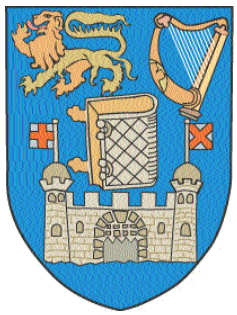
Quantitative Assessment of Perceptual, Motor and Cognitive Function in Parkinson's Disease and Their Contribution to Freezing of Gait



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A dissertation submitted to the University of Dublin for the degree of

Doctor of Philosophy

by

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Declaration

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Conor Fearon

17th March 2016

Abstract

Freezing of gait (FOG) is a paroxysmal motor symptom which manifests as episodic reduction or complete absence of effective stepping despite the intention to walk. It is a common and disturbing problem, affecting over 50% of people with Parkinson's disease (PwP), particularly in the later stages, when the disease becomes more complex and severe, leading to falls and nursing home placement. In spite of this, the pathophysiology of FOG remains very poorly understood. Associations with sensory and perceptual impairments, motor deficits and cognitive dysfunction have all been described in isolation in FOG but a common overriding mechanism has not been described to date.

The primary aim of this thesis was to develop quantitative measures of sensory, motor and cognitive function and to compare differences in these measures in PwP with and without FOG to determine whether true deficits in these domains exist. In particular, the measures were designed to be independent of the slowness of movement that is seen in PwP and would therefore be largely independent of the severity of the disease.

The secondary aim of the thesis was to develop an intervention which employed sensory, motor and cognitive strategies in order to train these domains simultaneously, and hence improve FOG in those people with Parkinson's disease who are affected by this symptom.

The main findings of the thesis are as follows:

1. **Sensory and perceptual processing speeds are different between PwP and age-matched healthy controls. In particular, there is a significant difference between auditory and visual reaction times in PwP (auditory faster than visual) which is not present in age-matched healthy controls. Furthermore, this relative difference between auditory and visual reaction times is significantly greater in those with FOG than those without FOG and also correlates with disease duration. The ability to combine auditory and visual stimuli (multisensory integration) is also impaired in PwP when compared to healthy controls.**
2. **Despite overall motor slowness, PwP on medication can refine goal directed movement to perform as quickly as age-matched healthy controls. There is no difference in control of goal directed movement in PwP with FOG compared to those without. However, PwP with left-sided onset PD have difficulty with goal-directed tasks initially compared with right-sided**

onset. This may relate to a higher order perceptual processing deficit which localised to the right frontostriatal network in patients with left-sided disease.

3. **Although there is evidence of an association between executive dysfunction and FOG, electrophysiological markers of cognitive function and decision making reveal no difference between those with and without FOG. However, cortical electrical potentials related to goal-directed movement preparation are significantly greater in amplitude in those with FOG suggesting excessive recruitment of cortical areas is required in order to perform a simple motor task.**
4. **Event-related potential analysis while stepping in place reveals attenuation of the N2b potential in those with FOG only. The N2b potential has close links with motor preparation and, in particular response inhibition and response conflict (which are closely associated with FOG). Whereas the N2b potential is present in freezers while sitting, it disappears while performing the simultaneous task of stepping in place. This potential is preserved while seated and stepping in those without FOG.**
5. **A virtual reality-based intervention which combined sensory, cognitive and motor training showed significant improvement in reaction time and gait parameters during single- and dual-tasks in patients with FOG, as well as improvements in cognitive flexibility and self-reported FOG.**

In summary, early sensory and motor signalling is impaired and inefficient in people with FOG. These processes, which are crucial to complex goal-directed tasks such as locomotion can be compensated for, at the expense of attentional and cognitive resources, which are limited. Therefore, when multiple motor and cognitive tasks take place in a complex sensory environment, this capacity to perform multiple or complicated tasks becomes easily depleted, probably leading to FOG. Fortunately, this capacity can be expanded with training, resulting in improvements not only in FOG but also overall gait performance and potentially cognition.

Acknowledgements

Firstly, I would like to thank my supervisors: Prof. Richard Reilly and Prof. Tim Lynch for their constant guidance, support, motivation and encouragement during the course of this PhD; for continuously making me think and re-think about this research in new and exciting ways; and for making the process greatly enjoyable and rewarding.

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Thanks to all of the staff at the Dublin Neurological Institute at the Mater Misericordiae University Hopsital, in particular Mr Brian Magennis who assisted with patient recruitment. Most importantly, I would like to express my deepest gratitude to all of the participants who volunteered their time and and energy in order for the studies in this work to be undertaken, especially the people with Parkinson's disease for their inspiring energy and commitment to promoting research in this area. Without them, no meaningful breakthroughs will ever be achieved.

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Abbreviations

ABBREVIATION	MEANING
BOLD	Blood Oxygen Level Dependent
COP	Centre of Pressure
CPP	Centroparietal Positivity
DTI	Diffusion Tensor Imaging
EEG	Electroencephalography
ERP	Event Related Potential
FMRI	Functional Magnetic Resonance Imaging
FOG	Freezing of Gait
GPE	Globus Pallidus Externa
GPI	Globus Pallidus Interna
LRP	Lateralised Readiness Potential
MRI	Magnetic Resonance Imaging
MSA	Multiple Systems Atrophy
PD	Parkinson's Disease
PPN	Pedunculopontine Nucleus
PSP	Progressive Supranuclear Palsy
PWP	People with Parkinson's disease
RT	Reaction Time
SNC	Substantia Nigra Pars Compacta
SNR	Substantia Nigra Pars Reticulata
STN	Subthalamic Nucleus
VBM	Voxel Based Morphometry
VE	Virtual Environment
VR	Virtual Reality

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Publications

International Peer-Reviewed Journal Papers

- C. Fearon*, J.S. Butler*, L. Newman, T. Lynch, R.B. Reilly. Audiovisual Processing is Abnormal in Parkinson's Disease and Correlates with Freezing of Gait and Disease Duration, Journal of Parkinson's Disease. 2015 Oct 17;5(4):925-36.
- C. Fearon*, I. Killane*, L. Newman, C. McDonnell, S. Waechter, K. Sons, T. Lynch, R.B. Reilly. Dual Motor-Cognitive Virtual Reality Training Impacts Dual-Task Performance in Freezing of Gait. IEEE Journal of Biomedical and Health Informatics, 2015 Nov;19(6):1855-61.
- C. Fearon*, J.S. Butler*, I Killane, S. Waechter, R.B. Reilly, T. Lynch. Getting Ready to Freeze: Motor Preparation Rather Than Decision Making Differentiates Parkinson's Disease Patients With and Without Freezing of Gait. Manuscript in Review
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- C. Fearon*, J.S. Butler*, I Killane, S. Waechter, R.B. Reilly, T. Lynch. Neurophysiological Correlates of Decision Making and Motor Preparation While Stepping in Parkinson's Disease Patients With and Without Freezing of Gait, Manuscript in Preparation.

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- C. Fearon, J.S. Butler, I Killane, S. Waechter, L. Newman, R.B. Reilly, T. Lynch. Motor Preparation and Decision-Making in Parkinson's Disease Patients With And Without Freezing of Gait: An Event-Related Potential Study. Irish Neurological Association Meeting. Limerick Ireland, May 2016.
- C. Fearon, J.S. Butler, L. Newman, R.B. Reilly, T. Lynch. Differential Audiovisual Processing in Parkinson's disease. Movement Disorder Society International Congress of Parkinson's Disease and Movement Disorders. San Diego, USA, June 2015.
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Disease Cohort with and without Freezing of Gait: An EEG and Behavioral Based Approach. Proceedings of 7th International IEEE EMBS Conference on Neural Engineering. Montpelier, France. April 2015.

- C. Fearon, L. Newman, J.S. Butler, B. Quinlivan, R.B. Reilly, T. Lynch. Motor Learning in Parkinson's Disease using an Action Acquisition Task. Irish Neurological Association Meeting. Galway, Ireland, May 2015.

- C. Fearon, L. Newman, B. Quinlivan, J.S. Butler, T. Lynch, R.B. Reilly, "Investigation of Motor Learning in Parkinson's Disease using an Action Acquisition Task". Association of British Neurologists Meeting. Harrogate, UK, May 2015.

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1. Introduction

1.1 Parkinson's Disease

Parkinson's disease (PD) is a neurodegenerative disorder characterised by loss of dopaminergic signalling in the basal ganglia, a complex structure in the basal forebrain with prominent functions in motor control as well as emotional, attentional, perceptual and motivational functioning. The symptoms of PD can be divided into motor and non-motor features. The motor symptoms of PD include tremor, bradykinesia (slowness of movement with decrement in amplitude), rigidity (stiffness or increased resistance to passive movement), stooped posture and gait disturbances such as postural instability and freezing of gait. Non-motor features, on the other hand, include constipation, depression, anxiety, cognitive impairment, autonomic instability, hallucinations and impulse control disorders.

The pathophysiology of PD is incompletely understood but environmental triggers are believed to play an important role in genetically predisposed individuals (Massano and Bhatia, 2012). The mainstay of treatment is dopamine replacement either with levodopa-carbidopa or dopamine agonists, with a further role for other medications such as monoamine oxidase inhibitors and anticholinergics. The goal of dopamine replacement is to restore circulating dopamine levels and at least partially reverse the above motor symptoms, a condition known as the "on" state. When patients are off medication, or the medications are not sufficient to create an "on" state, patients are described as being "off". More complex therapies such as deep brain stimulation of the subthalamic nucleus or globus pallidus interna or continuous infusions of levodopa (via a percutaneous gastrostomy) or a dopamine agonist (subcutaneously) can be undertaken when standard dopamine replacement therapy fails to work. In order to fully understand the current research questions and how they are relevant to PD, a detailed discussion of the anatomy of the basal ganglia in healthy subjects and in people with Parkinson's (PwP) is required.

1.2 Functional Anatomy of the Basal Ganglia.

The basal ganglia are a set of subcortical nuclei in the basal forebrain consisting of: the striatum (comprising the putamen and caudate nucleus), the globus pallidus interna (GPi) and externa (GPe), the subthalamic nucleus (STN) and the substantia nigra (pars reticularis (SNr) and pars compacta (SNc))

(Figure 1.1). The interactions among the basal ganglia nuclei and between the basal ganglia, cortex and thalamus are complex.

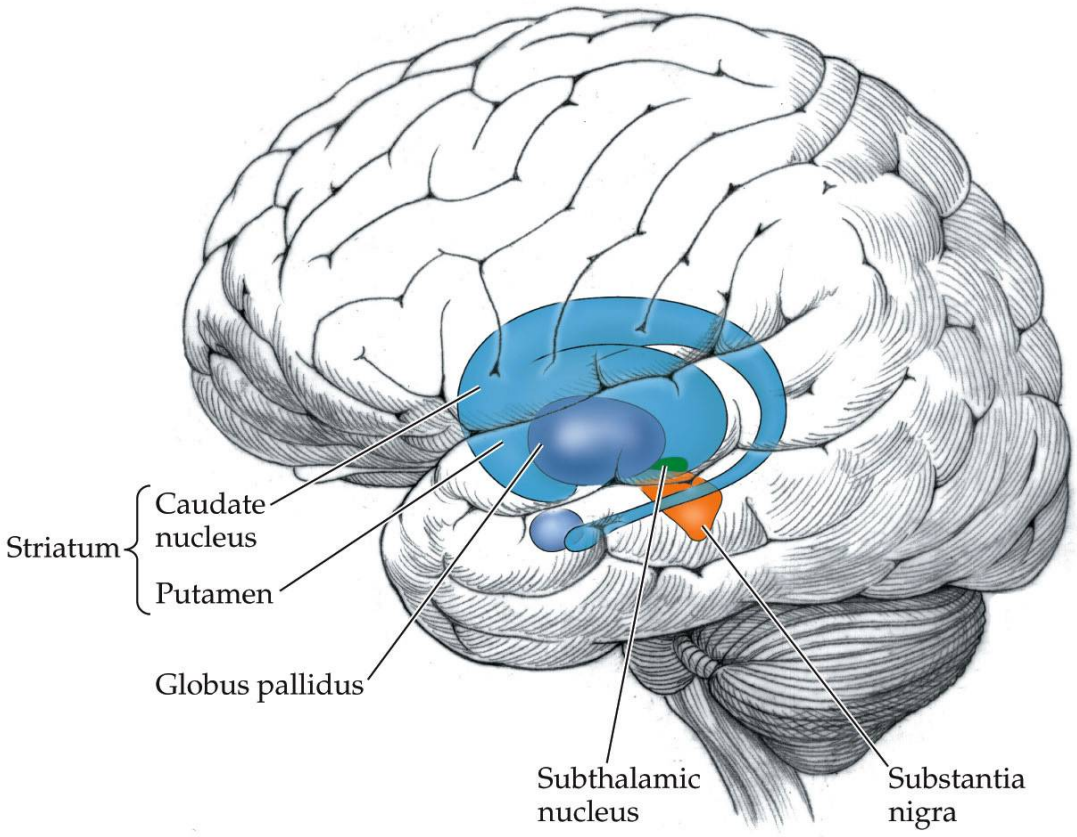


Figure 1.1 Basal Ganglia Anatomy *from* (Breedlove *et al.*, 2010)

The basal ganglia receive excitatory inputs from all areas of the cerebral cortex (and hence it is involved in a wide range of cortical functions) and they process this information via two different pathways: the direct pathway and the indirect pathway. The output from these pathways then projects back via the thalamus to the cortex, forming a complex feedback loop.

The direct pathway forms part of a net positive (excitatory) feedback loop, which receives input from the cortex at the striatum and sends inhibitory (GABAergic) signals to the GPi and SNr which in turn send inhibitory (GABAergic) projections to the thalamus (the combination of these two inhibitory signals having a net positive effect on the thalamus). The thalamus then sends excitatory (glutamatergic) projections back to the cortex forming a facilitatory feedback loop (Figure 1.2).

In the indirect pathway, the striatum sends inhibitory (GABAergic) signals to the GPe, which reduces the degree of inhibition on the STN. The STN is then allowed to send excitatory (glutamatergic) signals to GPi, GPe and SNr, activating all three structures. The GPi and SNr then have an increased inhibitory effect on the thalamus and therefore the cortex receives less excitation from the thalamus. The indirect pathway, thus, forms part of a net inhibitory feedback loop (Figure 1.2).

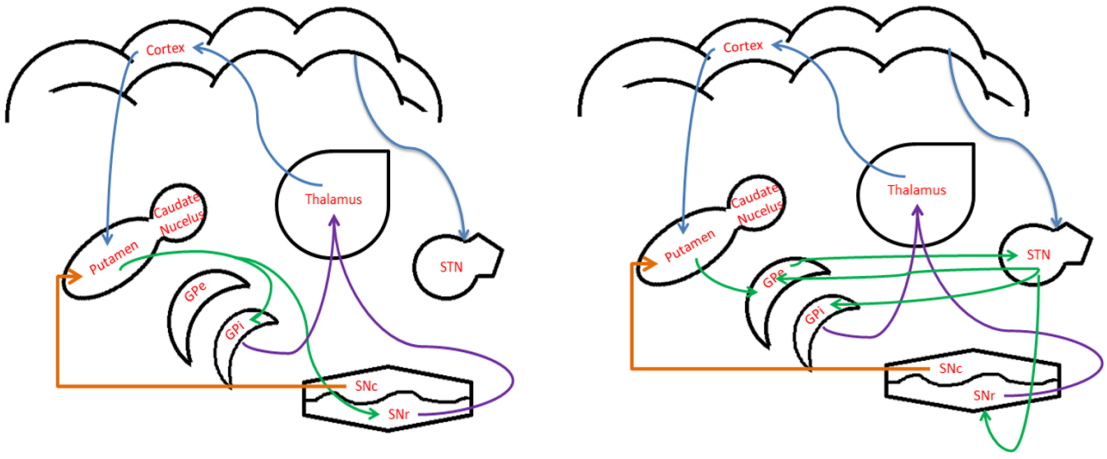


Figure 1.2 The direct and indirect pathways: *Direct pathway (left, green); Striatum receives inputs from the cortex (blue) and sends inhibitory signals to the GPi and to the SNr. This reduces their inhibitory outputs to the thalamus (purple). The disinhibited thalamus projects back to the cortex (blue) to complete an overall excitatory feedback loop. Indirect pathway (right, green): The striatum sends inhibitory signals to the GPe, leading to reduced inhibition of the STN. The STN then sends excitatory signals to the GPi, GPe and to the SNr. The net effect is that the GPi and SNr send a net inhibitory signal to the thalamus via GPi and SNr, reducing the excitatory thalamocortical connections (blue), forming a net inhibitory feedback loop. The striatum also receives dopaminergic input from the SNc (orange) which further modulates these interactions.*

In this way, the output of the basal ganglia is a balance between the excitatory output from the direct pathway and the inhibitory output from the indirect pathway allowing fine control of cortical responses via exquisite feedback control (Figure 1.3). This model, however, represents an oversimplification of the complex connections within the basal ganglia network. Recently, efforts have been made to better characterise the functional anatomy of the basal ganglia (Calabresi *et al.*, 2014), but a discussion of these models is beyond the scope of this thesis. Importantly, the output of the basal ganglia is now known to act on other nuclei in the brainstem, including the pedunculopontine nucleus (PPN) which is believed to have an important role in control of gait including gait initiation, turning, stopping and

avoiding obstacles (Mena-Segovia *et al.*, 2004; Windels *et al.*, 2015). Dopaminergic signalling from the SNc modulates these pathways considerably. In particular, the direct pathway (which contains predominantly D1-type dopamine receptors) is excited by dopamine and the indirect pathway (which contains predominantly D2-type receptors) is inhibited by dopamine. The effect of depletion of dopamine in the basal ganglia, as occurs in Parkinson's disease, will be discussed later.

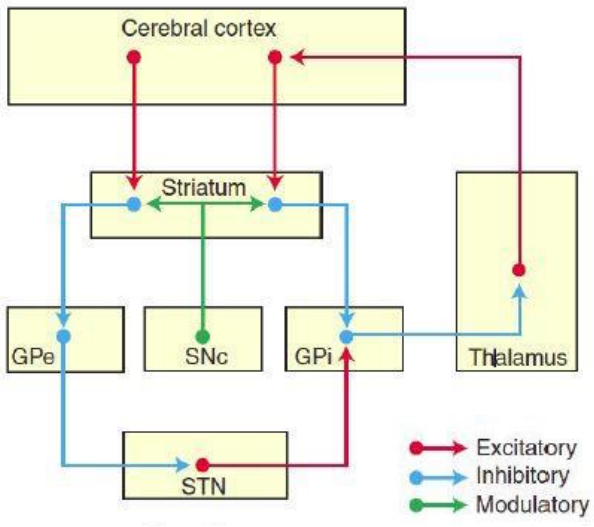


Figure 1.3: Basal ganglia – thalamocortical network: *The inhibitory and excitatory connections of the basal ganglia are shown along with the modulatory effect that the dopaminergic supply from the SNc provides.*

Given, the extensive connections that the basal ganglia have with almost every part of the cortex, it is not surprising that these nuclei have widespread roles in control of many different cortical responses. It has long been known that the basal ganglia has an important role in the control of movement (Graybiel, 2000). In the model above, the direct pathway facilitates movement while the indirect pathway inhibits it, much like a brake and accelerator. This antagonism allows selection of intended movements by selecting the desired action and suppressing unwanted ones. Traditionally, the basal ganglia was thought to comprise just two distinct pathways: the direct pathway, which was involved in selecting the desired movement and the indirect pathway, which suppresses unwanted movements. Recently, a third (hyperdirect pathway) was discovered consisting of a much more direct and, therefore, rapid connection between cortical areas and the STN (Nambu *et al.*, 2002). The hyperdirect pathway bypasses the striatum entirely and connects the cortex directly to the STN (Figure 1.4). The role of the hyperdirect pathway is to rapidly inhibit unwanted movement, especially movement which has been already

initiated. The crucial connection for this rapid response inhibition is between the right inferior frontal gyrus / supplementary motor area and the STN. Whereas the **supplementary motor area** is crucial for initiation of motor programs, the **right inferior frontal gyrus** also has an important role in dual task interference (Herath *et al.*, 2001). Thus, when a motor program is initiated, the hyperdirect pathway initially suppresses widespread motor programs, including the selected one. Thereafter, the direct pathway disinhibits the targets for the selected motor program and the indirect pathway inhibits competing motor programs. Thus, **response selection and response inhibition** is carefully balanced by a more complex network than originally thought.

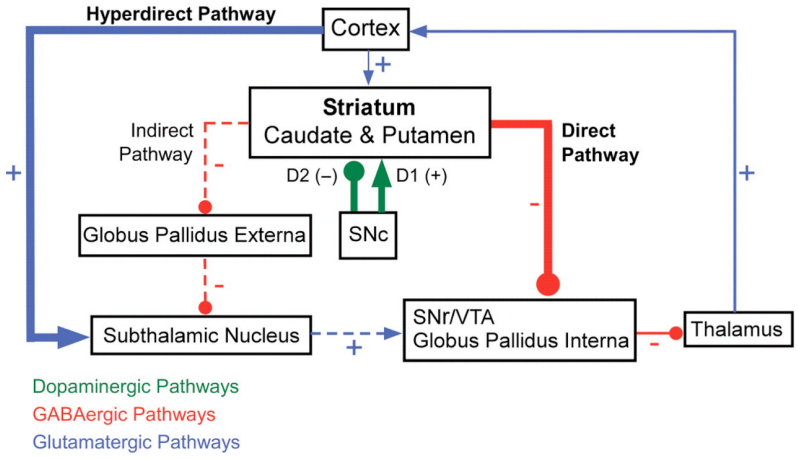


Figure 1.4. Anatomy of hyperdirect, indirect and direct pathways: *The hyperdirect pathway directly connects the cortex (in particular, the supplementary motor area and right inferior frontal gyrus) to the subthalamic nucleus, allowing rapid inhibition of unwanted movements. The direct and indirect pathways are also shown. From* (Fling *et al.*, 2014)*.*

The basal ganglia is not simply involved with control of movement, however. These nuclei have prominent roles in learning, attention, perceptual decision making and behavioural control (Ding and Gold, 2013). Neuroimaging studies have revealed abnormal basal ganglia activation in a wide range of neuropsychiatric disorders including obsessive-compulsive disorder (Hou *et al.*, 2013), Tourette's syndrome (Neuner *et al.*, 2014) and schizophrenia (Duan *et al.*, 2015), highlighting the important role that the basal ganglia plays in normal control of behaviour. Dopaminergic signalling is crucial to responses to reward in the brain and hence, plays a central role in behavioural learning (Anderson, 2015). Animal studies have shown that when an action results in an unexpected reward, phasic firing of

dopaminergic neurons in the striatum takes place and the animal subsequently repeats this action (Howe *et al.*, 2013). Thus, the basal ganglia regulate behaviour in a more general sense (far beyond motor control) balancing the selection and suppression of wanted and unwanted responses from the infinite range of possibilities. It is not surprising therefore that basal ganglia dysfunction has been associated not only with movement disorders, but also addiction, thought disorders and impulse control disorders.

The normal function of the basal ganglia nuclei can, therefore, be generalised as selection and control of behaviours (movement being a special case of behaviour). The cortex sends signals about the current status quo (motor, sensory, cognitive, limbic). The basal ganglia-thalamocortical network integrates this information together, then selects an action which, via feedback to cortical and subcortical networks, executes the response (Graybiel, 2000).

