos et al. 2014) in subjects with early Parkinson disease explored this approach by using an iron chelator (deferiprone) and evaluating its effect on UPDRS scores in a delayed start paradigm. Measured R_2* values from the substantia nigra served as a marker of disease progression. They observed a significant decrease in measures of iron in the substantia nigral reflected by a decrease in R_2* values that correlated with a slowing of progression reflected in UPDRS scores associated with the treatment. Given the lack of consensus in the literature on the relationship of measured R_2* values in the substantia nigra and disease progression, however, it would be prudent to be cautious in applying it as a biomarker in clinical trials.

The association between cognition and the emergence of FOG in Parkinson disease remains an important area of exploration. While neuronal loss in the pre-supplementary motor area was not shown to relate to these putative non-dopamine responsive features, the complex inter-relation of other functional and structural neural substrates is likely to contribute to the concurrent appearance of these disabling features.

Limitations

There are several limitations in the use of imaging markers to investigate brain mechanisms underlying motor or cognitive dysfunction. When imaging iron rich areas of the brain, such as the substantia nigra, ROI placement on R_2* maps may be challenging because of image distortion related to air-tissue interfaces. For any R_2* based approach, iron is only one contributor to the R_2* signal. In a longitudinal study, other processes, such as disease-related tissue changes including neuronal loss may result in an increase in free water, thereby altering R_2* values.

There are controversies surrounding the placement of the substantia nigra ROI as well as the definition of its sub-regions (Oikawa et al. 2002, Lotfipour et al. 2012, Manova et al. 2009) perhaps accounting for differences reported in R_2* and iron in the substantia nigra. Refined techniques and higher field strength systems providing better spatial resolution, may allow for more accurate delineation of these midbrain structures in the future.

Reliably placing ROIs in longitudinal studies in precisely the same location as in baseline scans can be challenging. Additionally, imprecise ROI placement on baseline scans will introduce imprecision throughout the study.

Other issues arising with the use of imaging markers include: motion artifact (particularly in Parkinson disease) rendering some scans unusable, unacceptable signal-to-noise ratios when measuring NAA/Cr ratios rendering spectra unusable, discomfort of participants prematurely ending scanning sessions and scanner reliability may impact scheduling. Several of these issues resulted in the exclusion of several subjects from analysis. Since there was no difference in the baseline characteristics of those who were excluded for any of these reasons, this decreased our sample size without impacting additionally on interpretation of results.

Retention of participants in a longitudinal study can be challenging. In our study, we had good retention rates in both the Parkinson disease and control cohorts. There was one death in the Parkinson disease group from causes unrelated to Parkinson disease after the 18 month visit. One other Parkinson disease participant withdrew consent after the baseline visit stating discomfort with the idea of being placed in a high magnetic field.

As with most studies, our Parkinson disease cohort was heterogeneous with respect to clinical presentation, eventual need for dopaminergic replacement therapy, and emergence of dopamine-resistant features. It has been suggested that some dopaminergic drugs act as a weak iron chelator and this may have had an effect on changes in R_2* values in those subjects on treatment (Youdim et al. 2000).

A further limitation of these studies is the duration of follow up. Parkinson disease is a disorder that slowly progresses over many years. One would anticipate relatively limited progression over the relatively short duration of this study. As clinical features worsen over time, compensatory symptomatic treatment is

required. As a result, in any longitudinal study of Parkinson disease, the symptomatic effect of medication becomes tightly intertwined with the symptoms of disease progression.

Future Directions

In these studies, we investigated the clinical, structural and functional correlates of Parkinson disease progression with respect to iron in the substantia nigra and altered pre-supplementary motor area NAA/Cr ratios. Other approaches to better understand the relationship between changes in brain structure and function in those with Parkinson disease that develop FOG include other structural imaging approaches like VBM, DTI and cortical thickness mapping. These approaches may further clarify the relationship shown between substantia nigral changes and the groups in our study. Larger patient populations followed for a longer duration would also help clarify the difference between early stage and later stage disease in these measures.

The quest for an imaging marker of disease progression in Parkinson disease is complex and challenging. It may be that multiple markers, rather than a single marker will be needed depending on the area of interest, for example, motor versus cognition progression or the specific presence of FOG. Multimodal imaging combining structural and functional approaches together with other biomarkers of disease may be needed as complex pathologic and neurochemical substrates may be different.

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