It was previously believed that the basal ganglia represented a single centre for pooled cortical information but we have since learned that the basal ganglia processes information in a highly specific manner (Redgrave *et al.*, 2010). The structure of the basal ganglia is anatomically segregated into regions which process information from specific cortical areas (Figure 1.5). The more posterior striatum (e.g. the caudal putamen) is concerned with processing sensorimotor information (which regulates automatic habitual control of movement) primarily from the premotor and motor cortices. The anterior striatum processes limbic (i.e. emotional) information from the medial and orbital prefrontal cortex and the areas between these anterior and posterior areas process associative (i.e. cognitive) signals from the dorsolateral prefrontal cortex. These associative signals govern consciously controlled, goal-directed movement. The basal ganglia, thalamus and cortex, therefore, form a series of independent feedback loops (rather than a single loop) for fine control of **sensory, motor, cognitive** and limbic processing. The functionally segregated nature of the basal ganglia has important implications for the effect that Parkinson's disease has on this system.

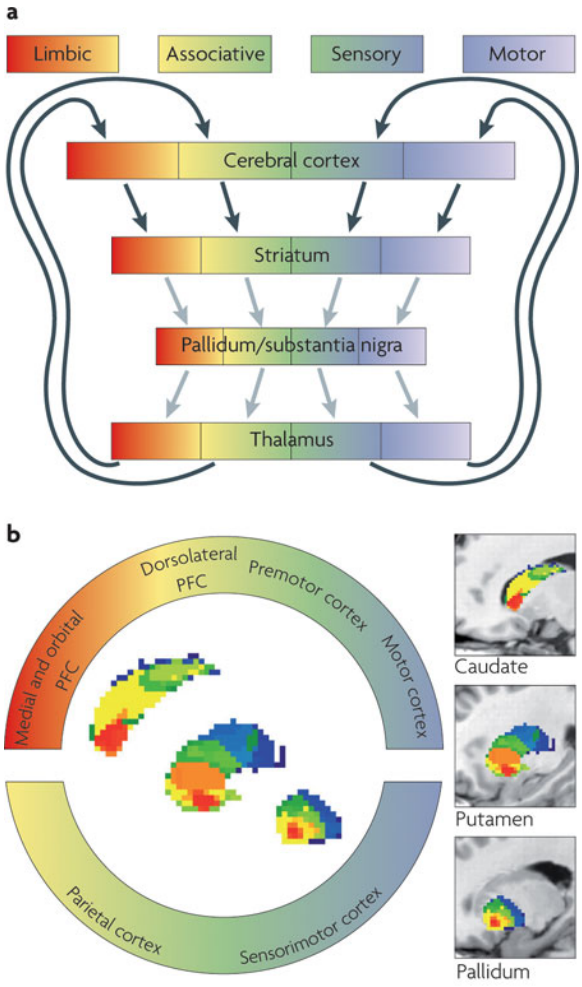


Figure 1.5 Functional segregation of the basal ganglia *(From* (Redgrave *et al.*, 2010)*): a) Feedback loops of limbic (red), associative (yellow-green) and sensorimotor (blue) information which operate as functionally independent circuits; b) spatial anatomy of basal ganglia: the outer ring shows the cortical areas concerned with limbic (red), associative (yellow-green) and sensorimotor (blue) information and how these are functionally connected to specific areas in the striatum.*

Dopamine has an important modulatory role in the basal ganglia model above. In Parkinson's disease, a reduction in dopaminergic signalling from the SNc simultaneously increases signalling in the inhibitory indirect pathway and reduces signalling in the excitatory direct pathway leading to excessive activation of basal ganglia output and a poverty or resistance to movement that characterises PD (Figure 1.6).

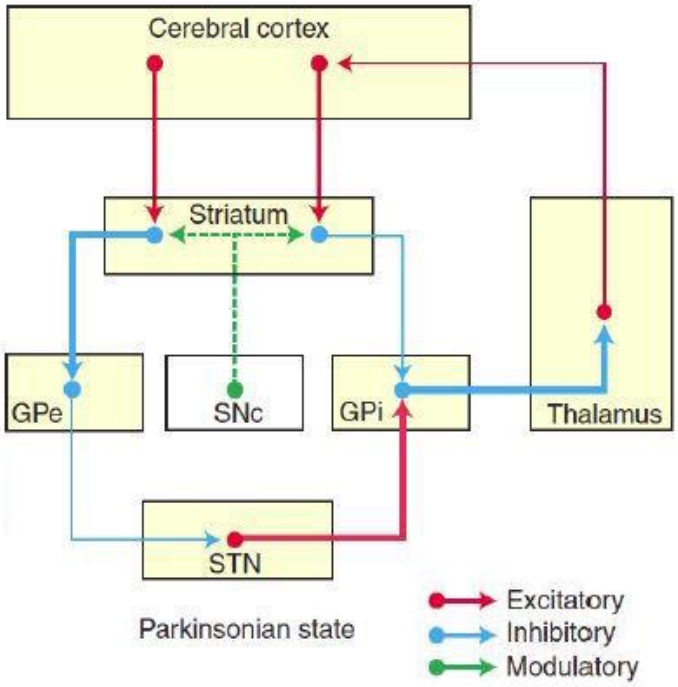


Figure 1.6: Basal ganglia–thalamocortical network in Parkinson's disease: *The inhibitory and excitatory connections of the basal ganglia are shown. The loss of dopaminergic outflow from the SNc to the striatum increases signalling in the inhibitory indirect pathway and reduces signalling in the excitatory direct pathway, leading to an increased inhibitory outflow from the basal ganglia to the thalamus.*

However, the loss of dopaminergic innervation to the striatum does not occur uniformly in Parkinson's disease. Denervation commences in the areas supplying the (sensorimotor) caudal putamen, which governs control of habitual/automatic movement (Redgrave *et al.*, 2010). As a result, people with early PD often report that automatic tasks become a little more effortful. In this situation, compensation for loss of habitual movement could occur by **recruiting frontal cortical areas** to provide more goal-directed movement, which passes through more anterior (associative) basal ganglia pathways (Figure 1.7). With disease progression, the loss of dopamine signaling may spread to involve more anterior regions of the basal ganglia involved in goal-directed behaviour. Hence, the balance between habitual and goal-directed control of movement becomes altered in PD.

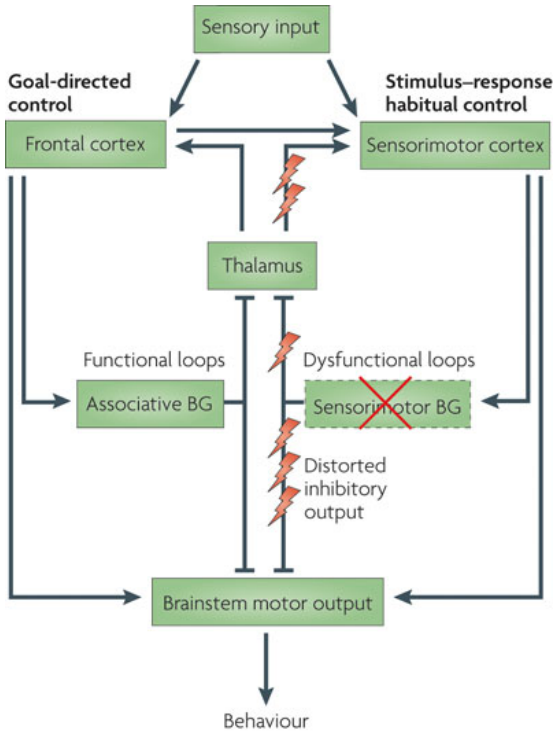


Figure 1.7 Dysfunction of the basal ganglia in Parkinson's disease *(From* (Redgrave *et al.*, 2010)*): a) Preferential loss of dopaminergic innervation in sensorimotor areas leads to loss of habitual control of motor output leading to an increased reliance on goal-directed control via associative loops.*

The question arises, therefore, what happens in advanced PD if associative loops are also involved and both habitual and goal-directed control of movement is affected? Could dysfunctional interactions of sensory, motor and cognitive processing networks lead to complete cessation of motor output? These questions have not been approached in the literature to date but will be further explored in Chapter 4.

1.3 Freezing of Gait

Freezing of gait (FOG) is a paroxysmal motor symptom which manifests as "brief, episodic absence or marked reduction in forward progression of the feet despite the intention to walk" (Nutt *et al.*, 2011). This careful definition was reached via a consensus at a workshop of FOG researchers in 2010. The difficulty in clearly defining FOG implicitly highlights many of the issues which surround research in this area. In clinical practice, the patient describes the feeling that their feet are "stuck" or "glued" to the floor and FOG is frequently preceded by severe gait festination (where step length and stride time shorten progressively until complete cessation of locomotion occurs). Freezing affects up to 60% of

patients with advanced PD (Giladi and Nieuwboer, 2008) but can also occur in early PD, progressive supranuclear palsy, multiple systems atrophy, vascular parkinsonism, normal pressure hydrocephalus and alone as a "pure freezing syndrome" (Giladi *et al.*, 1997). FOG most commonly occurs during turning, initiation of gait, in narrow spaces, in stressful or emotional circumstances and, particularly, while performing a second task simultaneously (dual-tasking).

A number of specific clinical features of FOG have been noted (Nutt *et al.*, 2011):

- During a freezing episode, the step height is severely reduced such that the feet barely leave the ground, if at all.
- Trembling of the legs often occurs (usually at a frequency of 3–8 Hz) (Moore *et al.*, 2008).
- Prior to a freezing episode, a deterioration of gait usually occurs in the form of hastening, with reduction in step length, symmetry, and rhythmicity.
- The patients often feel as though their feet are glued to the floor and episodes can be associated with intense anxiety.
- FOG can be provoked or relieved by various cues, which will be discussed in detail below.
- FOG is not always symmetrical and can affect one lower limb and can be initiated by turning in one direction but not another.

Despite advances in pharmacological therapy for Parkinson's disease, FOG remains a significant problem for those patients affected by it. Furthermore, there is a close association with falls and placement in nursing homes (Bloem *et al.*, 2004). In particular, dual-tasking is a significant contributor to falls in this population (Jacobs *et al.*, 2014). This highlights the importance of FOG, not only as a disturbing and disabling symptom for patients with PD, but also as a considerable cost to the healthcare system (Grimbergen *et al.*, 2004). FOG was felt to be the greatest unmet need in PD in a survey among neurologists at the European Federation of the Neurological Societies in 2013 (*Survey report: Unmet Needs in Parkinson's Disease*, 2013).

1.4 Investigating Freezing of Gait: Approaches and Challenges

There has been increased awareness of FOG in recent years. However, the underlying pathophysiological mechanisms still remain poorly understood. This lack of understanding is largely the result of the fact that freezing is inherently difficult to study. One cannot predict when a freezing episode might occur and, moreover, there exists no reliable way to initiate a freezing episode in a

controlled environment, allowing acquisition of FOG data to study it. Studies employing timed walk tests, e.g. Timed-Up-And-Go (TUG) tests, have had limited success (Hausdorff, Balash, *et al.*, 2003; Plotnik *et al.*, 2005). More complex walking tasks incorporating dual-tasking and obstacles have improved the sensitivity of initiating FOG but remain unreliable (Moore *et al.*, 2008; Schaafsma *et al.*, 2003; Shine, Naismith, *et al.*, 2013). Walking tasks which involve rapid turns, narrow spaces and dual-tasking provokes FOG more effectively than standard walking tests (Snijders *et al.*, 2012) (Table 1.1).

ONE task provoking at least one FOG episode		**(% of definite freezers)**
Task 1	Rapid full turns	84%
Task 2	Dual task gait trajectory	68%
Task 3	Normal speed gait trajectory	60%
Task 4	Normal speed full turns	48%
Task 5	Rapid speed gait trajectory	48%
Task 6	Narrow half turns	40%
Task 7	Wide half turns	0%
TWO tasks provoking at least one FOG episode		
Task 1 and 2	Rapid full turns, dual task gait trajectory	96%
Task 1 and 4	Rapid full turns, normal gait trajectory	92%
Task 1 and 3	Rapid full turns, normal speed full turns	88%
Task 2 and 3	Normal gait trajectory, dual task gait trajectory	84%
THREE tasks provoking at least one FOG episode		
Task 1, 2, 3	Rapid full turns, dual task gait trajectory, normal gait trajectory	100%

Table 1.1. Provoking Freezing of Gait: *Effectiveness of various manoeuvres in provoking FOG in a clinical environment. From* (Snijders *et al.*, 2012).

In addition, when freezing does occur, it is unpredictable in onset and duration. There is no clear demarcation between severe festination and the occurrence of a freezing episode. There is also significant inter-observer variability in identifying freezing episodes, even among experienced observers and correctly classifying these events can be problematic (Morris *et al.*, 2012). Three distinct patterns of freezing have been reported (Nutt *et al.*, 2011):

1. "Trembling in place": Rapid tremulousness of lower limbs with feet stuck in place.
2. Shuffling forward: Short rapid steps with no effective forward propulsion.
3. Complete akinesia.

Triggers for FOG can also be patient-specific such that initiation or turning may provoke freezing in one patient whereas freezing may only occur in stressful or emotional circumstances for another. This diversity implies that FOG may be a final common pathway for a number of different upstream impairments and makes designing studies difficult.

The majority of people who experience FOG, do so when they are in the "off"-state. The term "off-FOG" is reserved for those patients who experience complete resolution of freezing when "on". "Levodopa-unresponsive FOG" refers to those patients who predominantly freeze when "off" but who experience incomplete or no improvement with levodopa. In a small group of patients, FOG is induced or exacerbated by levodopa ("on-FOG"). For this reason, it is important to document medication status in FOG studies as the presence or absence of medication could have significant effects on the results. Many studies are undertaken on medication, primarily to facilitate patients being able to physically take part in the study and allow inclusion of those with more severe PD. The effects of withdrawal of longterm levodopa therapy on cognitive and motor function may be unpredictable and could introduce further confounders into a study that already has a complex relationship with medication. All studies described in this thesis were undertaken on medication.

Other issues with studying FOG include classification of participants as freezers or non-freezers. In most studies, participants are classified based on self-reported FOG over a recent arbitrary time period (usually one month). Distinguishing true freezing from severe festination or akinesia based on history alone can be problematic. It has been suggested that the classification of FOG could be refined by splitting freezers into 3 categories (Snijders *et al.*, 2012):

a. Self reported
b. Probable freezer (freezing confirmed by caregiver)
c. Definite freezer (observed freezing during testing)

It is not yet clear how these sub-classifications should be interpreted in studies, however. Should only definite freezers be included in studies? Furthermore, as outlined above we cannot predict those patients who will be definite freezers in an experimental setting. This creates significant problems for recruitment and powering of studies as well as leading to a lack of generalisability of results (Nieuwboer and Giladi, 2013). Moreover, it has been suggested that FOG should perhaps be considered as a continuous spectrum rather than a binary entity but, at present, there is no reliable scale for incorporating such a model into freezing studies (Nutt *et al.*, 2011). For these reasons, in the studies

described in this thesis, patients were classified as freezers based on the traditional, self-reported approach used in many studies.

Detecting and quantifying frequency and severity of FOG is similarly challenging. Body-worn sensors such as accelerometers (Moore *et al.*, 2008; Singh *et al.*, 2013), gyroscopes (Tripoliti *et al.*, 2013), goniometers (Singh *et al.*, 2013) and pressure-sensitive insoles (Plotnik *et al.*, 2005) have all shown the ability to automatically detect some freezing episodes, but not with sufficient accuracy to be clinically or experimentally useful. Shine et al. (2011) employed foot pedals to detect lower limb motor arrests while stepping (rather than true locomotion and FOG) and reported that these arrests correlated well with self-reported measures of freezing severity (Shine *et al.*, 2011). This paradigm was also used in a fMRI study of FOG (Shine *et al.*, 2013). Stepping in place on a force plate has similarly been utilised to examine freezing patterns but efforts to reliably detect freezing episodes automatically have been unsuccessful (Nantel *et al.*, 2011; 2012). For these reasons, the gold standard for detection of FOG remains the clinical observation of FOG episodes by an experienced observer whereas *severity* of FOG is often self-rated using one of a number of freezing of gait questionnaires: the Freezing of Gait Questionniare (Giladi *et al.*, 2000); the New Freezing of Gait Questionnaire (Nieuwboer *et al.*, 2008); or the Gait and Falls Questionnaire (Giladi *et al.*, 2000).

Even when groups are accurately divided, multiple confounders invariably exist, most notably disease severity and duration (Macht *et al.*, 2007). Freezing tends to occur late in the disease course of PD and, as a result, those patients who freeze tend to have more advanced and more severe parkinsonism and tend to be on higher doses of dopaminergic medication. When comparisons between freezers and non-freezers are undertaken, the freezing group tend to have significantly longer disease duration. Differences seen between groups may therefore reflect longer disease duration, rather than a specific freezing effect. Freezers and non-freezers are more likely to have different subtypes of PD: freezers tend to display the postural instability and gait deficit phenotype; non-freezers tend to have tremor-predominant PD (Nieuwboer and Giladi, 2013). Attempts at matching patients for these factors is also problematic as specifically recruiting early freezers or late non-freezers (both of which are reasonably rare) may be selecting out patients who have biologically different features and disease courses and are likely to bias a study. Because of greater disease duration and severity, FOG is often associated with significant slowing of motor responses. FOG studies which rely on motor output as a measure, inherently test both speed of information processing and speed of motor output. Drawing conclusions about freezing in PD in general from such studies could be misleading. Results should always be

interpreted in the context of these confounders and controlled for where possible. The studies outlined in this thesis employ, where possible, methods which are independent of speed of motor output.

The process of freezing is not purely an episodic phenomenon as patients with FOG show gait abnormalities in between episodes of freezing, including reduced step amplitude increased stride time variability and disordered bilateral coordination (Frazzitta *et al.*, 2012; Hausdorff *et al.*, 2003; Plotnik and Hausdorff, 2008). Moreover, patients with FOG demonstrate lack of control of bimanual upper limb movements (Nieuwboer *et al.*, 2009; Vercruysse *et al.*, 2012) pointing to a more general impairment of internal timing and motor control. One might therefore suspect that the underlying changes which cause freezing episodes to occur would be detectable, to some degree, at all times, rather than simply when freezing episodes occur. In addition, circumstances which tend to induce freezing in those who experience it (such as stress or dual-tasking), might make these changes more easily detectable.

1.5 Freezing, Executive Function and Dual-tasking

As mentioned above, freezers show persistent gait abnormalities even in the absence of freezing episodes. Furthermore, in spite of the episodic nature of FOG, persistent cognitive and behavioural differences exist between freezers and non-freezers. The strongest of these associations is with **executive dysfunction** (Amboni *et al.*, 2008). Executive function is a set of higher cognitive processes which integrates information from other cortical areas to produce behaviour and, in particular, goal-directed behaviour (Fuster, 1999). Three main divisions of executive function have been described (Miyake *et al.*, 2000). These are: the ability to shift between mental tasks (set-shifting); updating working memory to allow adaptive control of behaviour in response to the environment (**context-updating**); and inhibition of unwanted responses (**response inhibition**).

Whereas automatic (habitual) behaviour can be conducted without high-level neural control, tasks which are less automatic require conscious attentional supervision. Executive function is crucially important to the neural control of gait and walking through a complex environment requires conscious control from frontal areas of the brain (in particular, the dorsolateral prefrontal cortex) (Yogev-Seligmann *et al.*, 2008). From formulating the intention to walk and planning the required individual steps in order to achieve this goal to response monitoring (comparing ongoing movements with

intended plan) and response inhibition (including ignoring irrelevant sensory inputs), executive function is involved at every stage of locomotion.

Behavioural tests of executive function are closely linked with gait performance. The Wisconsin card sorting test (WCST), the Stroop test, the trailmaking test and Frontal Assessment Battery are all examples of neuropsychological tests of executive function (Dirnberger and Jahanshahi, 2013). In particular, **the trailmaking test**, a test of cognitive flexibility (set-shifting, in essence) which requires the participant to connect numbers and letters in an alternating, but increasing, sequence, correlates with gait speed in healthy older adults, especially with more complex walking tasks (Figure 1.8) (Ble *et al.*, 2005).

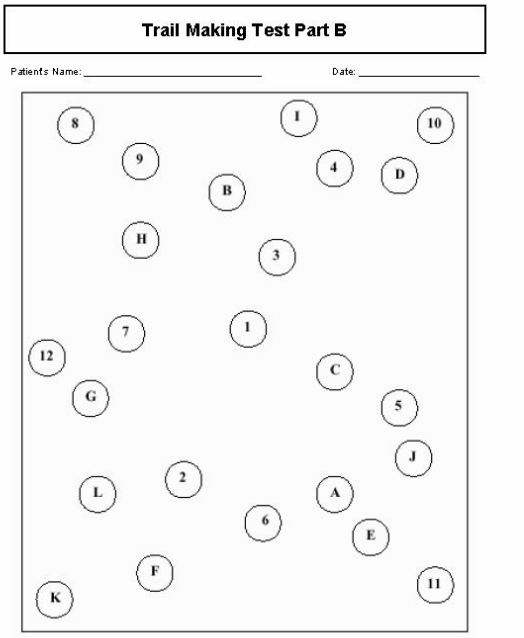


Figure 1.8. Trailmaking test part B: from (Strauss *et al.*, 2006)

As described previously in this chapter, in PD, the loss of dopaminergic innervation in the caudal striatum leads to relative loss of automatic control of behaviour and thus, most tasks are carried out in a goal-directed manner, requiring cortical control of even routine tasks. However, PD patients show deficits in the Wisconson card sorting test (WCST), the Stroop test, the trailmaking test and the Frontal Assessment Battery (FAB) (Dirnberger and Jahanshahi, 2013) suggesting that these cognitive functions are also impaired in PD. Furthermore, executive function deficits are particularly prominent in PD patients with freezing, suggesting that these deficits may play a role in the pathogenesis of FOG (Amboni *et al.*, 2008; Cohen *et al.*, 2014; Giladi *et al.*, 2007). Dysfunction of the frontal lobes or their connections

with the basal ganglia (frontostriatal connections) could, therefore, render these patients incapable of properly executing a complex motor program in response to a planned action.

FOG has been particularly associated with some specific aspects of executive function:

- Set-shifting
- Verbal fluency
- Implicit sequence learning
- Response inhibition
- Response conflict
- Attention and dual-tasking

Naismith et al. (2010) used the **trailmaking tests** to show that self-reported FOG severity correlates with deficits in set-shifting (Naismith *et al.*, 2010). The same group further showed that trailmaking performance correlated with occurrence of freezing episodes in a real environment (Timed Up-and-Go test) (Shine *et al.*, 2013). These deficits become particularly apparent **under time-pressure**.

Verbal fluency, another marker of executive function, is also impaired in freezers compared with non-freezers (Camicioli *et al.*, 1998) and Vandenbossche et al. showed that the speed with which freezers learn a sequenced task is impaired, especially when a second concurrent task is performed (Vandenbossche *et al.*, 2013).

Deficits in **response inhibition** (defined above) and **conflict resolution** (appropriate inhibition of unwanted responses when multiple actions are possible, allowing the intended act to be carried out) are very prominent in FOG (Vandenbossche *et al.*, 2012). The Stroop test (a behavioural test of response inhibition) is impaired in freezers compared with non-freezers (Amboni *et al.*, 2008). Vandenbossche et al. performed a choice reaction time test to examine function of three different attentional networks and found particular deficits in the ability to inhibit unwanted response in freezers (response inhibition) (Vandenbossche *et al.*, 2011). As a result, freezers make action errors when confronted with complex cognitive situations and particularly when presented with conflicting stimuli. It is suggested that this reliance on reflex-like reactions is an adaptive behavior developed in freezers. During locomotion, the individual is presented with frequent conflicts to resolve. For example, movement of one leg must be suppressed at the time that initiation of the contralateral leg is required.

These executive function deficits become most apparent when people with PD have to rely on internally generated attention to perform a cognitive task, as opposed to being guided by external cues. Attention is an important subset of executive function which appropriately allocates cortical resources in order that simultaneous tasks can be processed in parallel. In this way, attention regulates all other actions. Attention can be classified as follows (Yogev-Seligmann *et al.*, 2008):

- Selective attention: Focussing attention on specific information and suppression of irrelevant information
- Divided attention: Carrying out simultaneous tasks (also known as dual-tasking)
- Alternating attention: Rapidly shifting attention between tasks
- Sustained attention: Maintaining attention on a task over a period of time.

Dual-task paradigms divide attention by placing a cognitive load on the participant during a task such as gait and allow the effect of divided attention on the individual tasks (e.g., gait, cognitive tasks) to be studied. A number of models have been proposed to explain this dual-task effect and no consensus on an overriding model exists:

1. The capacity-sharing model: attentional capacity is limited so performance of multiple tasks uses up these limited resources, leading to deterioration in performance of one or more of those tasks (Tombu and Jolicoeur, 2003). This effect is exacerbated under time pressure.
2. The bottleneck model: Simultaneous tasks pass through a common neural processor which deals with each task in a serial fashion. Thus, the processing of each task only occurs after all other tasks are processed, leading to delays in ability to select responses for that task and hence, response times (Ruthruff *et al.*, 2001).
3. Multiple resource model: Different resources are needed for different tasks (Pashler, 1994). If two tasks do not require the same resources, they can be performed simultaneously without any deterioration in the performance of either task. However, if the resources required overlap, then deterioration of performance in one or both tasks will occur.

Difficulties with dual-tasking (reductions in gait speed, symmetry and rhythmicity during a dual-task (Hausdorff, Schaafsma, *et al.*, 2003; Yogev *et al.*, 2005)) are also reported in the Parkinson's disease population (Hausdorff, Balash, *et al.*, 2003) and particularly in those with FOG, highlighting that PwP with FOG have particular difficulty dividing attention (Pieruccini-Faria *et al.*, 2014; Spildooren *et al.*, 2010). During dual-tasking, freezers are more influenced by the second cognitive task than non-freezers

(Camicioli *et al.*, 1998). Gait parameters also deteriorate in freezers when the walking task becomes more complex such as walking through narrow spaces (Almeida and Lebold, 2010), avoiding obstacles (Pieruccini-Faria *et al.*, 2014; Snijders *et al.*, 2010) and turning (Spildooren *et al.*, 2012) and importantly if they have to adapt their movement plan during the walking task, suggesting that motor planning and preparation may be impaired also (Knobl *et al.*, 2011). In this way, **goal-directed movement planning** can act as a second cognitive task while walking.

Thus, FOG is associated with generalised executive dysfunction, specific deficits in cognitive flexibility, set shifting, verbal fluency, implicit sequence learning, dual-tasking, response inhibition and conflict resolution. However, the precise roles that these associations play in the pathophysiology of FOG remains unclear.

1.6 Pathophysiology of Freezing of Gait

Neural control of gait is complex, requiring contributions from the cerebral cortex, basal ganglia, limbic system, brainstem, cerebellum and spinal cord (Takakusaki, 2013). Execution of simple gait patterns is largely a habitual, automatic process requiring little cortical input and can be initiated by limbic projections to the brainstem e.g. in a fight or flight response. More complex, **goal-directed movement**, however, including conscious initiation of gait, requires a greater degree of activation of the cortex, primarily the frontal lobes where voluntary motor programs are stored in the premotor and supplementary motor areas (PMA, SMA) and pass to both brainstem and spinal cord (Takakusaki, 2013). Volitional movement requires focused attentional resources and intact working memory for planning of these complicated movements as well as feedback loops with the basal ganglia and cerebellum (Middleton and Strick, 2000). Throughout these goal-directed movements, however, further automatic adaptive control is employed in order to modify gait in real-time: rapid changes in motor programs in response to changes in sensory information are achieved via **sensorimotor integration** allowing fine adaptive control in a changing environment (i.e. **context updating**) (Andersen and Buneo, 2003) (Brandt and Dieterich, 1999); the limbic system, basal ganglia, cerebellum and brainstem allow predictive control of gait via ascending and descending pathways (Choi and Bastian, 2007); and anticipatory postural adjustments which originate in the SMA, continuously maintain posture during these changes (Jacobs and Horak, 2007). Coordination of these anticipatory postural adjustments with initiation of gait

is crucial and the fact that FOG occurs frequently during gait initiation suggests that some of these mechanisms are involved in its pathogenesis.

As a result of the limited understanding of the pathophysiology of FOG, few effective treatments are available for these patients. Until a greater understanding of the neural substrates of this phenomenon is achieved, it is unlikely that effective treatments will be found to ameliorate freezing. A recent review by Nutt et al. (2011) discussed the possible pathophysiology by examining a number of striking clinical features of FOG, as described below (Nutt *et al.*, 2011). These features suggest significant dysfunction of ***sensory/perceptual, motor*** and ***cognitive*** function in patients with FOG which will be explored throughout the body of work in this thesis. Six plausible explanations for the pathogenesis of FOG have been described:

1) Although FOG has been reported in the early stages of PD (Giladi, McDermott, *et al.*, 2001), it tends to be more prevalent in the "off" state and in advanced disease. This has led to the question of whether **striatal dopaminergic deficiency** underlies FOG. Dopaminergic therapy has been used in the majority of patients where the phenomenon occurs in the "off" state ("off-FOG") to reduce FOG by maintaining patients in the "on" state for longer periods (Schaafsma *et al.*, 2003). However, FOG can also occur during the "on" state and this can be poorly responsive or paradoxically exacerbated by levodopa therapy (Moretti *et al.*, 2011). There are many clinical differences between this "on-FOG" and the classic dopamine responsive form which makes it possible that separate pathological mechanisms exist as a similar clinical sign (Espay *et al.*, 2012). Dopamine agonists have been similarly shown to improve freezing severity in patients with "off-FOG" but increases in new freezing episodes following commencement of dopamine agonist therapy in early PD have also been reported (Parkinson's Study Group, 2002). The occurrence of "on-freezing", the suboptimal response of FOG to dopaminergic therapy as well as the fact that FOG occurs independently of tremor, rigidity and bradykinesia (Bartels *et al.*, 2003), all imply that the pathophysiology is unlikely to be related primarily to striatal dopamine depletion but that dopamine may modulate the tendency to freeze in these patients.

2) Another hypothesis is that FOG is a perceptual malfunction where patients suffer from an **inability to integrate external sensory input** to allow higher cortical centres execute subsequent motor output (Almeida *et al.*, 2005). Certain sensory inputs (e.g. narrow doorways) can precipitate FOG whereas others (e.g. walking up stairs, rhythmical sensory cues) can relieve it (Cowie *et al.*, 2010). As

patients with FOG approach a doorway, their gait speed and stride length reduce to a greater degree than those without FOG. This suggests abnormal motor response to visual inputs. However, pure perception of doorway width is not impaired in freezers, suggesting that integration of visual inputs with motor output or online adaptation of movement in response to visual inputs is the main deficit. However, the exact nature of the perceptual disturbance is unclear. Sensory and perceptual disturbances are common in Parkinson's disease (Martens and Almeida, 2011; Patel *et al.*, 2014). Integration of multiple environmental sensory inputs is crucial for locomotion and there is increasing evidence that these sensory deficits contribute to the pathophysiology of some of the abnormal motor features of PD (Abbruzzese and Berardelli, 2003), including FOG (Ehgoetz Martens *et al.*, 2013). The question of whether objective quantifiable sensory or perceptual differences exist between freezers and non-freezers has not been answered to date and this concept will be explored further in Chapter 3.

3) Other studies have proposed a frontal mechanism to FOG, attributing freezing to **executive dysfunction** in the setting of an additional cognitive demand (Amboni *et al.*, 2008; Maruyama and Yanagisawa, 2006). This theory, implicating dysfunction of the frontal lobe or its connections with the basal ganglia, has experimental evidence, particularly in dual-tasking paradigms where gait variability and symmetry are significantly worse when patients with FOG are asked to perform a second task while walking (Almeida, 2009; Yogev-Seligmann *et al.*, 2008). As described above, executive function is impaired in PD patients with FOG compared with those without FOG, specifically divided attention (as assessed by dual-tasking paradigms) (Spildooren *et al.*, 2010; Tard *et al.*, 2014), response inhibition (Cohen *et al.*, 2014), conflict resolution (Vandenbossche *et al.*, 2012) and set-shifting (Shine, Naismith, *et al.*, 2013). Although the association between cognitive dysfunction and FOG is strong, objective quantitative measures of cognitive function in FOG are lacking. This will be further explored in Chapter 5 and the role of cognition as a therapeutic target for FOG is dealt with in Chapter 7.

4) A fourth hypothetical mechanism centres on the loss of intrinsic generation of automatic motor execution due to dysfunction of the basal ganglia and its connections with the supplementary motor area (Iansek *et al.*, 2006). In healthy subjects, as motor tasks are learned, they stop becoming consciously controlled and become habitual or automatic. The basal ganglia have a crucial role in this process. As the basal ganglia degenerates in PD, this intrinsic initiation and control of habitual

movement becomes impaired and requires more attention or external stimuli to initiate, control and learn movement. Implicit learning and automatic task performance may be more impaired in patients with FOG and thus, require more attention to perform (Hallett, 2008). This **loss of automaticity** would require compensation via other pathways to select appropriate actions for smooth control of complex movements such as locomotion. Thus, cortical and/or cerebellar pathways are activated to compensate for this deficit. If this is the case, one would expect that learning a skilled movement would be more impaired in PD patients with FOG compared to those without. Although loss of automaticity is postulated to play a role in FOG, there is a paucity of studies testing this hypothesis formally in FOG, let alone quantifying it. Studies investigating the acquisition or refinement of automaticity are even scarcer. As automaticity is learned, activity in frontostriatal circuity reduces when performing the task, reflecting a learning process (less computation is required to perform the task) (Poldrack *et al.*, 2005). Vandenbossche et al. (2013) showed that the speed with which freezers learn a sequence task is impaired, especially when a second concurrent task is performed (Vandenbossche *et al.*, 2013). However, this study tests both cognitive function and motor response time and does not control for the greater degree of slowness usually seen in freezers. The study described in Chapter 4 formally investigates whether learning and refinement of skilled movements is more impaired in those with FOG (independent of bradykinesia) and the study presented in Chapter 5 examines the neurophysiological markers of movement initiation.

5) The presence of persistent gait abnormalities such as lack of symmetry and rhythmicity in patients with FOG, even in the absence of freezing episodes, suggests a constant problem with the coordination of rhythmical movement of upper and lower limbs in freezers (Nieuwboer, Vercruysse, *et al.*, 2009). The "sequence effect", whereby a progressive decrement in motor output occurs in PD, may lead to the progressive deterioration in gait parameters which occurs prior to FOG episodes. It has been suggest that this spatiotemporal disruption may be due to **abnormal output from central pattern generators in the spinal cord** (Nutt *et al.*, 2011).

6) Finally, rhythmic knee trembling is frequently seen during freezing episodes in many (but not all) patients with FOG. It is believed that these represent excessive anticipatory postural adjustments, based on fine adjustments in lower limb muscle groups controlled by connections involving the supplementary motor area and the mesencephalon which maintain balance before movement

(Jacobs *et al.*, 2009). Healthy controls perform one or two of such anticipatory adjustments before initiating a step. However, in FOG, failure of step initiation at the level of the supplementary motor area (as outlined above) and defective basal ganglia which assists in preparation of normal automatic gait function could lead to **decoupling of anticipatory postural adjustments with normal gait** function giving rise to intermittent breakdown of gait (Lewis and Barker, 2009).

Although the neural mechanisms outlined above have all been implicated in FOG to date, no single explanation has been shown to be unifying. The associations which have the most consistent support in the literature are those of impairments in **executive function/dual tasking**, **automatic motor initiation** and **sensorimotor integration**. The main focus of this thesis, therefore, was to progress research in FOG specifically probing these **sensory, motor and cognitive** processes in PD patients with and without freezing of gait in order to further understand and localise the mechanisms which underpin freezing. Most of the attempts to localise freezing to date have come from neuroimaging studies.

1.7 Neuroimaging of Freezing of Gait

In order to further understand and localise the mechanisms of FOG, a number of imaging studies (both structural and functional) have tried to elucidate which regions are affected in patients with FOG. These brain imaging studies offer excellent spatial resolution, allowing detailed study of small subcortical regions of interest such as the basal ganglia. The temporal resolution of these methods are not, however, ideal to study paroxysmal events such as FOG. In particular, with functional MRI (fMRI), where the time taken for the BOLD haemodynamic response to be expressed, as well as the time taken for the net magnetisation of voxels and the acquisition time for all slices in a sequence to be performed, instantaneous events are difficult to capture accurately (Huettel *et al.*, 2009). Findings in these studies may therefore reflect compensatory responses rather than the neural substrates of FOG. Another significant limitation in the use of neuroimaging to study gait is that the majority of methods require immobilisation of the participant's head, precluding the study of natural gait. The majority of MRI studies are, therefore, performed in the resting state, using motor imagery (imagining walking without actual execution) or using a surrogate tasks (e.g. stepping or cycling). The major imaging studies in FOG to date have recently been reviewed (Fasano *et al.*, 2015). The structural imaging studies will be discussed first, followed by the functional imaging studies.

1.7.1 Structural Neuroimaging

Kostic et al. employed voxel-based morphometry (VBM) to examine distribution of grey matter atrophy in patients with and without FOG (Kostic *et al.*, 2012). Freezers and non-freezers displayed significant atrophy of numerous cortical areas including the dorsolateral prefrontal cortex, medial and lateral temporal lobe, inferior parietal cortex, and occipital cortex compared with healthy controls implying that **executive function** and **sensorimotor control pathways** may be affected in all PwP. The frontal grey matter atrophy also correlated with freezing of gait questionnaire scores. Importantly, a greater degree of atrophy was seen in freezers in three cortical areas (left inferior frontal gyrus, left precentral gyrus, and left inferior parietal gyrus) when compared to the non-freezing group. These areas have roles in **sensorimotor processing** and **dual task performance**. Specifically, the left inferior parietal gyrus has a role in shifting attention during **simultaneous multisensory stimulation** (Collette *et al.*, 2005) whereas the left inferior frontal gyrus has been shown in functional MRI studies to be important in **resolving interference during dual-tasking** (D'Esposito *et al.*, 1999). This suggests a specific pattern of grey matter loss in freezers involving frontal and parietal areas which correlate with severity of FOG and executive function.

Other VBM studies, on the other hand, have found freezers displayed selective grey matter atrophy compared with non-freezers in other regions: the left cuneus (which correlated with severity of FOG), precuneus, lingual gyrus, and posterior cingulate cortex (Tessitore *et al.*, 2012); inferior parietal lobe and angular gyrus and bilateral caudate nuclei (Herman *et al.*, 2014); mesencephalic locomotor region(Snijders *et al.*, 2011); and thalamus (Sunwoo *et al.*, 2013). Thus there is great variability among these studies which implicate a wide range of cortical and subcortical regions.

More recently, diffusion tensor imaging (DTI) has been employed to examine white matter changes in FOG. Freezers display reduced connectivity between the PPN and the cerebellum but increased connectivity between the cortex and the pons (Schweder *et al.*, 2010). Another diffusion tensor imaging study revealed a reduced structural connectivity along the **hyperdirect pathway** between the SMA and the right subthalamic nucleus (rSTN) in people with Parkinson's disease which was similar in freezers and non-freezers suggesting that response inhibition and control may be affected in PD in general (Fling *et al.*, 2014). Vercruysse et al. showed more extensive white matter alterations FOG involving frontostrial tracts and connections between the cerebellum and STN/PPN bilaterally (Vercruysse *et al.*, 2015).

The role of attention in FOG was explored by Peterson et al. (2014) who performed diffusion tensor imaging on patients with and without FOG to examine structural connectivity of the PPN, which has been associated with both FOG and attentional control (Peterson *et al.*, 2014). Attention relies not only on cortical areas (such as the dorsolateral prefrontal cortex) but also subcortical areas such as the PPN, ventral striatum and insula. The degree of asymmetry of PPN structural connectivity was shown to correlate with stride length difference between single and dual tasks (a measure known as the **dual task cost or interference**) as well as **reaction time and accuracy** on a No-Go task. This highlights the role of another **subcortical nucleus** in the pathophysiology of FOG and a further association with difficulty performing dual tasks.

1.7.2 Functional Neuroimaging

Functional MRI studies have allowed **task-related brain responses** to be studies in freezers and non-freezers. Many of these studies have utilised **virtual reality (VR) environments** to provide visual flow within the scanner and also to present the subject with triggers of FOG such as narrow spaces and cognitive tasks. Thus, these tasks present the participant with a situation which may trigger a freezing episode making it more likely that the brain responses seen might be similar to those which occur during FOG in a real environment. Shine et al. (2013) performed functional MRI analysis of 18 freezers using a virtual reality based stepping task which places a cognitive load on the patient to provoke FOG and compared BOLD responses during periods of stepping arrest (which correlate with FOG) to periods of normal motor output (Shine *et al.*, 2013). This revealed reduced activation in **sensorimotor cortical regions** and an increased response within **frontoparietal cortical regions** (dorsolateral prefrontal cortex, posterior parietal cortex and anterior insulae) during freezing episodes. In addition, there was a significantly decreased response in **subcortical** regions (bilateral caudate head, anterior thalamus, globus pallidus interna and subthalamic nuclei). This suggests that altered activation in frontoparietal regions (which are crucial to sensory, motor and cognitive function) and/or paroxysmal increases in inhibitory basal ganglia output may be the cause of FOG. Increased activation of frontal areas and reduced subcortical activity is also seen in freezing of upper limb movements (Vercruysse *et al.*, 2014). It is not clear whether these cortical responses seen have a primary role in FOG or are merely a compensatory adaptation to freezing episodes. It appears that physical execution of movement is required to see these changes as activation of frontal and posterior parietal areas is reduced when freezers simply imagine rather than execute gait (Snijders *et al.*, 2011).

Functional MRI can also determine connectivity between regions of interest in the resting state by inferring that simultaneously activated areas are functionally connected. Resting-state studies have identified a number of networks involved in sensory, motor and cognitive processing (Friston, 2011). These include a cognitive control network, a basal ganglia network, an attentional network, a sensorimotor network and visual and auditory networks. Connectivity between the attention and visual networks is impaired in freezers (Tessitore *et al.*, 2012) suggesting **altered visual processing** may play a role in FOG (see Chapter 3). Shine et al. studied functional MRI activation and connectivity in a number of regions of interest in freezers and non-freezers both using their VR-based task (Shine, Matar, Ward, Bolitho, Pearson, *et al.*, 2013; Shine, Matar, Ward, Frank, *et al.*, 2013). While stepping in the supine position, all PwP showed activation of the left cognitive control network (which includes the dorsolateral prefrontal cortex and the posterior parietal cortex) and the ventral attention network (anterior cingulate and anterior insula) and also showed increased connectivity between the cognitive control networks bilaterally. However, freezers demonstrated less ability to recruit specific cortical and subcortical regions within the cognitive control network **during the performance of simultaneous motor and cognitive tasks** and furthermore, demonstrated functional decoupling between the basal ganglia network and the cognitive control network in each hemisphere. This decoupling was also associated with freezing episodes suggesting that FOG is associated with **impaired recruitment of cognitive cortical networks and communication between basal ganglia and frontoparietal cortical networks,** especially in the setting of increased cognitive load. Cognitive and attention networks are involved in dual-tasking during locomotion and these networks interact with the basal ganglia but may be impaired in patients with FOG. However, the cognitive control network is also involved in the **processing of novel information and decision-making**, rather than merely activating during tasks requiring increased cognitive demand (Cole and Schneider, 2007).

Fling et al. (2014) performed functional connectivity analyses on 15 PD patients with and without FOG and 14 healthy controls (Fling *et al.*, 2014). The resting state analysis in this study showed increased functional connectivity in freezers between the SMA and mesencephalic locomotor and cerebellar locomotor regions (MLR, CLR) compared with non-freezers but less functional connectivity in the hyperdirect pathway between rSTN and SMA. It has been proposed that the communication between the STN and SMA fails as PD progresses and some patients try to compensate via alternative routes of communication (such as the SMA-MLR/CLR pathway) resulting in FOG. This functional reorganisation is proposed to represent a maladaptive response in freezers as connectivity between the left CLR/MLR-SMA pairs was correlated with both objective and subjective measures of FOG.

In an effort to overcome the ambiguity of inferring neuronal activity from the BOLD response, other functional imaging methods such as positron emission tomography (PET, which examines cerebral glucose metabolism) and single photon emission computed tomography (SPECT, which reveals regional cerebral blood flow) have been employed. PET studies have revealed reduced glucose metabolism at rest in **frontal and parietal** regions as well as the basal ganglia in freezers compared with non-freezers and healthy controls (Bartels *et al.*, 2006; Imamura *et al.*, 2012). In particular, **right sided cortical hypoperfusion** is seen in freezers (anterior cingulate cortex and visual cortex) (Bartels and Leenders, 2008) consistent with the previous finding that patients **left-onset PD** (predominantly affecting the right hemisphere) are at greater risk of developing FOG and are more likely to experience FOG when walking through doorways (Cohen *et al.*, 2012; Giladi *et al.*, 2001). It should be noted that a SPECT study on patients with parkinsonism and FOG caused by vascular disease (rather than degenerative parkinsonism such as PD) found reduced perfusion in the right parietal cortex compared to patients with vascular disease but no FOG, suggesting that **right sided cortical dysfunction** is associated with FOG independent of the cause of parkinsonism (Terashi *et al.*, 2012).

Functional imaging methods employing more specific ligands (to investigate neurotransmitter differences between freezers and non-freezers) have also been applied. Given the central role of dopamine in parkinsonism and the frequent finding of reduced activation of the basal ganglia in fMRI studies, a number of studies have investigated dopaminergic imaging. An initial dopaminergic PET study showed reduced basal ganglia dopaminergic activity in freezers (Bohnen *et al.*, 2014). However, this may simply have been due to a longer disease duration in the freezing group as progression of dopaminergic denervation with disease progression is well documented in PD. Nevertheless, reduced dopaminergic activity in the right caudate nucleus in freezers has also been confirmed by another study (Bartels *et al.*, 2006). Again, this may be due to longer disease duration as loss of dopamine in PD tends to begin in the caudal putamen and progress anteriorly with time to affect the caudate nucleus at a later stage. As will be discussed in Chapter 4, the more anterior areas of the basal ganglia such as the caudate nucleus are intricately connected with prefrontal areas and are therefore associated with initiation of goal-directed, volitional movement. Loss of dopaminergic innertvation in this area may therefore contribute to the pathophysiology of FOG. Cholinergic denervation has been associated with falls in PD. FOG is more common in patients with cholinergic denervation and cortical amyloid deposition (Bohnen *et al.*, 2009). This may be due to loss of cholinergic output from the PPN, an area central to gait control which has previously been linked with FOG both via functional imaging (Fling *et al.*, 2013) and the response of FOG

to deep brain stimulation of the PPN (Thevathasan *et al.*, 2011). These findings imply that non-dopaminergic mechanisms may be involved in the development of FOG.

In summary, recent imaging has shown both structural and functional changes in patients with FOG which implicate both cortical and subcortical structures. They suggest a close association between **sensory** processing, **cognitive** function (including attention and response inhibition) and **motor** control. Taken together, these studies suggest that interactions between cognitive and motor networks are impaired in freezers, in particular during **dual motor-cognitive tasks**. An inability to recruit cortical and subcortical areas required for complex tasks such as gait and, in particular, overactivity of the prefrontal cortex have been consistently shown in those with FOG. However, the precise nature of these impaired interactions remains unclear.

1.7.3 Electroencephalography-based approaches

The imaging studies above studies do not fully answer the question of where the primary deficit lies in FOG. Are the changes seen causative in FOG or are they merely an adaptive response to FOG? Although functional MRI has a high spatial resolution, determining the processes that occur immediately prior to a freezing episode require an acquisition method with good temporal resolution. EEG represents a promising alternative to functional MRI as it can resolve events which are closely related in time to a stimulus or event. Electroencephalography (EEG) measures brain activity by detecting surface electrical potentials from the scalp, which are summed postsynaptic potentials from pyramidal cells in the cortex. Potentials from deeper brain structures are attenuated and are therefore not detected by surface EEG. Thus, EEG activity almost exclusively represents cortical activity only. Whereas functional MRI lacks the temporal resolution to accurately discern areas of activation during brief episodes at the onset of a freezing episode, EEG can reveal the dynamics of these cortical potentials with a resolution of milliseconds, but this is at the expense of lower spatial resolution. EEG can thus be used to analyse temporal dynamics across widespread regions of the brain allowing a greater understanding of information transfer and processing between disparate brain regions. Few EEG-based studies in FOG exist to date (Handojoseno *et al.*, 2012; 2013; Shine *et al.*, 2014; Singh *et al.*, 2013; Thevathasan *et al.*, 2012; Toledo *et al.*, 2014; Velu *et al.*, 2014). Many of the EEG studies to date have focussed on identifying a signature of FOG from EEG recordings during **motor** activity (normal walking and freezing episodes).

Wavelet-based methods were the first employed to investigate whether energy in different frequency bands (spectral energy) is different during freezing episodes and normal walking in 26 patients with FOG (Handojoseno *et al.*, 2012; 2013). Power in the delta (<4Hz), theta (4-7Hz) and alpha (8-15Hz) frequency bands were all found to be different at onset of freezing (5 seconds prior to freezing occurring) and during freezing than during normal walking implying significant changes in cortical activity during a freezing episode (Handojoseno *et al.*, 2012). Furthermore, total wavelet energy was shown to be a marker for changes between onset of a freezing episode and the freezing episode itself with greater total energy before and during a freezing episode compared with normal walking. A neural network classifier incorporating the total energy was used to reliably detect FOG (76% accuracy) from EEG data alone. The sensitivity for predicting FOG was further enhanced by examining a space-time-frequency model which included wavelet cross-frequency energy ratios (Handojoseno *et al.*, 2013). This implies that cortical activity increases significantly during a freezing episode, in keeping with the functional MRI findings described above. Moreover, the cortical activity occur early in the evolution of a freezing episode making a compensatory response to the freezing state less likely.

The same group examined the transition between normal walking and freezing more closely showing that the transition is associated with increased theta power in central and frontal regions and (to a lesser extent) beta power in parietal regions compared with normal locomotion and thus could be an alternative signature of FOG (Shine *et al.*, 2014). Since theta frequencies have been associated with response conflict (Cohen and Donner, 2013) and beta frequencies have been associated with motor preparation (Little and Brown, 2014), the authors suggest that these findings support dysfunction in a frontoparietal network involved in conflict-related signals. The authors propose that subcortical dysfunction drives the increase in cortical theta oscillations during a freezing episode. However, no analysis of the spatial distribution of this data was performed but the authors suggest that "further studies would benefit from characterizing the electrographic signal associated with freezing across more scalp locations, helping to determine with more precision the precise spatiotemporal dynamics underlying freezing behaviour" (Shine *et al.*, 2014).

In order to assess the contribution of **sensory** information to the dynamics of these cortical potentials, Velu et al. investigated the effect of visual cues on spectral connectivity in two patients with Parkinson's disease and FOG (one who responded to visual cues and one who did not) (Velu *et al.*, 2014). They hypothesised that visual cues would result in an increased connectivity between visual/parietal areas and the motor cortex in the pre-movement time period. The method employed virtual reality (VR) based

visual cues generated by VR glasses while the subject walked a set path with synchronous 64-channel EEG recording. They found that the patient who responded to visual cues had reduced power and increased information flow in occipital to central electrodes and occipital to parietal electrodes in the beta range along with a reduction in delta and alpha-band powers. The results suggest that visual feedback cues affect information flow in the occipital-parietal-motor network facilitating movement, although the sample size was small and no comparison was made between freezers and non-freezers.

EEG studies are limited as they can only analyse activity from cortical regions. Given the significant interaction with the basal ganglia in proposed models of FOG, EEG studies can only present a limited picture of cerebral activity. The implantation of deep-brain stimulating (DBS) electrodes for treatment of PD, has allowed recording of local field potentials (LFP) at the sites of stimulation in the basal ganglia, such as the subthalamic nucleus (STN) and globus pallidus interna (GPi). Such studies have confirmed that increased beta oscillations in the STN are associated with the "off"-state in PD but local field potential studies have found signatures of FOG in deep brain nuclei also. Toledo et al. showed higher resting-state beta power in the STN in freezers when in "off"-state compared with non-freezers which decreased when they switched to "on"-state with associated reduction in FOG (Toledo *et al.*, 2014). This was consistent with the findings of Singh et al. who found that low beta frequencies were enhanced in freezers while walking/standing, but especially so during the swing phase of walking (Singh *et al.*, 2013). There has been extensive work investigating the role of beta oscillations in motor impairments in Parkinson's disease (Little and Brown, 2014). Initiation of voluntary movement during spontaneous increased cortical beta activity leads to slowness of movement. Beta power is suppressed by levodopa and STN-DBS and this suppression correlates with motor improvements. Furthermore, beta power correlates with bradykinesia and rigidity supporting its proposed role as a "promotor of motor status quo". Beta oscillations therefore lead to an increase in tonic activity, inhibiting free voluntary movement. It is possible that FOG could result from changes in beta power to a threshold where movement does not occur. However, these changes may merely represent differences in severity of PD rather than a specific FOG effect.

As mentioned previously, another important nucleus in control of gait is the pedunculopontine nucleus (PPN) and Thevathasan et al. performed a similar LFP study in 7 patients who underwent DBS of the PPN and showed that activity in alpha frequency bands (rather than beta frequencies) in a network involving that PPN and cortex correlated with gait speed in PD (Thevathasan *et al.*, 2011). Only a single patient in this study experienced freezing episodes post-surgery, but the onset of these episodes were associated

with attenuation of alpha power. The authors propose that since alpha power has been associated with allocation of attentional resources, and importantly suppression of competing processes, these findings suggest freezing my be associated with deficits in focused execution of a task at the level of the PPN. Importantly no changes in beta oscillations were seen in the LFPs recorded from the PPN in this study.

As described above, the current state of the neuroimaging literature in FOG supports many of associations and proposed pathophysiological mechanisms outlined previously. These studies do not, however, shed light on a clearly unifying model to explain the occurrence of FOG in some patients with PD and its absence in others.

1.8 Models of Freezing of Gait

In an effort to reorganise the disparate nature of the multiple proposed pathophysiological mechanisms above, Nieuwboer and Giladi (2013) reviewed the FOG literature and proposed four distinct models of FOG to summarise the current thinking on freezing: the threshold model; the interference model; the cognitive model; and the decoupling model.

Models of FOG	Principle
Threshold (Plotnik et al., 2012)	Accumulation of motor deficits occurs until threshold is reached => FOG
Interference (Lewis and Barker, 2009)	Motor, cognitive and limbic inputs compete for central processing resources causing interference => FOG
Cognitive (Vandenbossche et al., 2012)	Impairment in processing of response conflict induces block => FOG
Decoupling (Jacobs et al., 2009)	Decoupling between motor programs and motor response induces block => FOG

Table 1.2: Models of FOG: *Adapted from* (Nieuwboer and Giladi, 2013).

Plotnik et al. proposed the "threshold model" as follows: in parkinsonism, tasks involving repetitive bilateral coordination (such as locomotion) progressively deteriorate towards a threshold where a complete breakdown of coordination occurs, leading to failure of motor output (Plotnik *et al.*, 2012). Freezers have a greater impairment in baseline gait parameters (step length, rhythmicity, symmetry, bilateral coordination) compared with non-freezers. For this reason, freezers are more likely to cross the threshold in situations which interfere with gait (e.g. concurrent cognitive load, online adaptation of gait, off levodopa) and less likely to cross the threshold in the "on"-state or in the setting of rhythmical

cues (Figure 1.9). This model can be used to provoke freezing in an experimental setting by shifting those gait parameters towards the breakdown threshold. Asking patients to take rapid small steps which reduces stride length or rapid 360° turns in a small space which necessitates asymmetrical step sizes frequently causes freezing (Snijders *et al.*, 2008). Any situation which forces a reduction in those gait parameters outlined above drives overall gait towards the threshold. The baseline gait parameters, as well as the influence of the provoking factor, determines the probability that this will lead to FOG in this population. This point will be dealt with in Chapter 7 where an intervention is shown to bring about an improvement in baseline gait parameters in freezers and ultimately, FOG.

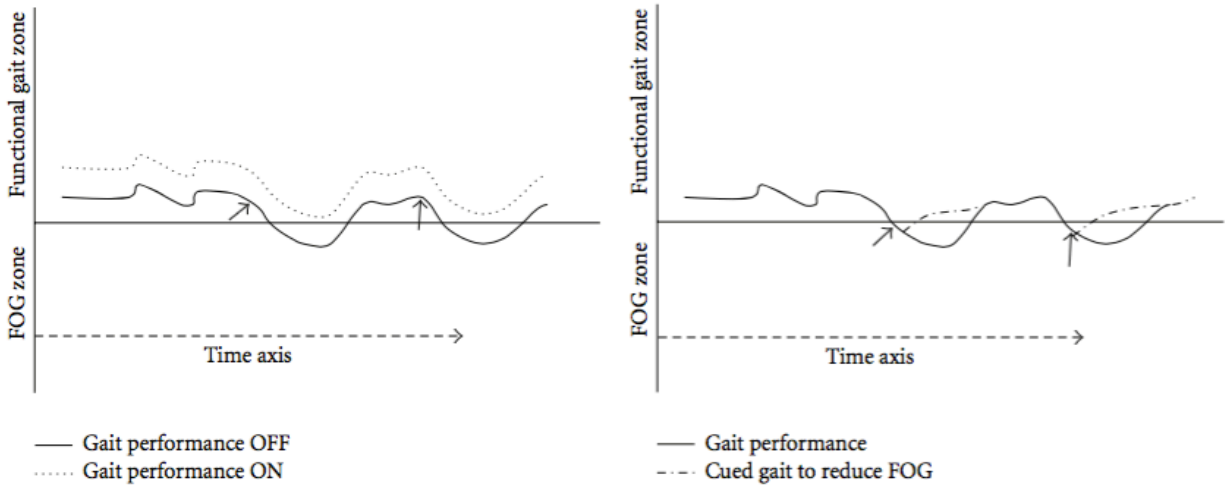


Figure 1.9: Threshold model of FOG: *Breakdown in gait parameters (y-axis) in PD reaches threshold where freezing may occur (horizontal line). The effects of "on"- vs "off"-state and cueing clearly show how these affect this model. From* (Plotnik *et al.*, 2012).

Lewis and Barker proposed the "interference model" which suggests that when competing cognitive, limbic and motor demands are processed in parallel through a defective basal ganglia, paroxysmal interference/obstruction can occur (Lewis and Barker, 2009). This, in turn, leads to bursts of excessive inhibition arising from the basal ganglia output nuclei leading to FOG (Figure 1.10). The stratified nature of the basal ganglia allows parallel processing of information from separate cortical areas simultaneously in healthy older adults (Middleton and Strick, 2000). However, degeneration of the basal ganglia in PD leads to a decreased neural reserve. Lewis and Barker propose that this creates a bottleneck for information processing at the level of the basal ganglia. Dual-task experiments in PD support this model, whereby concurrent cognitive and motor demands provoke FOG, especially those

tasks with a greater cognitive load (Shine, Matar, Ward, Bolitho *et al.*, 2013; Spildooren *et al.*, 2010). The neuroimaging evidence outlined above also supports this model where increased frontoparietal and reduced basal ganglia activity occurs during FOG, especially when a cognitive load is applied.

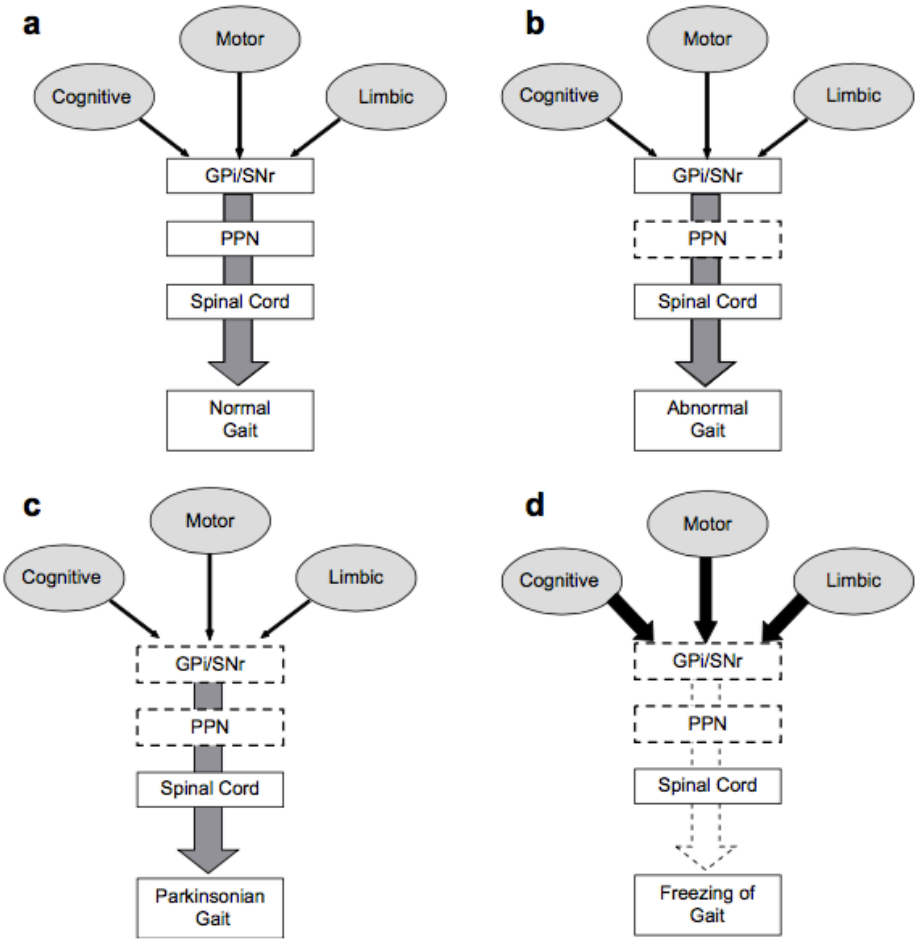


Figure 1.10. Interference Model of FOG: *a) Normal healthy gait – combination of inputs from corticstriatal processes facilitates PPN output to spinal cord; b) Cholinergic loss in settings such as cognitive impairment affects PPN function resulting in abnormal gait; c) In PD, striatal dopaminergic denervation leads to activation of the output nuclei of the basal ganglia which inhibits the dysfunctional PPN leading to a parkinsonian gait; d) Convergence of motor, cognitive and limbic circuits on the basal ganglia causes excessive momentary inhibition of the PPN triggering freezing episodes.From* (Lewis and Barker, 2009).

The "cognitive model" is based on the assumption of a deficit in conflict resolution which becomes apparent when **response decision** is required (Vandenbossche *et al.*, 2012). Conflict resolution is impaired in PD in general, but more significantly in freezers (Vandenbossche *et al.*, 2011). Response

selection involves both activation of the desired response as well as inhibition of unwanted responses. Freezers demonstrate impaired inhibition of unwanted responses and this becomes more prominent under time pressure. Frontostriatal networks and the hyperdirect pathway (incorporating the supplementary motor area, right inferior frontal gyrus and subthalamic nucleus) are essential for response selection and inhibition (Coxon *et al.*, 2012). When a conflict occurs, these areas delay response selection by raising the decision threshold in the globus pallidus interna, preventing a response until the conflict is resolved (Frank *et al.*, 2007). Conflict resolution becomes more difficult in the setting of frontal executive dysfunction, which occurs more frequently in freezers. The loss of automaticity of responses which occurs in PD can be compensated for with cognitive control to a degree, but once this cognitive control is lost in freezers, conflicting situations could lead to FOG. Vandenbossche et al. further suggest that rather than simply being a either a deficit in automatic habitual control of movement or cognitive control of movement, it may be an **interplay between control of cognitive and automatic motor processes** (Vandenbossche *et al.*, 2012). Automatic regulation of movement at the level of the basal ganglia spares cognitive resources which are only required then for complex or dual tasks. In PD, the loss of automatic control of movement means that cognitive resources are required for even simple tasks. This shift from subcortical to cortical control of movement compensates to a certain degree. However, the pressure this places on cortical processes, along with concurrent impairment in cognitive processing, leads to FOG. This model is supported by both multiple structural neuroimaging studies which show grey matter atrophy in FOG in regions associated with resolving conflict (Kostic *et al.*, 2012) as well as structural and functional connectivity changes involving the hyperdirect pathway (Fling *et al.*, 2014).

Attention is implicitly involved in the cognitive model. Once diversion of attention away from cognitively controlled locomotion occurs, motor output can be interrupted, leading to FOG. Thus, dual-tasking leads to breakdown of gait parameters and, ultimately, FOG. The less automatic gait becomes, and the more conscious control is relied on for locomotion, the greater the effect of divided attention. However, freezing does not solely occur in situations where attention is divided or cognitive demand is high and focussing attention toward walking is not always helpful in reducing freezing, implying other mechanisms are also at play.

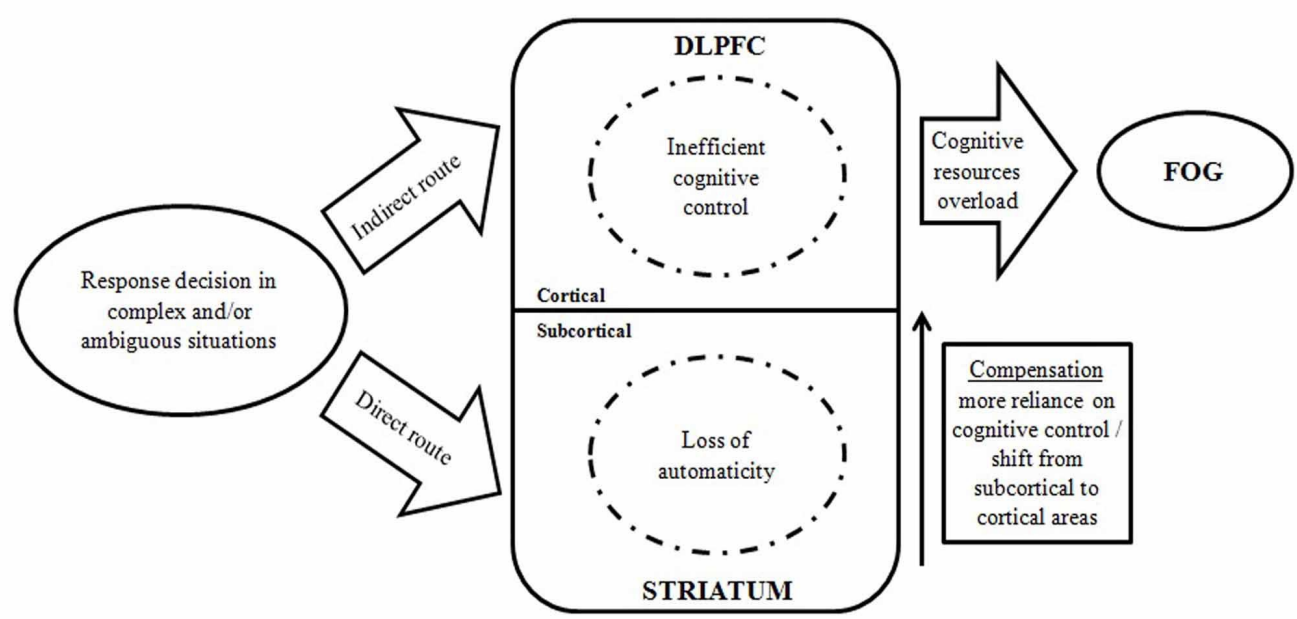


Figure 1.11. Cognitive Model of FOG: *interplay between automatic and controlled cognitive dysfunctions in the occurrence of FOG episodes. From* (Vandenbossche *et al.*, 2012). *DLPFC: dorsolateral prefrontal cortex.*

Lastly, the "decoupling model" proposes that FOG results from the momentary inability to couple a planned motor program with **initiation of movement** (Jacobs *et al.*, 2009). Anticipatory postural adjustments (APAs) are small automatic preparatory adjustments which are triggered by the supplementary motor area prior to step initiation in anticipation of the destabilization which may occur with the planned voluntary movement. Freezers often shift their weight between the leg they plan to step with and back to the other leg repeatedly. Thus, timing of these APAs is impaired in PD due to progressive SMA dysfunction and in freezers, these APAs are frequently seen in association with delayed or failed step initiation. It is felt that the rhythmical knee trembling which is frequently seen during freezing episodes is a burst of dysfunctional APAs which is not coupled the planned motor output. This abnormal posture-gait suggests the freezers are unable to control inhibition of the postural adjustments and release step initiation.

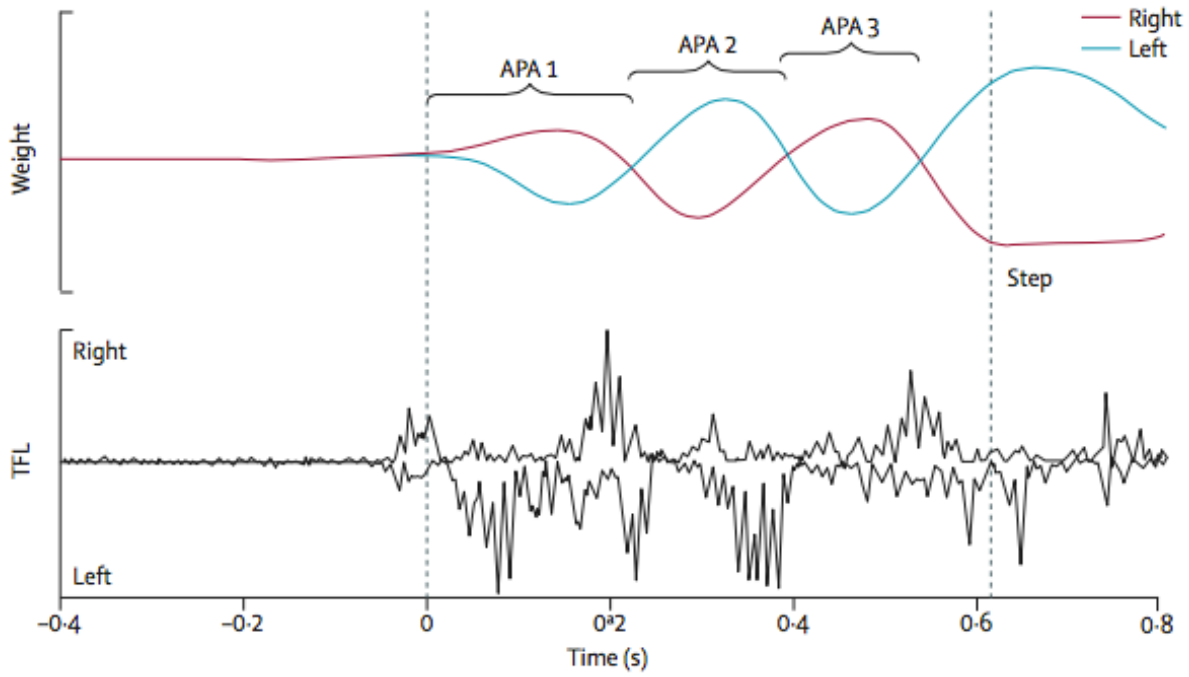


Figure 1.12. Anticipatory Postural Adjustments (APAs) in FOG: *Patient with FOG displaying multiple anticipatory postural adjustments in the form of lateral shifting of weight (upper plot) and EMG activation of right and left hip abductors prior to a step at 0.6 seconds. From* (Nutt *et al.*, 2011)*. TFL: tensor fascia lata.*

Although some overlap of these models is seen with some explanations of FOG, no single study to date has unified all four models of FOG. The above models will be referred to throughout this thesis.

1.9 Interventions for Freezing of Gait

The lack of understanding of the precise neural mechanisms causing FOG has meant that treatment strategies targeting freezing are limited. The use of pharmacological approaches for the treatment of FOG has been controversial. Giladi outlined the current evidence for medical treatment of freezing (Giladi, 2008). The majority of freezing episodes are related to the "off"-state (off-FOG) and, as a result, medications that reduce the period of time in this state will, in turn, reduce the frequency and duration of freezing episodes (Schaafsma *et al.*, 2003). The response of "off"-FOG to levodopa is often predictable in early stages of PD but FOG can become more resistant in the later stages (Bartels *et al.*, 2003). FOG can, however, also occur during the "on"-state (Espay *et al.*, 2012). In these patients, where the other symptoms of PD tend to be well controlled, treatment of FOG can be problematic. Although levodopa

can be effective in "off"-FOG, it has been shown to paradoxically induce FOG in patients who experience "on"-FOG (Ambani and Van Woert, 1973). It is clear, therefore, that use of medications for Parkinson's disease has a more complex effect on FOG than on other symptoms of PD such as tremor, rigidity and bradykinesia. Other pharmacological strategies have been tested in an attempt to directly improve FOG including botulinum toxin injection (Giladi, Gurevich, *et al.*, 2001), selegiline (Shoulson *et al.*, 2002), amantadine (Giladi, Treves, *et al.*, 2001) and methylphenidate (Pollak *et al.*, 2007), with variable results.

Deep brain stimulation of the subthalamic nucleus (STN-DBS) can improve FOG in selected patients (Brozova *et al.*, 2009; Moreau *et al.*, 2008). Vercruysse et al. showed that STN-DBS reduces severity and occurrence of "off"-FOG at 6 and 12 months but nearly half of those that underwent surgery continued to experience FOG (Vercruysse, Vandenberghe, *et al.*, 2014). Furthermore, a small number of patients without FOG pre-operatively developed freezing following surgery. Stimulation of the PPN has also shown modest benefits in FOG (Thevathasan, Cole, *et al.*, 2012) but not via improving overall gait parameters. There is ongoing debate as to which stimulation site is superior for FOG (PPN vs STN vs GPi) (Moreau *et al.*, 2009). Although isolated case reports and small case series argue for one stimulation site over another, no large randomised trial has been undertaken.

Given the variable response of FOG to pharmacological agents and the risks associated with invasive neurosurgery, much attention has been focused on non-invasive therapeutic approaches. A wide range of physiotherapy techniques is available for management of PD in general. A recent systematic review and meta-analysis of these techniques showed short-term efficacy for this approach (follow-up less than 3 months) (Tomlinson *et al.*, 2013). However, the majority of the trials included in this meta-analysis were small and of low methodological quality.

Although physiotherapy approaches alone have not been shown to have a specific effect on FOG, recent interest in the effect of physiotherapy combined with sensory cueing on freezing have shown promising results. Cueing consists of "applying temporal or spatial external stimuli associated with the initiation and ongoing facilitation of motor activity" (Nieuwboer et al., 2007). It had initially been noted that PD patients, even those with severe disease can occasionally have near normal locomotion under certain circumstances (e.g. climbing stairs), whereas other situations (e.g. walking through a narrow doorway) will induce freezing (Glickstein and Stein, 1991). Von Wilzenben first reported the use of cueing to improve gait in patients with post-encephalitic PD in 1942 (Wilzenben, 1942).

Auditory cueing, for example using rhythmic beeps, metronomes, clicks delivered via headphones or rhythms embedded in music, has been used in a number of pre-experimental studies and randomized controlled trials to improve gait parameters such as walking speed, stride length and cadence and significantly reduce FOG (Arias and Cudeiro, 2010). Similarly, there have been numerous studies investigating the effect of visual cueing on FOG and gait in PD (Azulay *et al.*, 2006; Griffin *et al.*, 2011; Lewis *et al.*, 2000; Vitório *et al.*, 2014). The most widely used method of visual cueing is the placement of lines/stripes on the floor. As far back as 1967, Martin showed that use of transverse lines were effective in improving gait in patients with PD (Martin, 1967). Even at that stage, it was clear that only specific patterns of visual cues were effective. Indeed, Martin noted that lines of contrasting colour, 18 inches apart and one inch or more wide were optimal. With recent developments in technology, there has been a move towards using **virtual reality** to provide visual cues. A comparison of real and virtual visual cues showed that real cues proved more effective in improving gait and reducing freezing frequency compared with virtual cues (Griffin *et al.*, 2011).

Thus, cueing can improve gait in patients with PD and may also have some effect on reducing frequency and severity of freezing episodes. The RESCUE trial, a crossover trial of 63 patients with FOG, randomized patients to either a 3-week home cueing program followed by 3 weeks without training or the same 3-week periods in the reverse order (Nieuwboer et al., 2007). Participants trained using their preferred cueing modality (auditory, visual or somatosensory). The full cohort showed a small improvement in the freezing of gait questionnaire scores after 3 weeks as well as in severity of freezing, gait speed and step length. A subgroup analysis of this trial showed that turning speed was greater for auditory cues than in visual or somatosensory cues in all patients (with and without FOG) but there was no evidence that one cueing modality is more effective than another in reducing freezing episodes (Nieuwboer, Baker, *et al.*, 2009). Unfortunately, any gains seen after the 3-week training period were absent at 6 week's follow-up. This lack of carry-over is also supported by other studies in PD (Brichetto *et al.*, 2006; Sidaway *et al.*, 2006; Thaut *et al.*, 2001). This has significant implications for rehabilitation and may require regular follow-up in a clinical setting or the ability to monitor gait parameters and FOG remotely at home.

As described in section 1.3, virtual reality paradigms have been employed in a number of studies in order to study FOG (Delval *et al.*, 2010; Feasel *et al.*, 2011; Naismith and Lewis, 2010; Park *et al.*, 2011; Shine, Matar, Bolitho, *et al.*, 2013; Shine, Naismith, *et al.*, 2011) and have been combined with functional MRI techniques in order to probe the underlying pathophysiology of FOG (Shine, Matar,

Ward, Bolitho, Gilat, *et al.*, 2013; Shine, Matar, Ward, Bolitho, Pearson, *et al.*, 2013; Shine, Matar, Ward, Frank, *et al.*, 2013). Virtual reality cue-based interventions have also shown improvements in gait parameters (stride length and walking speed) in patients with PD (Badarny *et al.*, 2014; Griffin *et al.*, 2011). However, more general virtual reality-based interventions have been examined in PD cohorts, showing improvements in dynamic balance (Liao *et al.*, 2014), reaching movements (Ma *et al.*, 2011), gait speed in challenging situations (Mirelman, Maidan, *et al.*, 2011), mood and activities of daily living (Lee *et al.*, 2015), and sensory integration and attention (Yen *et al.*, 2011). It appears that optic flow is an important component of training programs in PD, at least in real environments (Almeida and Bhatt, 2012). It has also been postulated that abnormalities in perception of optic flow may be linked to FOG, especially in those with left-sided dominant PD (Cohen *et al.*, 2012). Optic flow in itself, as part of a virtual reality-based intervention may therefore have beneficial effects on FOG. To date, no virtual reality based intervention has been designed specifically for FOG.

In recent years, off-the-shelf gaming products such as Kinect (Microsoft, USA) and Wii (Nintendo, Japan) have allowed testing of such virtual reality with a view to home-based intervention. Numerous studies have shown the benefit of these products on gait and balance in PD (Barry *et al.*, 2014; Esculier *et al.*, 2012; Herz *et al.*, 2013; Liao *et al.*, 2014; Mhatre *et al.*, 2013). Given the lack of carry-over seen in the cueing trials outlined above and the general immobility of the Parkinson's disease population, any future intervention should be designed for regular use in a home-based setting. These platforms allow this design strategy to be realised.

1.10 Summary

In summary, FOG is a common and disturbing symptom in Parkinson's disease. It is clinically important as it is associated with falls and nursing home placement. The pathophysiology is poorly understood. Although many associations with perceptual, motor and cognitive abnormalities have been demonstrated by behavioural and neuroimaging studies, no clear unifying deficit has been identified. Without a clear model for pathogenesis of FOG, treatment options will remain limited. A greater understanding of the pathophysiology of FOG, however, is likely to lead to new and promising therapies. Given the strong evidence for deficits in sensory, motor and cognitive processing in FOG, the focus of this thesis is to probe these mechanisms in PD patients with and without FOG in an effort to develop a therapeutic intervention for FOG.

2. Thesis Objectives

This section details the specific research questions based on the literature review, in addition to the primary focused aims of the research.

2.1 Research Questions

After an analysis of the literature on FOG as outlined in Chapter 1, it is clear that a number of research questions still remain unanswered in this research field. In particular, objective quantitative measures of sensory/perceptual, motor and cognitive differences between freezers and non-freezers are lacking. Furthermore, few therapeutic strategies which specifically target underlying deficits exist. The research questions to be considered are as follows:

1) **Can a multimodal approach to the investigation of FOG be employed to successfully probe the pathophysiological mechanisms of FOG, specifically in the perceptual, motor and cognitive domains**
    1. Using a combination of neurocognitive assessments, clinical assessments, gait analysis, EEG and behavioural measures in patients with and without FOG, can we see differences between groups with respect to sensory/perceptual, motor and cognitive processing, controlling for overall speed of motor responses?
2) **Given the findings in previous studies that strong SENSORY/PERCEPTUAL effects may be seen in PD and particularly in FOG, are there quantifiable differences in the way patients with PD/FOG process sensory information and combine information from different modalities?**
    1. By examining response times to stimuli from different modalities in freezers, non-freezers and healthy controls, is there a measurable difference in sensory processing between these modalities?
    2. Do patients with PD integrate sensory information differently to healthy controls and, if so, is there a difference between freezers and non-freezers?
3) **If progression of dopaminergic denervation in the basal ganglia contributes to FOG, are there quantifiable differences in performance of goal-directed MOTOR behaviour between freezers and non-freezers?**

1. Using a task designed to examine acquisition of a motor task, are there differences in performance and refinement of goal-directed movement between freezers and non-freezers?
2. Given their over-reliance on goal-directed behaviour, do people with PD perform this goal-directed task better or worse than healthy controls?

4) <u>Since certain sensory environments trigger FOG while walking, can a virtual reality (VR) based paradigm while stepping in place be used to provoke FOG as reliably in an experimental setting?</u>

1. By stepping in place to navigate through a virtual reality environment consisting of triggers of FOG, can motor arrests be reliably induced in patients who experience FOG?
2. Do these motor arrests while stepping in place in the VR environment correlate with New Freezing of Gait Questionnaire (NFOG-Q) scores and gait performance in a real environment (such as gait speed and number/duration of FOG episodes on a standardised walking test)?
3. Are these motor arrests while stepping in place a reasonable surrogate marker for FOG? How does a VR based paradigm compare to current clinical measures of inducing FOG in an experimental setting?

5) <u>Can this same VR paradigm be used to examine temporal gait parameters in these patients during stepping in place under different environmental conditions?</u>

1. By recording centre of pressure while stepping, does calculation of temporal gait parameters such as step frequency, gait symmetry and rhythmicity show differences between freezers and non-freezers which are comparable to those seen in real environments?
2. What is the effect of optic flow in the VR environment on these gait parameters and freezing frequency/duration in freezers and non-freezers?
3. What is the effect of virtual visual cues in the VR environment on freezing and gait parameters in these patients?
4. Can freezing episodes be detected automatically and differentiated from volitional cessation of gait?

6) <u>By examining dual-tasking in the VR environment, can we quantify the effect that dual-tasking has on freezers and non-freezers?</u>

1. Is there a difference between groups in performance of a COGNITIVE task? Does performance of this task deteriorate while simultaneously stepping in place compared with sitting? If so, is this deterioration greater in freezers compared with non-freezers, suggesting a greater effect of dual-tasking in freezers?
2. Is there a difference between groups in dual-task MOTOR performance, i.e. do gait parameters deteriorate while stepping in place and performing a secondary cognitive task simultaneously? Is this effect greater in freezers compared with non-freezers?

7) <u>Can event-related potentials generated by a cognitive task while seated demonstrate differences in SENSORY, MOTOR and COGNITIVE cortical mechanisms between freezers and non-freezers and thus elucidate the cortical mechanisms of FOG?</u>

1. Using high-density electroencephalography during a two-stimulus oddball task while seated in freezers and non-freezers, the following hypotheses can be tested:
    - Are there visual evoked potential differences between groups, further supporting basic SENSORY processing differences?
    - Are there differences in cortical markers of MOTOR readiness while preparing for a motor response in freezers compared with non-freezers?
    - Are there differences in event-related potentials which are associated with COGNITIVE function (such as the P3b) between groups?

8) <u>Can ambulatory EEG while stepping in place show cortical activity while walking in freezers and non-freezers?</u>

1. Event-related potential studies have not been performed while walking or stepping in Parkinson's disease. Can evoked responses be reliably detected in people with Parkinson's disease while stepping in place?
2. Are there differences in amplitude and latency of evoked responses between freezers and non-freezers while stepping?
3. Are there differences in amplitude and latency of evoked responses while sitting vs stepping in place?
4. Are there differences frequency-specific power between freezers and non-freezers, particularly in the beta frequency band which has been shown to be associated with initiation of voluntary movement.

9) <u>What are the neural correlates of rhythmical sensory cueing in these patients?</u>

1. Using functional MRI during normal stepping in place through the VR environment in the scanner (e.g. using foot pedals) and comparing activation patterns with and without virtual visual cues in the environment, do the differences reveal the neural substrates which facilitate gait in patients who respond to visual cues?

10) **What local field potential changes occur in the basal ganglia during freezing episodes and do these correlate with cortical EEG changes?**
    1. By examining local field potentials from the stimulating electrode of deep brain stimulators, can we find a signature of freezing in the subthalamic nucleus?
    2. Comparing these local field potential changes with surface electrode recordings of cortical signals, can we deduce whether the primary deficit occurs at the level of the basal ganglia (with a resulting cortical response) or at the level of the cortex which is then filtered through a deficient basal ganglia?

11) **Can the underlying deficit which causes FOG be trained using a combination of PERCEPTUAL, COGNITIVE AND MOTOR training?**
    1. Does a VR based training program which combines perceptual, cognitive and motor elements lead to improvements in freezing and other gait parameters, ability to perform dual tasks, measures of cognitive function, and overall quality of life?

2.2 Research Focus

The main aims of this thesis are thus as follows:

- **To design quantitative measures to assess SENSORY/PERCEPTUAL, COGNITIVE and MOTOR function in PwP with and without FOG.**
- **To examine whether true SENSORY/PERCEPTUAL processing differences exist between patients with and without FOG**
- **To examine whether goal directed MOTOR behaviour performance is different between patients with and without FOG**
- **To develop a virtual reality based paradigm which reliably provokes FOG in an experimental setting and to use this paradigm in order to examine behavioural and neurophysiological**

PERCEPTUAL, COGNITIVE and MOTOR differences between patients with and without FOG while performing single- and dual-tasks

- **To conduct a pilot study examining the effects of combined sensory, motor and cognitive, training on patients with FOG**

Many of the research questions in the chapters that follow have not been previously addressed in the literature. As such, the studies presented in this thesis are entirely novel in this patient group.

In particular, the experimental methods have been designed to test these specific hypotheses in a manner which is independent of motor output. Although many of the findings are significant, the breadth of this thesis has allowed only the primary questions to be answered. Many further research questions have arisen as a result of the findings in these studies. The paradigms employed within this thesis were designed specifically so that these future studies can be undertaken using the same paradigms, allowing these research questions to be answered in the context of future work.

3. Audiovisual Processing is Abnormal in Parksinson's Disease and Correlates with Disease Duration and Freezing of Gait

3.1 Introduction

As described in Chapter 1, sensory and perceptual disturbances are common in Parkinson's disease (Konczak *et al.*, 2012; Martens and Almeida, 2011; Patel *et al.*, 2014). Subtle deficits of the sensory system, often not detected by routine examination, occur in PwP. From simple anosmia and impaired kinesthetic perception, to more complex visual hallucinations and spatiotemporal perceptual abnormalities, altered sensory processing in PD is found across multiple modalities (Armstrong, 2011; Doty, 2007; Klockgether *et al.*, 1995; Troche *et al.*, 2012; Vercruysse *et al.*, 2011). Online integration of multiple sensory inputs from our environment is crucial for a refined but complex goal-directed motor output (such as locomotion through a crowded environment). It is not surprising, therefore, that there is increasing evidence suggesting that these sensory deficits contribute to the pathophysiology of some of the abnormal motor features of PD (Abbruzzese and Berardelli, 2003; Demirci *et al.*, 1997; Keijsers *et al.*, 2005), including FOG. Although the underlying pathophysiology FOG is incompletely understood, sensory mechanisms have also been proposed as the core factor underlying this motor symptom (Ehgoetz Martens *et al.*, 2013).

There are many studies quantifying sensory deficits in Parkinson's disease with respect to a single modality (unisensory). Simple reaction time tests are helpful when exploring sensory responses, as they require little cognitive processing which make interpretation difficult in a patient population where cognitive impairment is common. Indeed, it has been shown that reaction time increases in patients with PD as the cognitive complexity of the reaction time task is increased (Cooper *et al.*, 1994). Simple reaction times to auditory and visual stimuli are delayed in PwP compared with healthy controls (Bloxham *et al.*, 1987; Cooper *et al.*, 1994; Evarts *et al.*, 1981; Jordan *et al.*, 1992; Low *et al.*, 2002; Paunikar *et al.*, 2012; Pullman *et al.*, 1988; Yokochi *et al.*, 1985). However, the execution of motor output in response to sensory stimuli requires both sensory processing and sensorimotor integration. Therefore, it is not surprising that simple unisensory reaction times are delayed in PwP since they are slow (bradykinetic) and reaction times do not directly assess sensory differences in these patients, the response being a combination of motor and sensory processing pathways. Quantitative assessment of

sensory processing speeds therefore requires examination of relative differences in response times between conditions with a similar motor output. Nevertheless, premotor delays in processing have been shown via movement-related potentials (Low *et al.*, 2002; Praamstra *et al.*, 1996) as well as auditory, visual and somatosensory evoked potentials (Gawel *et al.*, 1981; Özden Sener *et al.*, 2001; Rossini *et al.*, 1993; Silva Lopes *et al.*, 2014). This suggests that unisensory processing is impaired in Parkinson's disease, independent of integration with motor output.

Multisensory integration is the ability of the brain to integrate sensory information from multiple modalities into a single coherent percept, leading to increased speed and accuracy of identification (Freiherr *et al.*, 2013). When reaction times to multisensory stimuli are compared to those of individual component unisensory stimuli, the responses are significantly faster than would be predicted based on the unisensory reaction times. By comparing relative response times to unisensory and multisensory stimuli, quantitative assessment of the effect of multisensory integration can be examined, taking into account common variations in motor response times in PD.

Multisensory integration is enhanced in healthy elderly populations (Laurienti *et al.*, 2006) and older adults display greater multisensory facilitation of reaction times than young adults (Peiffer *et al.*, 2007). This may be a mechanism to compensate for loss of unisensory feedback (Laurienti *et al.*, 2006). However, inefficient multisensory processing is linked with falls in healthy older adults, highlighting the importance of controlled multisensory integration in locomotion (Setti *et al.*, 2011). Multisensory integration is crucial for effective sensorimotor integration and motor control (Elliott *et al.*, 2010; Tagliabue and McIntyre, 2014) including orientation (Forsythe, 2011) and speed estimation during movement (Sun *et al.*, 2003). Adaptive movement, therefore, requires, not only intact afferent sensory information, but also the ability to efficiently integrate that information. Locomotion is a highly complex multisensory process involving contributions from visual, vestibular, kinesthetic, somatosensory and auditory systems. Given that progressive gait impairment frequently occurs in Parkinson's disease, abnormal multisensory processing may occur in PD. Both cortical and subcortical areas have been implicated in multisensory integration including the superior colliculus, insula, hippocampus and frontal, temporal and parietal areas (e.g. superior temporal sulcus, intraparietal sulcus, fusiform gyrus and orbitofrontal networks). More recently, single cell animal studies have highlighted the basal ganglia as an important multisensory hub (Nagy *et al.*, 2006; Reig and Silberberg, 2014). Given that PD is a basal ganglia disorder, with evidence of widespread sensory abnormalities, it is hypothesized that multisensory integration would be altered in PD.

Few studies to date have explicitly examined multisensory integration in PD (Sabaté *et al.*, 2008). Sensorimotor integration is known to be altered in PD and multisensory effects have been implicitly embedded in studies examining interactions between proprioceptive and visual information (Adamovich *et al.*, 2001; Demirci *et al.*, 1997; Keijsers *et al.*, 2005; Klockgether and Dichgans, 1994). These studies have primarily examined pointing to remembered targets in the presence and absence of visual feedback in PD and have suggested that PwP have an over-reliance on visual information in order to compensate for early loss of proprioceptive feedback. Other studies have implicitly investigated multisensory integration in PD when studying relationships between kinesthesia and auditory stimuli on motor output (Sabaté *et al.*, 2008) and kinesthesia and vestibular processing on spatial orientation (Barnett-Cowan *et al.*, 2010). The above studies have employed spatial accuracy as a parameter of sensory integration. Few studies have examined response *speed* as a measure of sensory integration in PD. Although the multisensory effects with respect to visual and proprioceptive modalities have been well established in PD, it is difficult to study reaction speed in response to a proprioceptive stimulus as a joint must be moved over a finite period of time making instantaneous stimulus presentation effectively impossible. By far the most dramatic sensory effect seen in Parkinson's disease is that of sensory cueing on gait (Spaulding *et al.*, 2013) and, in particular, on FOG. The strong evidence for visual and auditory cueing in PD suggesting that addition of a stimulus from these modalities improves motor function. However, the multisensory interactions between auditory and visual stimuli have not been studied in PD to date. In this study, PwP and age-matched healthy controls performed a reaction time task in response to unisensory (auditory-alone, visual-alone) and multisensory (audiovisual) stimuli. Because of the inherent delays in motor responses seen in PD, *relative* unisensory and multisensory processing delays were assessed as these take into account the motor delays which are invariably present in PwP. This paradigm was then used to explore associations between sensory deficits and FOG status.

In this study efforts were made to limit the effect of attention by comparing relative differences between auditory-alone, visual-alone and audiovisual response times. In this way, each participant acts as his or her own control. Thus any differences in performance represent relative differences in either processing of different modalities or shifts in modality-specific attention between groups. Given the widespread sensory abnormalities in PD, it was hypothesized that multisensory integration is also altered in PwP. The reaction time task was used in order to:

1. Assess differences in unisensory (auditory and visual) processing speed in PwP and age-matched healthy controls.

2. Correlate *relative* differences in unisensory (auditory vs visual) processing in PwP with disease duration and FOG status taking into account the known motor delays in PD.

3. Compare relative differences in multisensory processing between PwP and age-matched controls.

3.2 Methods

3.2.1 Participants

39 patients with idiopathic Parkinson's disease (as defined by the Parkinson's Disease Brain Bank Criteria (Hughes *et al.*, 1992); Hoehn and Yahr stage II-IV) were recruited from the Movement Disorder Clinic at the Dublin Neurological Institute at the Mater Misericordiae University Hospital. Ethical approval was granted from the hospital ethics committee and informed consent was obtained from all participants. All patients underwent clinical and neuropsychological testing including Montreal Cognitive Assessment (MoCA), Frontal Assessment Battery (FAB) and Unified Parkinson's Disease Rating Scale III (UPDRS III). FOG status was recorded for all patients based on Question 1 of the New Freezing of Gait Questionnaire ("Did you experience a freezing episode over the past month?") (Nieuwboer *et al.*, 2008). All participants had normal corrected vision and hearing and were tested in the "on"-state with timing of last medication dose recorded. 17 age-matched healthy controls were recruited for comparison. The control group had no neurological comorbidities and normal cognition, vision and hearing.

3.2.2 Stimuli

Participants performed a simple reaction time task consisting of three stimulus conditions: "auditory-alone" (A), "visual-alone" (V) and "audiovisual" (AV). Stimuli were presented using Presentation software. The auditory condition consisted of a 1000-Hz tone (duration 60 msecs; 75 dB; rise/fall time 5 msecs), which was presented via inbuilt speakers of a laptop (Dell Latitude E5530). The visual condition consisted of a red disc with a diameter of 3.2 cm (subtending 1.5 degrees in diameter at a viewing distance of 122 cm) appearing on a black background, which was presented on the laptop screen for 60 milliseconds. The disc was located 0.4 cm superior to central fixation along the vertical meridian (0.9 degrees at a viewing distance of 70 cm). A small cross marked the point of central fixation on the

monitor. The audiovisual condition consisted of the auditory and visual conditions presented simultaneously.

Figure 3.1. Unisensory and multisensory stimuli: *Stimuli for auditory-alone (A, left), visual-alone (V, middle) and audiovisual (AV, right) conditions. The auditory condition consisted of a 1000-Hz tone (75 dB; rise/fall time 5 msecs). The visual condition consisted of a red disc with a diameter of 3.2 cm (subtending 1.5 degrees in diameter at a viewing distance of 122 cm) on a black background. The audiovisual condition consisted of the auditory and visual conditions presented simultaneously. All stimuli were presented for 60 milliseconds.*

3.2.3 Procedure

Participants were seated comfortably in front the laptop and instructed to press a button with their right thumb as quickly as possible when they saw the red circle, heard the tone, or saw the circle and heard the tone together. The same response key was used for all three stimuli. The stimulus conditions were presented with equal probability and in random order in blocks of 100 trials. Inter-stimulus-interval (ISI) varied randomly between 1000 and 3000 milliseconds according to a uniform (square wave) distribution. Participants completed 3 blocks, resulting in 100 repetitions per stimulus condition. Breaks were encouraged between blocks to help maintain concentration and reduce restlessness or fatigue. The range of reaction times accepted was determined at the individual participant level with the slowest cut off at 150 milliseconds and fastest 2.5% of trials excluded. The experiment was programmed using Presentation software (Neurobehavioral Systems, Inc., Albany CA). Versions of this paradigm have successfully been used to interrogate multisensory processing in older adults, patients with epilepsy,

autism and other developmental disorders (Andrade *et al.*, 2014; Brandwein *et al.*, 2015; Laurienti *et al.*, 2006; Mercier *et al.*, 2013).

3.2.4 Analysis

Data were processed and analyzed using MATLAB (Mathworks, Natick, MA) where custom scripts were developed and SPSS 22 (IBM, Chicago, IL).

3.2.4.1 Reaction Time Analysis

Mean reaction times for each condition were calculated for all participants. A mixed one-way analysis of variance (ANOVA), with the factors of stimulus condition (auditory-alone, visual-alone, audiovisual) and group (PwP and control participants) was performed to compare the reaction times of the three stimulus conditions between PwP and controls. Post-hoc comparisons between the conditions were performed to test for the presence of relative differences between the unisensory conditions as a well as faster reaction times in the multisensory condition.

3.2.4.2 Relative Sensory Processing and Freezing of Gait Status

To investigate the relationship between relative sensory processing (controlling for motor delays) and FOG status, the PwP group was subdivided by Question 1 of the New Freezing of Gait Questionnaire, as described above (Nieuwboer *et al.*, 2008). The reaction times were subtracted from each other to account for variable motor delays in PwP. In this way, the results relate to *relative* changes in sensory processing rather than reflecting slower motor responses with disease severity. A mixed repeated ANOVA was performed with the within-participant factor of relative reaction time (auditory-visual vs audiovisual-visual vs audiovisual-auditory) and between-participant factor of FOG status (freezers vs non-freezers).

3.2.4.3 Correlation Analysis of Disease Duration

Correlation analyses were performed on the PwP group to assess the extent to which the relative differences of reaction times for the three conditions, (auditory-visual, audiovisual-visual, audiovisual-auditory), are associated with disease duration (years since symptoms onset).

3.2.4.4 Miller Race Model

In order to quantitatively assess the degree to which multisensory integration contributes to response times for the audiovisual condition, the Miller race model was employed (Miller, 1982). Faster reaction times to the multisensory stimuli could be the result of participants responding to whichever stimulus is processed fastest, even in the absence of any interaction between the individual sensory stimuli. In this way, sensory processing could be considered a race between two modalities (auditory and visual in this case) on a trial-by-trial basis. This is known as the "redundant signal effect". The race model proposed by Miller is a commonly used behavioral index of multisensory integration which takes into account this redundant signal effect (Andrade *et al.*, 2014; Brandwein *et al.*, 2011; 2013; 2015; Mercier *et al.*, 2013; Molholm *et al.*, 2004). According to Miller's race model, reaction times are still expected to be faster in the multisensory condition compared with the unisensory state. This is because there are now two inputs, which can trigger a response, as opposed to just one. Whichever input is fastest, triggers a response, making a faster response more likely in the multisensory condition than if only a single stimulus was present. Miller's race model defines an upper limit for multisensory responses in this simple linear model based on the sum of the cumulative probabilities of each unisensory stimulus triggering a response. If the recorded multisensory reaction time is faster than this upper limit then violation of the race model has occurred and it must be assumed that the unisensory inputs interacted during processing (i.e. multisensory integration occurred). Failure to violate the race model, however, does not prove that the unisensory inputs did not integrate, but implies that the recorded multisensory reaction time could be explained by simple summation of unisensory probabilities.

3.3 Results

3.3.1 Demographics

The demographic and neurocognitive data for the Parkinson's disease cohort (divided by FOG status) is given in Table 3.1 below. The 17 healthy control participants (10 male) had a mean age of 66 +/- 9.7 years (range 52-80).

	All PwP	Freezers	Non-Freezers
N	39	23	16
Age	67.4 (9.8)	68.7 (9.7)	66.7 (10.05)
Gender (M:F)	23:16	15:8	8:8
H&Y stage	2.65 (0.58)	2.8 (0.65)	2.4 (0.36)
Disease Duration (years)*	11.6 (9.7)	15.5 (10.5)	6.1 (4.6)
UPDRS	35 (14)	38 (14)	30 (14)
MOCA	25 (3.6)	24 (3.3)	26 (3.6)
FAB	16 (2.6)	15.2 (2.9)	17 (1.5)

Table 3.1 Patient Demographics by FOG status for audiovisual task: *Means shown with standard deviation in parentheses. * indicates statistically significant difference between groups.*

3.3.2 Hit Rate Analysis

Hit rates (proportion of stimuli responded to) were consistently high across all groups (Table 3.2). No significant hit rate differences were found between first and last blocks of trials for any group.

Group	A	V	AV
PwP (N=39)	**0.94 (0.08)**	**0.92 (0.09)**	**0.97 (0.03)**
Controls (N=17)	**0.98(0.05)**	**0.94 (0.06)**	**0.98 (0.02)**

Table 3.2. Mean hit rate and standard deviation for control group and PD group. *A=auditory-alone, V=visual-alone, AV=audiovisual.*

3.3.3 Reaction Time Analysis

PwP were significantly slower than controls for all conditions compared with healthy controls. Table 3.3 and Figure 3.2 show the mean reaction times and standard deviations for each condition (auditory-alone, visual-alone, audiovisual) and group (PwP and control participants). The mixed repeated ANOVA revealed a significant difference between the conditions' reaction times ($F_{2,108} = 84.32$, $P < 0.001$) with the fastest reaction times for the audiovisual condition. The analysis revealed significant difference

between groups ($F_{1,53}$ = 24.1, P < 0.001) with faster reaction times for all stimulus conditions in the control participants than in the participants with PD. No gender effect was seen.

Group	A	V	AV
PwP (N=39)	374.1 (74.0)	403.8 (67.6)	325.2 (68.0)
Controls (N=17)	295.2 (47.9)	315.1 (36.9)	245.1 (29.7)

Table 3.3. Mean and standard deviation of reaction times for control group and PD group. *A=auditory-alone, V=visual-alone, AV=audiovisual.*

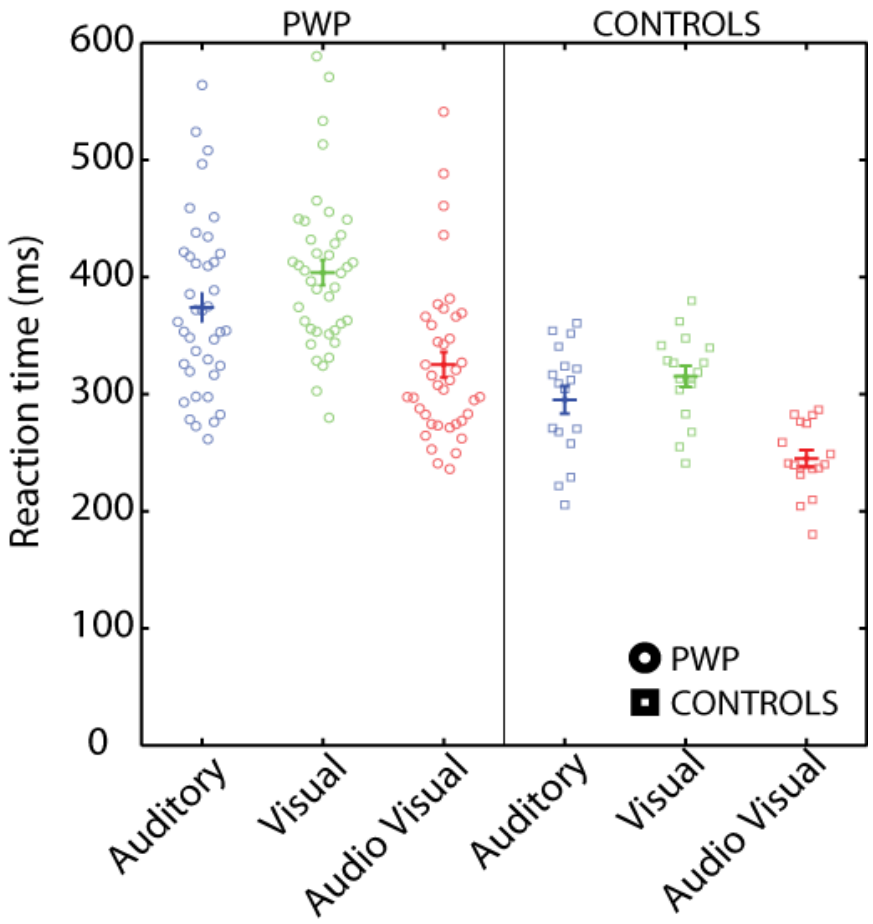


Figure 3.2. Reaction times for audiovisual task: *Reaction times for the auditory (blue), visual (green) and audio-visual (red) conditions for both the PwP (circles) and control participants (squares). The horizontal line and errorbars depict the mean and standard error of the mean.*

To investigate the significant effect of condition above, the data were submitted to a follow-up within-group, between-stimulus conditions analysis. The paired t-tests revealed that the reaction times in the audiovisual condition (AV) were significantly faster than the reaction times for the auditory-alone (A) and visual-alone (V) conditions in the control group (auditory-alone vs audiovisual $p<0.001$; visual-alone vs audiovisual $p<0.001$) and the PwP group (auditory-alone vs audiovisual $p<0.001$; visual-alone vs audiovisual $p<0.001$). This shows a redundant target effect, resulting in a faster reaction time for the audiovisual condition than the unisensory conditions for both groups. The analysis in the patients with Parkinson's disease revealed significant differences between the unisensory conditions with faster reaction times for the auditory-alone condition (auditory-alone vs visual-alone ($p<0.001$)), while in the control participants there was no significant difference between the unisensory auditory-alone and visual-alone conditions ($p=0.26$).

3.3.4 Freezing of Gait Status and Disease Duration Analysis

To investigate the relationship between *relative* sensory processing (controlling for motor delays) and FOG status, the PwP group was subdivided by Question 1 of the New Freezing of Gait Questionnaire (Nieuwboer *et al.*, 2008), as described above (Table 3.1). A mixed repeated ANOVA was performed with the within-participant factor of *relative* reaction time (A-V, AV-V vs AV-A) and between-participant factor of FOG status (freezers vs non-freezers). The reaction times were subtracted to account for variable motor delays in PwP, which allows for the analysis of *relative* sensory reaction times, taking into account variable motor delays seen in PwP. In this way, the results reflect true changes in sensory processing rather than simply slower motor responses in freezers. The analysis revealed a significant difference between the relative reaction times ($F_{2,74}$ = 67.663, $P < 0.001$). There was a significant interaction of FOG status and relative reaction time ($F_{2,74}$ = 3.37, $P < 0.05$). The analysis revealed no significant difference between groups across relative reaction times ($F_{1,37}$ = 2.39, P =0.131). The interaction effect was driven by a statistical difference (t_{37}= 2.037, $p < 0.05$) of the relative difference between the auditory and visual unisensory reaction times (i.e. A-V) in the freezers (M = -43.3, SD = 55.13 ms) compared with non-freezers (M = -10.32, SD = 40.23 ms). As FOG tends to occur late in the course of the idiopathic PD, we conducted a follow up unpaired t-test of disease duration between the freezers and non-freezers which revealed a statistical difference between the groups (t_{37}= 3.331, $p <$ .005).

To explore the relationship between relative sensory processing (controlling for motor output delays) and disease duration (years since symptom onset) three post-hoc correlation analyses were performed on the PwP group (Figure 3.3). Correlation analyses were performed between years since symptom onset (x-axis) versus 1) auditory-alone reaction times minus visual-alone reaction times (A-V); 2) audiovisual reaction times minus visual-alone reaction times (AV-V); and 3) audiovisual reaction times minus auditory-alone reaction times (AV-A). Again, the reaction times were subtracted to account for variable motor speed in PwP. Thus any differences are due to true sensory processing differences rather than slower motor responses with disease progression.

The correlation between the subtraction of mean reaction time of auditory from visual (A-V) conditions and years since symptom onset revealed a significant relationship (r_{37} =−0.351, P <0.05). A similar significant relationship was found between the subtraction of mean reaction time of audiovisual from visual (AV-V) conditions and years since symptom onset (r_{37} =-0.415, P < 0.0125). In contrast, there was no significant correlation between the subtraction of mean reaction time of auditory and visual (A-V) conditions and years since symptom onset (r_{37} =0.0952, P = 0.56). The analysis suggests that relative delays in visual processing correlate with disease duration.

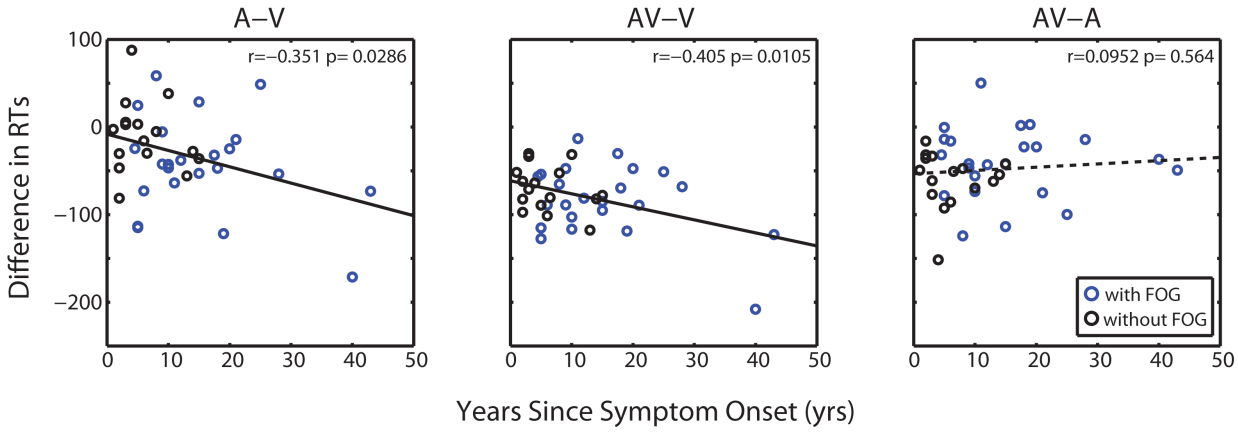


Figure 3.3. Correlation of disease duration and relative sensory processing: *Scatterplots displaying, on the x-axis, years since symptom onset and, on the y-axis of the left panel, the subtraction of visual from auditory reaction times; middle panel, the subtraction of visual from audiovisual reaction times; and right panel, the subtraction of auditory from audiovisual reaction times. Each circle represents a PwP, r-values and p-values are shown for significant (solid lines) and non-significant (dashed lines) regression analyses.*

3.3.5 Miller Race Model

To test the race model, reaction time range was calculated across the three stimulus types for each participant. Reaction times were sorted from fastest to slowest and the reaction time distribution was then divided into quantiles from the 5th to the 100th percentile in increments of 5% (e.g. as shown in Figure 3A and Figure 3B). At the individual level, a participant was said to have shown race model violation if the cumulative probability of his reaction times to the audiovisual stimulus was larger than that predicted by the race model at any quantile. We expect violations to occur in the quantiles which contain the fastest reaction times since, the faster the multisensory response, the more likely it is that multisensory facilitation has occurred. Conversely, the quantiles relating to slower multisensory reaction times are less likely to violate the race model. Testing of the Miller race model outlined above is also independent of variable motor responses as the multisensory response times are compared directly to the individual unisensory response times. In this way, each participant acts as his or her own within-subject control.

Figure 3.4A and 3.4B shows the cumulative probability for the auditory-alone (blue), visual-alone (green), audiovisual (red) and the cumulative probability predicted by Miller's race-model (black-dotted) for PwP and aged matched controls, respectively. The PwP group has a broader cumulative probability distribution for all three conditions with onsets later than their aged matched controls. Figure 3.4C and 3.4D shows the subtraction of the value predicted by the race model from the audiovisual cumulative probability curve, known as the Miller inequality, as a function of reaction time divided into percentiles. Miller inequality values statistically greater than zero (dashed horizontal line) signify race-model violation. To test for within-group violation of the race model, the Miller inequality values at each of the reaction times for the first half of the distribution were submitted to one-tailed t-tests (greater than 0, dashed line). The analysis revealed significant violation of the race model (shaded areas) for PwP (Figure 3.4C) and aged-matched controls (Figure 3.4D), thus both groups show multisensory reaction time benefits. Interestingly, there was no significant difference in race model violation between freezers and non-freezers.

Figure 3.4E illustrates the Miller inequality as a function of percentile for the PwP group (dark grey) and control group (light grey). To investigate differences in multisensory processing between the PwP and controls, taking into account reaction time differences, the Miller inequalities at each percentile were submitted to unpaired t-tests. The analysis revealed significantly larger Miller inequality and a larger

number of percentiles violating the race model (dashed line) in the control group (shaded area) than the PwP group. Thus, the PwP group have less enhanced multisensory processing compared with aged matched controls, as measured by violation of the race model.

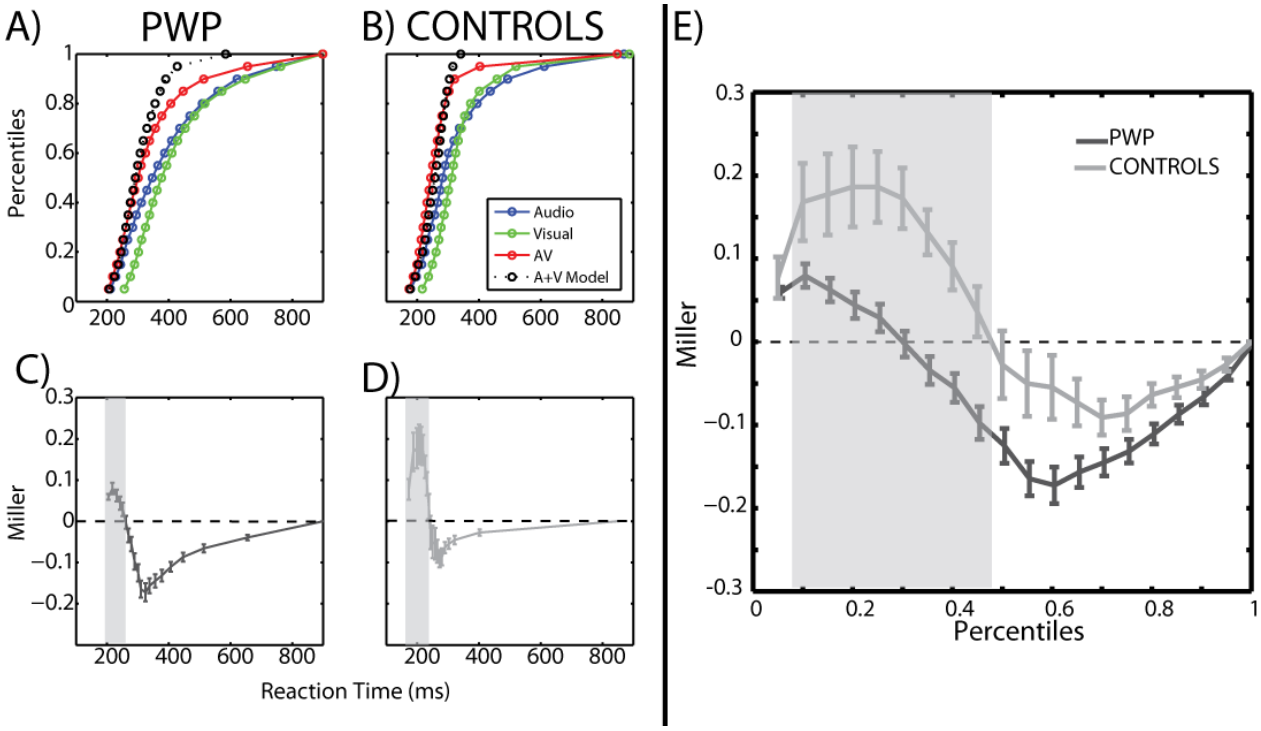


Figure 3.4 Miller Race Model. *A) & B) Cumulative Probability distributions for the auditory-alone (blue), visual-alone (green), audio-visual (red) and the cumulative probability predicted by the race model (black dotted) as a function of reaction time for Participants with Parkinson's diseases (PwP) and aged matched controls, respectively. C) & D) illustrates the subtraction of the multisensory cumulative probability and the cumulative probability predicted by the race model, known as the Miller inequality, as a function of reaction times for PwP (left) and aged matched controls (right), the errorbars depict standard error of the mean. The shaded areas indicate Miller inequality values statistically greater than zero (dashed horizontal line) signify race-model violation. E) The Miller inequality as a function of percentiles for PwP (dark grey) and aged matched controls (light grey). The shaded area indicates percentiles where the Miller inequality is greater than zero (dashed horizontal line) for the control group that are also significantly greater than the PwP.*

3.4 Discussion

Sensory and perceptual disturbances are prominent in PD and probably contribute to motor deficits such as bradykinesia and gait disturbance (Abbruzzese and Berardelli, 2003; Demirci *et al.*, 1997; Keijsers *et al.*, 2005). Our results show delays in response times to visual, auditory and audiovisual stimuli in PwP compared with age-matched healthy controls. This is not surprising, given the prominence of bradykinesia in PD. However, by comparing auditory-alone, visual-alone and audio-visual responses, differences in relative sensory processing suggest that sensory processing is inherently altered in PD. Importantly, these changes correlate with both FOG status and disease duration, consistent with an effect that is specific to PD progression and, moreover, provides a link between these sensory abnormalities and a motor feature of PD. Specifically, there is a significant difference between auditory and visual reaction times in PwP which is not present in age-matched healthy controls. This relative difference between auditory and visual reaction times is significantly greater in those with FOG than those without and also correlates with disease duration. Although multisensory facilitation occurs in PD, it is significantly less enhanced than in healthy controls. Reaction time tests represent a simplistic model for assessing sensorimotor function and cross-sensory function but it allows quantitative assessment of deficits which underpin more complex abnormalities of sensorimotor function in PD using a simple portable paradigm.

There is an extensive literature describing sensory deficits in PD, predominantly in response to a single stimulus. Few studies have quantitatively examined multisensory integration in PD and no study to date has investigated the interaction of auditory and visual modalities and their effect on reaction time. This study has shown that both unisensory and multisensory processing abnormalities are present in patients with Parkinson's disease. The unisensory and multisensory findings of the current study will be discussed separately.

3.4.1 Unisensory

Unisensory processing deficits have been noted in all modalities in PD (Armstrong, 2011; Doty, 2007; Klockgether *et al.*, 1995; Troche *et al.*, 2012; Vercruysse *et al.*, 2011). This study showed that unisensory responses to both auditory and visual stimuli are slower than healthy controls. However, within the PD group, the responses to visual stimuli were significantly slower than in the auditory modality. This was not the case for healthy controls suggesting that auditory and visual processing are differently affected in PD.

There is extensive clinical, behavioural, electrophysiological and imaging evidence, showing abnormal visual processing with PD progression. Assessment of visual processing in Parkinson's disease has revealed deficits at multiple levels from retina to visual cortex (Archibald *et al.*, 2009; Cardoso *et al.*, 2010). Gait parameters of PD patients deteriorate significantly in the absence of visual feedback (Martens and Almeida, 2011) and FOG occurs most often when visual feedback is omitted (Ehgoetz Martens *et al.*, 2013). Neuroimaging and neurophysiology lend further support to aberrant visual processing in PD. Retinal nerve fibre layer thickness is reduced on optical coherence tomography (OCT) in PwP compared with controls (Hajee *et al.*, 2009) and this correlates with both disease duration (Garcia-Martin *et al.*, 2014) and severity (Altintaş *et al.*, 2008). Functional imaging studies have shown reduced activation of the primary visual cortex in response to simple checkerboard tasks in PD patients without visual symptoms (Cardoso *et al.*, 2010) and reduced connectivity along ventral visual pathways in PD (Zhang *et al.*, 2015). Visual evoked potential (VEP) latencies are closely correlated with bradykinesia (Sener *et al.*, 2001) and disease duration (Wang *et al.*, 1999) and delays in early latency potentials also correlate with visual hallucinations in PD (Matsui *et al.*, 2005). Thus, there is evidence that visual processing deficits correlate with both disease duration and specific motor symptoms in PD.

There is a less extensive literature on auditory processing in PD. Early, middle and long latency auditory evoked potentials are abnormal in PD, providing evidence of both early and late information processing deficits in this modality (Çelik *et al.*, 2000; Gawel *et al.*, 1981; Pekkonen *et al.*, 1998; Philipova *et al.*, 1997; Raudino *et al.*, 1997; Wright *et al.*, 1996). In a similar manner to the visual modality, the amplitude and latency of long latency evoked potentials correlate with degree of cognitive impairment (Katsarou *et al.*, 2004; Matsui *et al.*, 2007; Nojszewska *et al.*, 2009; Pang *et al.*, 1990). Thus, mirroring other modalities, there is evidence of abnormalities of both simple and complex auditory processing in PD.

Motor responses to sensory stimuli test not only sensory processing, but also sensorimotor integration and motor performance. Existing reaction time studies, which examine each modality separately, therefore, reflect sensorimotor effects rather than purely perceptual ones. By comparing relative differences between reaction times to auditory and visual stimuli over a large number of trials, the current study examines perceptual responses independent of common motor output. This study shows that, although both auditory and visual responses are slower in PD than in healthy controls, the visual modality was significantly slower compared with auditory reaction time. Moreover, the difference between auditory and visual response times was strongly correlated with FOG status and disease duration. The relative differences between freezers and non-freezers appears to be due to a greater

reduction in auditory reaction time (i.e. faster response) in the freezers compared with non-freezers, rather than being driven by differences in visual reaction times. This suggests a possible adaptive response in PwP where auditory processing becomes faster relative to visual processing. This difference becomes more marked with disease duration and the development of FOG. Such an adaptive process is consistent with a recent neuroimaging study which found functional reorganization of locomotor networks in PD patients with FOG, postulated to be a maladaptive compensatory mechanism in freezers (Fling *et al.*, 2014).

Since FOG occurs more commonly in late stage Parkinson's disease, one should always be cautious when interpreting associations involving disease duration and FOG as they are closely correlated. This confounder is present to some degree in all studies of FOG. Nevertheless, these results support a disease-specific effect, independent of motor performance, rather than a corollary of multiple other neurological deficits seen in this group.

3.4.2 Multisensory

Few studies have explicitly examined multisensory integration in PD and this is the first study to explicitly examine audiovisual multisensory integration in this group. This study has shown that, although multisensory facilitation occurs in PD, it is significantly less enhanced compared with both age-matched healthy controls.

A number of studies have implicitly examined multisensory integration in PD with respect to other modalities. Investigations into interactions between proprioceptive and visual information and their effect on spatial estimation have focused on spatial orientation and inherently invoked the investigation of spatial working memory, which complicates the effect of multisensory integration in PD (Adamovich *et al.*, 2001; Demirci *et al.*, 1997; Keijsers *et al.*, 2005; Klockgether and Dichgans, 1994; Martens and Almeida, 2011; Martens, Ellard, *et al.*, 2013). As mentioned previously, these studies have focussed on accuracy of response rather than speed but showed a dependence on vision which starts in early PD in order to compensate for loss of proprioception. Multisensory integration, and its effect on motor output in PD, has been more formally examined by Sabaté et al. who examined the integration of auditory and kinesthetic stimuli and their effect on real and imagined movements (Sabaté *et al.*, 2008). Again the focus was on accuracy of response and they found that type and speed of a motor task determined the effect of unisensory and multisensory integration on motor output with the greatest multisensory benefit for slow continuous movements.

Animal studies have shown that kinesthetic sensory processing deficits correlate with degree of dopamine loss in the basal ganglia. However, with minor dopamine loss (e.g. in caudate nucleus only), this deficit can be overcome with the integration of visual information (Cools *et al.*, 1983). This effect has similarly been seen in clinical studies in PwP (Keijsers *et al.*, 2005). It is proposed that, as striatal dopamine loss worsens, the ability to compensate by integrating sensory information is also lost. Single cell recordings in mouse and cat have isolated large populations of multisensory neurons in the caudate nucleus and substantia nigra (cat) and dorsomedial striatum (mouse) (Nagy *et al.*, 2006; Reig and Silberberg, 2014). These suggest that the basal ganglia (like the superior colliculus) are a multisensory hub, crucial for integration of complex sensory stimuli from multiple modalities during execution of motor output. The striatal multisensory responses can be facilitatory or inhibitory. It is probable that a similarly large proportion of human striatal neurons have the capacity for multisensory integration, refining the response to multisensory stimuli and allowing very fine motor control from complex sensory inputs. The progressive loss of striatal dopaminergic innervation in PD affecting these populations of neurons explains this reduced multisensory facilitation in Parkinson's disease. Furthermore, as progressive loss of these neurons occurs over time, the sensorimotor responses could become less refined, approaching an all-or-nothing response. In this case, certain complex sensory environments could lead to dramatic augmentation of motor output by leading to a net crude facilitatory response whereas others (e.g. doorways, noise, crowds) could cause dramatic inhibition of motor output by leading to a net crude inhibitory response, causing akinesia or freezing of gait. This is consistent with existing models of FOG, which suggest that intense sensory stimulation overloads integrated parallel processing network within the basal ganglia leading to overactivity of the output nuclei of the basal ganglia causing FOG (Lewis and Barker, 2009; Lewis and Shine, 2014; Shine, Moustafa, *et al.*, 2013). Cowie et al. compared the gait of PwP and healthy controls walking through doorways and showed progressive scaling of gait parameters as PwP walked through increasingly narrow doorways (Cowie *et al.*, 2010). As FOG frequently occurs at doorways (Cowie *et al.*, 2012), it is possible that a perceptual deficit underpins the pathophysiology of FOG (Ehgoetz Martens *et al.*, 2013; Nantel *et al.*, 2012). A recent study has suggested that impaired visuo-proprioceptive integration causing a pause in processing of sensory information in order for a recalibration of inputs to occur (Beck *et al.*, 2015). During this period the authors suggest that motor output ceases during this recalibration, leading to FOG. The current study suggests that there may be a more global sensory integration impairment in patients with FOG and that these sensorimotor effects may occur due to multisensory interactions between visual and non-visual sensory inputs, rather than simple unisensory deficits.

The most dramatic multisensory effect seen in Parkinson's disease is that of sensory cueing on gait (Spaulding *et al.*, 2013) and, in particular, on FOG (Lee *et al.*, 2012). Sensory cueing (i.e. the use of a temporal or spatial stimulus to facilitate motor output) is used widely in PD as a strategy to improve gait. The fact that FOG can be strikingly relieved by the addition of a rhythmical sensory stimulus, provides further evidence that there are significant multisensory effects in PD. Given that locomotion is a highly complex multisensory task, the improvements in gait achieved by using specific sensory stimuli are probably via alterations in integration of those stimuli with motor output (Campos *et al.*, 2012). It should be noted that attention is a powerful modulator of these sensory effects, in particular, sensory cueing. Indeed, attentional cues alone can reduce freezing and improve gait. Recent meta-analyses and systematic reviews have confirmed the effect of visual and, in particular, auditory cueing as a rehabilitation strategy for gait in Parkinson's disease (Lim *et al.*, 2005; Rocha *et al.*, 2014; Spaulding *et al.*, 2013). The finding of the current study (that multisensory integration is less enhanced in PwP than in healthy controls) could be considered to be at odds with the observation that PwP appear to gain significant benefit from additional sensory information such as in rhythmical cueing. It is important to highlight that the results of the current study show that multisensory integration is reduced *but present* in PD. We must consider the possibility that intact but diminished multisensory integration may be beneficial, as the over integration seen in older adults has been linked with falls (Setti *et al.*, 2011). Finally, the multisensory changes seen here do not correlate with either disease duration or FOG status. This suggests that altered multisensory processing occurs even in early PD and may, therefore, be a potential biomarker for the disease. Multisensory deficits have similarly been suggested as a potential biomarker in other neurodegenerative disorders, such as Niemann Pick Type C, using a similar paradigm (Andrade *et al.*, 2014).

3.4.3 Limitations and Future Work

Rehabilitation strategies which incorporate sensory feedback have been shown to be of significant benefit in PD (Baram, 2013; Lefaivre and Almeida, 2015; Mirelman, Herman, *et al.*, 2011; Pelosin, 2014; Sage and Almeida, 2010; van den Heuvel *et al.*, 2013; Zalecki *et al.*, 2013). Specific strategies targeting multisensory integration have also led to behavioural and imaging changes in healthy cohorts (Bernstein *et al.*, 2013; Butler *et al.*, 2011; Powers *et al.*, 2009; Setti *et al.*, 2014) providing evidence that these deficits can be improved with training. Such multisensory strategies have led to improvements in balance and posture in older adults (Alfieri *et al.*, 2010; 2012; Hu and Woollacott, 1994; Kristinsdottir and Baldursdottir, 2014) as well as in rehabilitation following spinal cord injury and stroke (Johansson,

2012; Yen *et al.*, 2014). Further exploration of the role of multisensory training in PD may lead to promising therapeutic strategies for mobility, safety and FOG.

Tard et al. recently examined attention in FOG using unisensory reaction times and showed no difference between freezers and non-freezers in simple reaction times when corrected for disease duration (Tard *et al.*, 2015). However, when a divided attention task was performed freezers were slower. This suggests that divided attention is impaired in FOG. Thus, there is significant difficulty in separating attention effects from sensory effects in these types of studies. Multisensory integration is intricately linked with attention and it is likely that attentional effects may contribute to the results seen above. Performance on attentional tasks are correlated with FOG, in particular when performed under temporal pressure (Naismith *et al.*, 2010; Shine, Naismith, *et al.*, 2013). A recent study by Beck et al. explored the interactions between attention and sensory mechanisms in freezers and non-freezers while walking towards a doorway (Beck *et al.*, 2015). By examining gaze behaviour and gait in the presence and absence of visual cues and a dual task, they concluded that whereas increasing cognitive/attentional demand significantly affects FOG, there must also be an sensory effect to account for the improvements seen when visual cues are presented to freezers. They postulate that visual cues may decrease the demand on cognitive processing resources by operating through alternative pathways (e.g. cerebellar pathways). It was also noted that freezers do not look at upcoming doorways as much as non-freezers do, tending to focus their attention on their feet or the pathway instead. It is proposed that freezers do this in order to reduce the amount of sensory information they process in order to prevent an information overload during online sensorimotor integration. However, **decision making and motor preparation crucially rely on the accumulation of sensory information** (Kelly and O'Connell, 2013) and thus a mismatch between sensory information requirements and the ability to process and integrate that information for motor output could lead to FOG. If this is true, then it may be possible to train freezers' ability to process sensory information while walking. By presenting them with visual flow and complex visual triggers of FOG (such as turns, doorways and narrow spaces) while walking or stepping in place, the capacity to simultaneously process this sensory information might expand. This may ultimately be beneficial in improving FOG through sensory/perceptual training as will be discussed in Chapter 7.

The multisensory findings presented here could be explained by inequality of unisensory response times. It has been shown that equivalence of unisensory responses of individual modalities lead to optimal multisensory facilitation when combined (Alais and Burr, 2004; Ernst and Banks, 2002). If one

modality dominates (as auditory does in the PD cohort), then there is less opportunity for multisensory facilitation. The auditory response times in this study are closely correlated with multisensory facilitation, which supports this. In contrast, the healthy control group displays approximately equal responses to auditory and visual stimuli. This may explain the greater multisensory integration seen in this group compared with the PD group. Alterations in visual processing in PD described above may, therefore, be contributing directly to the diminished multisensory enhancement seen here. To account for this difference, the visual and auditory stimuli could be titrated for each participant to allow equivalent unisensory response times, thus eliminating this dominance effect. Secondly, there may be a ceiling on how quickly PwP can react in the multisensory condition. Thus even though multisensory facilitation is occurring, there may be a limit to how quickly a response can be generated in PwP giving rise to an apparent diminution of the multisensory effect in PwP.

Future work should include examining the effect of dopaminergic therapy on the above findings. All patients were tested in the "on"-medication state. It would be necessary, however, to confirm that our multisensory findings are similar off medication. Future studies should also include variation of detectability of unisensory stimuli to allow for optimum multisensory gain, inclusion of other sensory modalities and more complex stimuli as well as variation of timing between stimuli to examine the effect of temporal window of integration. Although the discussion here is in terms of specific modalities (visual and auditory), there may be a more global effect of relative sensory differences also affecting other modalities. Finally, the neural substrates of multisensory intergration may be different in PwP than in healthy controls, correlation of this task with functional neuroimaging may shed light on this.

3.5 Conclusions

The current study has shown that:

1. Both unisensory and multisensory delayed reaction times exist in patients with PD, in line with previous findings.

2. Relative differences in auditory and visual processing occur in PwP and correlate with FOG and longer disease duration.

3. Multisensory integration of auditory and visual stimuli is significantly less enhanced compared with age-matched healthy controls, adding to the literature supporting both simple and higher-order sensory processing abnormalities in PD. This finding is independent of FOG status and disease duration and may have a potential role as a biomarker in PD.

Parkinson's disease is, therefore, associated with widespread sensory deficits: peripheral and central; simple and complex; unisensory and multisensory. The precise interaction that these impairments have with gait and motor control is incompletely understood. It is, however, likely that a greater understanding of these processes will have positive implications for therapeutic targets and rehabilitation. In particular, recent literature suggests that impaired sensory integration and resultant information overload may be a core factor in the occurrence of FOG. Expanding this capacity to process sensory information while walking may be beneficial in improving FOG and such an intervention should therefore include complex visual flow during locomotion as well as those sensory environments which frequently trigger FOG such as narrow spaces and doorways. In this way, encouraging freezing patients to look at complex sensory environments which they usually exclude may improve their ability to concurrently process sensory information while walking and may have knock-on effects on FOG. This concept is taken into consideration when designing an intervention for FOG in Chapter 7. However, such training would be futile if there was also a motor output impairment in freezers preventing improvements in motor performance.

4. Motor Skill Acquisition and Goal Directed Behaviour in Parkinson's Disease and Freezing of Gait

4.1 Introduction

As discussed in Chapter 3, intact sensory processing in crucial for motor control (Mesulam, 1998), but the performance and learning of skilled movements is complex, involving multiple structures including cortex, hippocampus, cerebellum and the basal ganglia. Chapter 1 described the classical model of the basal ganglia (consisting of the direct and indirect pathways) and also more recent anatomical and functional revisions of this model. In particular, the organization of the basal ganglia is now known to contain topographically organised segregations which connect with functionally separate cortical areas forming limbic, associative and sensorimotor loops between the cortex and basal ganglia (Figure 4.1). Cortical areas associated with emotional processing (such as the medial and orbital prefrontal cortex) project to ventromedial portions of the basal ganglia to form the limbic loop. The premotor and motor areas which govern automatic control of movement connect to the dorsolateral areas of the basal ganglia (e.g. the caudal putamen) as the sensorimotor loop. Finally, the dorsolateral prefrontal cortex, which is essential for initiation and control of goal-directed movement, projects to the zone between the limbic and sensorimotor areas (predominantly the caudate nucleus) within the basal ganglia forming the associative loop. The substantia nigra provides ascending dopaminergic innervation to the striatum to modulate these processing loops. This innervation takes the form of both constant (tonic) and pulsed (phasic) signaling. Tonic firing allows *selection* of behaviours (including movement) at the level of the basal ganglia whereas phasic signaling uses sensory inputs to inform reward-based *learning* of behaviour. Rewards reinforce the connections of habitual responses during goal-directed behaviour and allow transition of this response from primarily goal-directed control (from the dorsolateral prefrontal cortex) to more automatic habitual control with a resultant reduction in the amount of activation required from multiple cortical areas including the dorsolateral prefrontal cortex (Hallett, 2008).

4.1.1 Basal Ganglia, Goal-Directed and Habitual Movement

In idiopathic Parkinson's disease there is early loss of dopaminergic innervation to the caudal putamen, which governs control of habitual movement (Redgrave *et al.*, 2010). As a result, tasks which were previously automatic cannot be controlled in a habitual fashion. In this situation, compensation for movement can occur by recruiting frontal cortical areas to provide more goal-directed motor control,

which passes through more anterior basal ganglia loops (Figure 4.1). With disease progression, the loss of dopaminergic signaling may spread to involve these anterior regions of the basal ganglia associated with goal-directed behaviour. If, with advanced disease, these areas become affected and contribute to FOG, performance of goal-directed tasks would be expected to be impaired to a greater degree in a cohort of freezers than in a group without FOG. Although loss of goal-directed movement in advanced PD has been suspected (Redgrave *et al.*, 2010), it has never been shown experimentally. No study of goal-directed motor control has been undertaken in FOG previously.

Goal-directed movement is complex and incurs significant computational cost but it allows refined, elaborate control of movements in an adaptive, flexible fashion (Rangel and Hare, 2010). Automatic movement requires little computational processing but little, if any, adaptive control of movement is possible. In healthy people, goal-directed and habitual movement work in parallel allowing delicate motor control, and the corticobasal-cortical loops allow rapid switching between conscious and automatic control. The exact point where motor processing reaches its final common pathway is unclear but it is believed that the basal ganglia is essential in switching between habitual and goal-directed modes. In PwP, this balance sways in favour of goal-directed movement as habitual control is essentially lost and PwP are "trapped in goal-directed control" (Redgrave *et al.*, 2010). The natural state of the output of the basal ganglia is inhibitory and the loss of sensorimotor areas will further keep this output in a tonic inhibitory state. Overcoming this tonic inhibition may be possible by increasing cortical activity in those areas which initiate goal-directed, rather than habitual movement but this is at the expense of increased computation processing. One might, therefore, expect early PD patients to be severely impaired when performing automatic tasks but may be able to perform goal-directed tasks as proficiently as a healthy person (provided they have not lost innervation to these more anterior associative basal ganglia zones). In more advanced PD (where FOG is commonly seen), goal-directed behaviour may also be affected. Although there has been disagreement in the literature, PwP on medications have been shown to perform and learn goal-directed movements as well as their healthy counterparts, as is discussed in the next section.

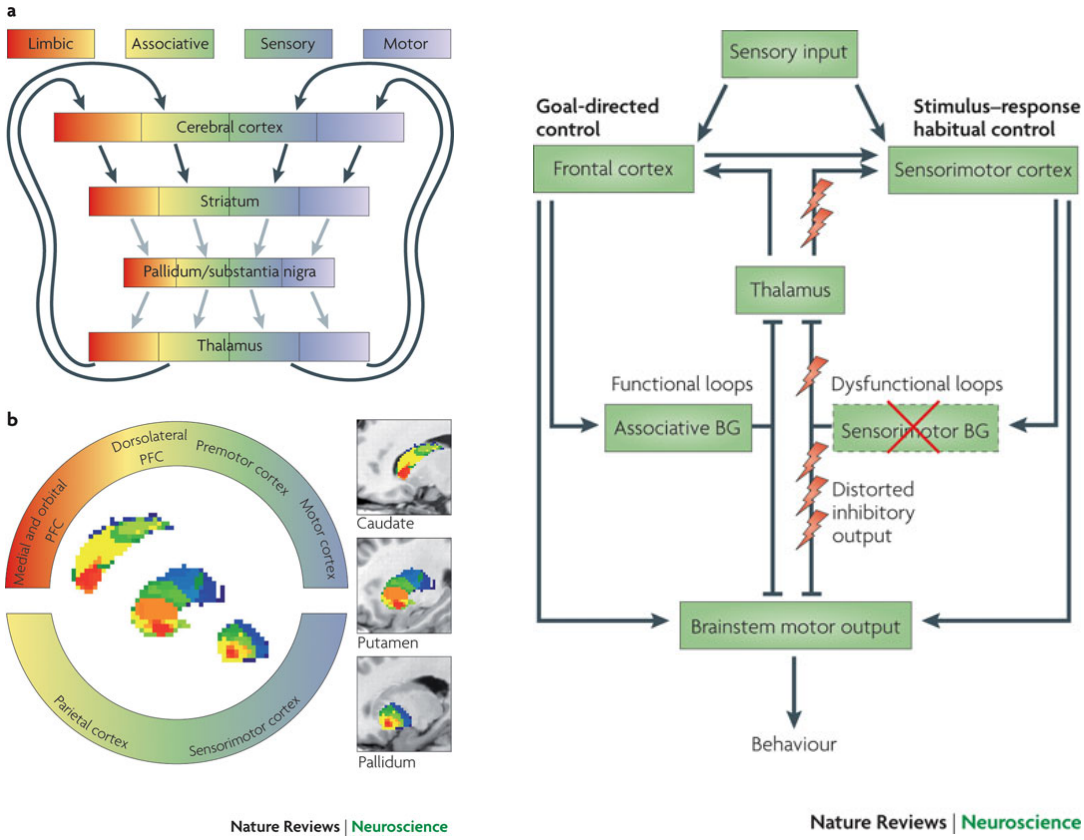


Figure 4.1 Functional anatomy of the basal ganglia and disruption of sensorimotor loops in Parkinson's disease *(From* (Redgrave *et al.*, 2010)*): Left a) Feedback loops of limbic (red), associative (yellow-green) and sensorimotor (blue) information which operate as functionally independent circuits; Left b) spatial anatomy of basal ganglia: the outer ring shows the cortical areas concerned with limbic (red), associative (yellow-green) and sensorimotor (blue) information and how these are functionally connected to specific areas in the striatum; Right: Loss of habitual control in Parksinon's disease due to loss of innervation to sensorimotor areas of the basal ganglia with resultant disruption of sensorimotor loops.*

4.1.2 Basal Ganglia and Motor Learning

The basal ganglia is integral in the learning and adaptation of skilled movements (Graybiel, 2000). Miyachi et al. have shown that when associative areas of the basal ganglia are inactivated, monkeys retain the capacity to perform previously well-learned tasks (Miyachi *et al.*, 1997). However, they are unable to acquire any new motor skills. This suggests that areas of the basal ganglia which govern goal-directed movement are crucial in motor skill acquisition. Conversely, when sensorimotor areas within the basal ganglia are inactivated, monkeys are able to learn new motor skills but the ability to perform

well-learned skills is lost. Given the loss of phasic dopamine signaling to the striatum in PwP, motor learning may be impaired in PD. In early PD, where sensorimotor areas of the basal ganglia are disrupted, acquisition of new motor skills should remain intact (based on the animal model above). Therefore, one would expect goal-directed learning and motor skill acquisition to be impaired In PD only if associative areas are disrupted. The primary aim of this study is to develop a method which examines goal-directed motor control and motor learning and to use this to examine whether goal-directed movement is impaired in PD compared with age-matched healthy controls or goal-directed behaviour is preserved due to compensatory reliance on these networks in the face of loss of habitual control of movement.

Two types of motor learning exist: motor skill acquisition, which is defined as adaptive motor control which favours improvements in speed *and* accuracy of a movement; and motor skill adaptation, in which improvements in speed *or* accuracy occur in a trade-off fashion (Redgrave *et al.*, 2013). Motor skill acquisition is a more robust marker of true motor learning as it is possible to adapt by focusing attention on one element of a task, at the expense of others. The study presented in this chapter will examine motor skill acquisition. Previous studies of movement learning in PD have shown conflicting results with some studies showing impaired motor learning in PwP(Harrington *et al.*, 1990; Muslimovic *et al.*, 2007; Smiley-Oyen *et al.*, 2006; Soliveri *et al.*, 1997) and others showing no difference compared with healthy controls(Agostino *et al.*, 1996; Jessop *et al.*, 2006; Jordan and Sagar, 2009). Disagreement has arisen because of variability in the tasks used to assess motor learning, study methodologies and disease severities assessed (Redgrave *et al.*, 2013).

4.1.3 Designing a Motor Skill Acquisition Task

The current literature pertains to motor learning in PD in general. A well-designed paradigm to study goal-directed movement and motor skill acquisition is lacking. A number of study design problems are evident when examining the literature on motor learning in PwP:

1. Patient features: The PD cohorts studied varied in disease severity and cognitive function. Redgrave et al. proposed that with more advanced disease, loss of associative areas of the basal ganglia will lead to progressive impairment in goal-directed behavior. However, this has never been shown conclusively to

be the case. Tests conducted at varying stages of disease severity may lead to variable interplay between goal-directed and habitual control mechanisms (Nieuwboer, Rochester, Müncks, *et al.*, 2009).

2. Contribution of bradykinesia: When examining speed (rather than accuracy) of a motor skill in PD, outcome variables must be independent of speed of movement to allow for slowness of movement in PwP. Many studies have focused on accuracy of movement rather than speed or have normalized measures with respect to the participants first attempt. When retention of a motor skill is examined a period of time after de novo skill learning, the natural fluctuations in motor speed may lead to misinterpretation of results (Ghilardi *et al.*, 2003; Pendt *et al.*, 2011). For this reason, the study presented here examines motor skill acquisition relative to performance on a similar non-learning motor task. In this way, the results are independent of overall motor speed in a given participant, at the time of study.

3. Medications: The effect of dopamine replacement on goal-directed and habitual movement is not straightforward and relies, to a degree, on the amount of residual endogenous dopaminergic innervation that person retains (Redgrave *et al.*, 2013). Exogenous dopamine may restore signaling required for movement learning or may flood the basal ganglia in areas where near-normal dopamine levels persist leading to inappropriate reinforcement learning. Studies off medication however, are less ecologically valid and do not account for adaptive changes that may have occurred with longterm dopamine replacement therapy. For this reason, the studies in this chapter were performed on medication but doses and timings of medications were recorded for group comparisons. As a result, caution should be taken in over-interpreting the results herein as medication is likely to have a significant effect on outcomes.

4. Task complexity and methodology:

- Motor sequence learning: Many studies examine only the learning of motor sequences (which involves at least some degree of cognitive input to remember and recall the motor sequence to be executed) (Dan *et al.*, 2015; Doyon, 2008; Ghilardi *et al.*, 2003; Hayes *et al.*, 2015; Smiley-Oyen *et al.*, 2006). The executive function deficits seen in PD in general, might therefore impact on performance in a way which is not directly related to pure motor learning. Furthermore, the cerebellum plays a much more dominant role in motor sequence learning than the basal ganglia does(Doyon *et al.*, 2002). As a result, these tasks may reflect cerebellar performance rather than primarily basal ganglia-based learning.

- Contributions of visuospatial information: When motor tasks (rather than motor sequences) are examined, these tend to consist of tracking of visual inputs with a cursor or joystick(Day *et al.*, 1984; Frith *et al.*, 1986; Harrington *et al.*, 1990; Schugens *et al.*, 1999) or require other visuospatial judgements(E. D. Anderson *et al.*, 2013; Pendt *et al.*, 2011). Since visual processing and sensorimotor integration are abnormal in PwP, these studies do not examine true motor skill acquisition in isolation. To examine true learning of a movement skill participants should not be asked to follow, track or trace a target in visual space. No study has been designed to examine the performance and refinement of a skilled goal-directed movement in the absence of visual input in PwP.
- Effect of response inhibition and habitual control: A number of motor learning studies in PD employed mirror drawing (where the actions of the participant were reversed by a mirror) in an effort to force the participant to used goal directed control (Agostino *et al.*, 1996; E. D. Anderson *et al.*, 2013; Schugens *et al.*, 1999). However, response inhibition deficits in PwP will have significant effect on mirror drawing where the motor response is reversed compared with that expected by the participant. When the outcome of the participant's movements is reversed, as in mirror drawing, the participant must suppress the normal expected habitual aspects of control in order to succeed with the task. Response inhibition is impaired in PwP (and particularly has been associated with FOG). Inhibition of the expected response is crucial to performing the mirror drawing task and therefore, interpreting the results of mirror drawing studies in PD is complex and does not purely assess goal-directed control.

Stafford et al. have described a method to examine motor skill acquisition in healthy subjects which will be described in detail in the next section. This method has been adapted to study motor skill acquisition in PwP herein. In addition, goal-directed movement and motor skill acquisition have not been studied in FOG to date. Since FOG tends to occur in advanced PD, this could be explained by loss of dopaminergic signaling from these more anterior areas of the basal ganglia. One would expect, therefore, to see impaired goal-directed movement in FOG. As a secondary goal of this study, a the PwP cohort studied was divided into freezers and non-freezers. Performance of the small group of freezers in this study was compared to the rest of the PD cohort to test for differences between groups.

4.1.4 Motor Control and Side of Onset of Parkinsonism

Another variable in the study of motor control in PD is the side of onset of parkinsonism. The right and left cortex have different roles in motor control. The left hemisphere is dominant for motor control of either hand but the right hemisphere plays a dominant role in spatial memory, learning and orienting, which are all crucial for goal-directed behavior (Serrien *et al.*, 2006). PwP with left-sided onset of symptoms (in which the right hemisphere is most affected) have been reported to show a left visuospatial neglect (A. C. Lee *et al.*, 2001; Villardita *et al.*, 1983). Line bisection tasks and visual attention tasks show that left-sided predominant PwP tend to search initially in right hemispace and neglect the left hemifield consistently (to a minor degree at least) (Ebersbach *et al.*, 1996; A. C. Lee *et al.*, 2001; Villardita *et al.*, 1983). More recently, however, this has been shown to be a left-sided neglect-like syndrome due to higher order attention rather than true neglect (Norton *et al.*, 2015) and patients with predominantly left-sided disease perform less well on visuospatial testing than those with right-sided disease (Seichepine *et al.*, 2015). Side of onset of PD has also previously been implicated in FOG whereby those with left-side predominant disease experienced an increase in freezing episodes in the presence of optic flow while walking towards a doorway (Martens, Pieruccini-Faria, *et al.*, 2013). This did not occur in right-side predominant subjects, suggesting that left-side predominant patients have deficits in perceiving or processing optic flow when passing through doorways, further supporting a link between perceptual disturbance, adaptive motor control and FOG. It has been suggested that reduced visual feedback from optic flow which occurs as FOG patients enter a narrow corridor could provoke FOG (van der Hoorn *et al.*, 2010). The role of such asymmetry in FOG has also been supported by neuroimaging studies (Bartels and Leenders, 2008; Fling *et al.*, 2013). Thus, the most affected side of parkinsonism may also determine performance in goal-directed tasks although this has never been investigated previously. Furthermore, since side of onset of parkinsonism also influences relative sensitivity to rewarding and aversive stimuli (an essential component of motor learning) (Maril *et al.*, 2013) motor skill acquisition may also be abnormal in left-side predominant PwP. A third hypothesis tested in the current study is that left- and right-sided predominant PD will affect performance on this goal-directed task.

4.1.5 Aims of the Current Study

Acquisition of motor tasks and refinement of goal-directed behavior will have significant implications for rehabilitation in Parkinson's disease and particularly in FOG. PwP can learn new locomotor patterns and this forms the basis of gait rehabilitation (Roemmich *et al.*, 2014). The ability of patients with FOG to perform and refine a motor skill has not been considered previously. Any rehabilitation strategy in FOG will rely crucially on this ability. The primary aim of this chapter was to develop a method which examines goal-directed movement in isolation and to use this paradigm to examine early refinement of goal-directed movement in PwP and (in a preliminary fashion) in those PwP with FOG. To eliminate the contribution of sensory stimuli to goal-directed performance, a goal-directed movement paradigm must be independent of visuospatial factors as well as controlling for overall speed of movement. This study is designed to quantitatively assess performance of goal-directed movement and acquisition of motor tasks in a manner that controls for both the effect of visuospatial processing on these tasks as well as the slowness of movement which is characteristic of PD.

The aims of this chapter are to:

1. Design an experiment in which participants perform a goal-directed task and which allows performance of that task to be assessed quantitatively in a manner that is independent of slowness of movement and visual processing. In this way, the experiment should examine higher order cognitive control of movement rather than simply motor speed, which is likely to be more impaired among PwP in general and particularly, in freezers who have more advanced disease.

2. Use this paradigm to assess goal-directed movement and motor skill acquisition in PD and compare performance with healthy age-matched controls. Some studies (e.g. mirror drawing studies) have suggested that medication PwP can perform goal-directed tasks as well as healthy controls. Unlike mirror drawing, however, the paradigm should not require inhibition of habitual responses as part of the goal-directed movement. By examining how movements are refined in order to locate seen and unseen targets, rate of motor skill acquisition with and without sensory feedback, can be quantitatively assessed, controlling for bradykinesia.

3. Undertake a preliminary study to compare the performance of freezers and non-freezers on this task. If progression of PD leads to progressive loss of dopaminergic innervation to areas of the basal ganglia

that govern goal-directed behaviour, could selective loss of this innervation, and subsequent disruption of cognitive control of movement, lead to FOG?

4. Compare the performance of predominantly right- and left-side affected PwP on this task. Given the the asymmetry of motor control and reward physiology (which is essential to motor learning) as well as the association between asymmetry of PD and FOG, performance of these two groups may lend important insights into goal-directed control in PD.

4.2 Methods

4.2.1 Participants

21 PwP (H&Y stage II-III; age 64 ± 12.5 years; 7 with FOG as defined previously; 14 RHS-predominantly affected) and 10 age-matched healthy controls (age 61.5 ± 9.7 years) were recruited from the Movement Disorder Clinic at the Dublin Neurological Institute at the Mater Misericordiae University Hospital. All patients were tested in the "on-medication" state. As before, participants underwent clinical and neuropsychological testing including Montreal Cognitive Assessment (MoCA), Frontal Assessment Battery (FAB) and Unified Parkinson's Disease Rating Scale III (UPDRS III). FOG status was recorded for all patients based on Question 1 of the New Freezing of Gait Questionnaire ("Did you experience a freezing episode over the past month?") (Nieuwboer *et al.*, 2008). All participants had normal corrected vision.

4.2.2 Experimental Design

This method has been adapted from that described by Stafford et al (Stafford *et al.*, 2012). The primary aim of the paradigm is to encourage participants to discover a specific hand movement that will elicit a reward stimulus as quickly as possible by manipulating a joystick. Initially the participant "explored" all possible hand movements and once the desired movement is discovered, they repeated this movement until it was refined. Rather than examining free movement as Stafford et al. did, participants were trained prior to the experiment on how to manipulate the joystick and the procedure of repeating these desired movements. The exact movement required in the experiment, however, was not the same as in the training phase and thus, it is this precise movement which needed to be learned by the participant (rather than the behaviour of manipulating the joystick in order to discover a reward stimulus).

The experimental setup is shown in Figure 4.2. Participants were seated in a comfortable chair 70cm from a laptop computer (Latitude E5530, Dell) in a quiet room and asked to complete a training period followed by two separate experimental conditions. During the training period, participants were asked to repeatedly manipulate a joystick (Extreme Pro 3D, Logitech) in order to move a cursor on a computer monitor within a search space (10.8°, visual degrees) until they located a target location highlighted by a grey annulus (3.2°). Stimuli were presented using Presentation software (Neurobehavioral Systems, Inc., Albany CA). When the target location was reached, a white annular reinforcement signal was presented at the centre of the search space for 30ms. The cursor then re-appeared at the centre of the search space with the participant instructed to find again the target location. A white cross-hair was presented at the center of the search space throughout to give participants a focal point during the trials. To complete a block of trials, the participant was required to reach the same target location 15 times. At the beginning of each block a new target location was selected at random. The hand and joystick was hidden from view in order to ensure an egocentric frame of reference was determining hand movements. A further training exercise block (15 trials) was undertaken which was identical to the above, except that the cursor was hidden during the trials while the target remained visible. The participants therefore used the joystick to move an unseen cursor towards a seen target. Two further training exercise blocks were undertaken whereby the cursor was present but the target location was hidden (the participants therefore had to find the location of the target by moving the cursor around the space), and finally a training exercise where both the cursor and target were hidden. All of the above was undertaken for explanation purposes in order to minimise any effect of learning of the experimental setup / joystick rather than motor skill acquisition during the experimental trials.

Figure 4.2 Experimental setup for motor skill acquisition task*. The cursor and target are shown in this training exercise on a black background. Participants are asked to manipulate the joystick (hidden from view) in order to move the cursor into the target space in order to obtain the reinforcement signal. The cursor and/or target are shown or hidden, depending on the exact training exercise. During the Continuous Cue Experiment, the cursor is hidden and the target is shown. During the Movement Learning experiment, both cursor and target are hidden.*

The training period was followed by two separate experimental conditions:

1. Continuous Cue (CC)
2. Movement Learning (ML)

These conditions are shown in Figure 4.3. In the CC condition, the participant manipulated the joystick to move the unseen cursor to a seen target location (shown as a grey annulus). In the ML condition, both the cursor and target were hidden and so the participants had to learn the movements required to obtain the reinforcement signal. The ML task requires action selection at the level of the basal ganglia. To complete a block of trials, the participant was required to reach the target location 15 times. At the beginning of each block a new target location was selected at random. Participants completed 3 experimental blocks (3x15 trials) with right and left hands for both conditions as was tolerated due to patient fatigue. Trajectories of the cursor were sampled by the Presentation software at 60Hz and time to reach the target was recorded for each trial and plotted to show a mean learning curve (of the 3 blocks) for each task (time vs trial number).

In the ML task no visual feedback is provided to the participant to guide their movements. This allows assessment of pure motor control which can rely only on proprioceptive feedback independent of the visual processing abnormalities outlined in Chapter 3. However, the ML task alone does not account for overall speed of movement. PwP would be penalized for bradykinesia since the outcome measure of interest is time to find the target. In the CC task, however, no movement learning is required as the target location is given. Participants simply move the joystick towards the direction of the target, allowing assessment of baseline speed of movement when no action selection is required. For this reason, the time to find the target is normalized with respect to performance on the CC task.

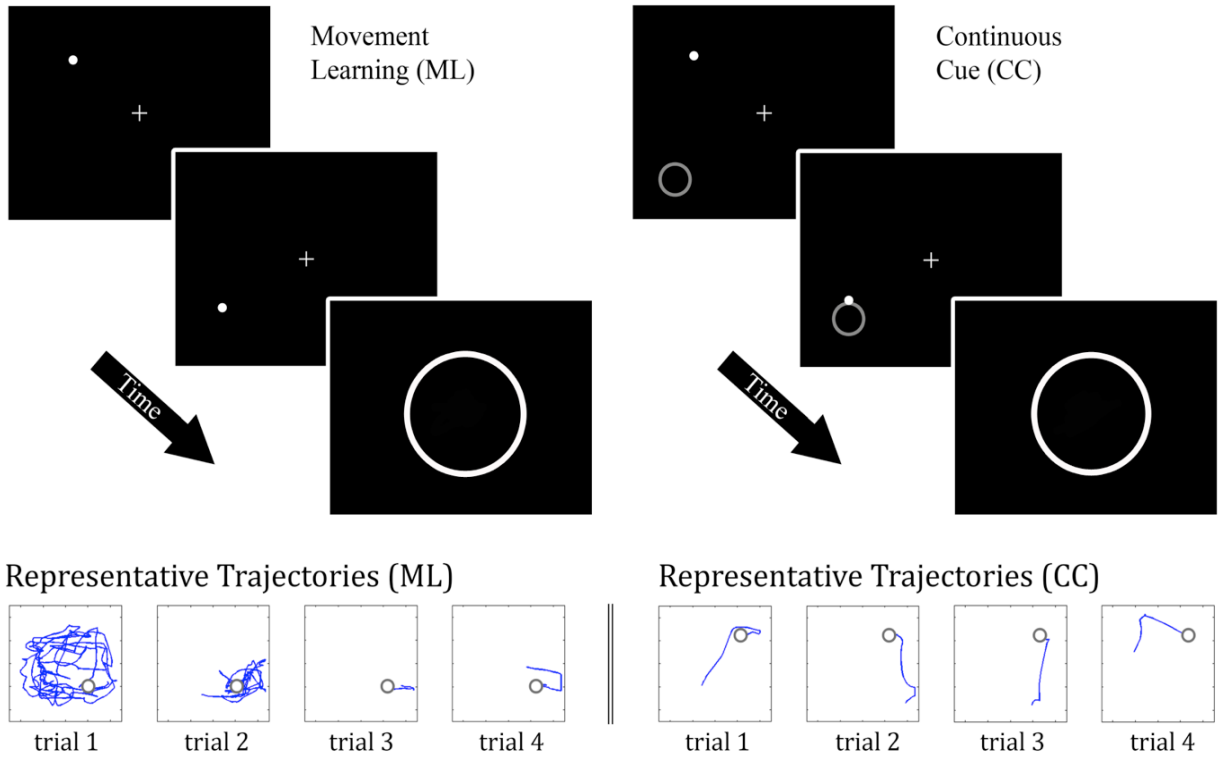


Figure 4.3. Sample frames from the Movement Learning and Continuous Cue condition of the task performed by participants. *For demonstration purposes, the cursor is shown here but is not visible to the participant in either condition. The target is hidden in the ML condition but is visible in the CC condition. The figure highlights the progression of the unseen cursor from the start of a single trial to the location of the target and, finally, the presentation of the 30ms feedback stimulus. Exemplar trajectories for each condition, for trials 1 to 4, are displayed underneath. The blue line represents the movements of the cursor and the grey annulus represents the target location*

The validity of the task as a goal-directed learning task is best assessed by examining the learning curves which will follow a power law if goal-directed behaviour is being performed. As performance improves with practice, the time taken to find the target should decay exponentially to an asymptotic point. The time taken on the first trial is important for two reasons: firstly, it represents a (goal-directed) spatial exploration phase and also determines the final performance. Further trials are refinements of this initial exploration phase. The greater the initial exploration, the better the performance on later trials. Therefore, the initial performance, rate of improvement and final performance are all important behavioural measures of goal-directed learning and these can easily be deduced by inspecting the learning curves plotted for each condition. Figure 4.4 shows an example of such a curve where distance to find a target was the outcome measure from (Stafford *et al.*, 2012).

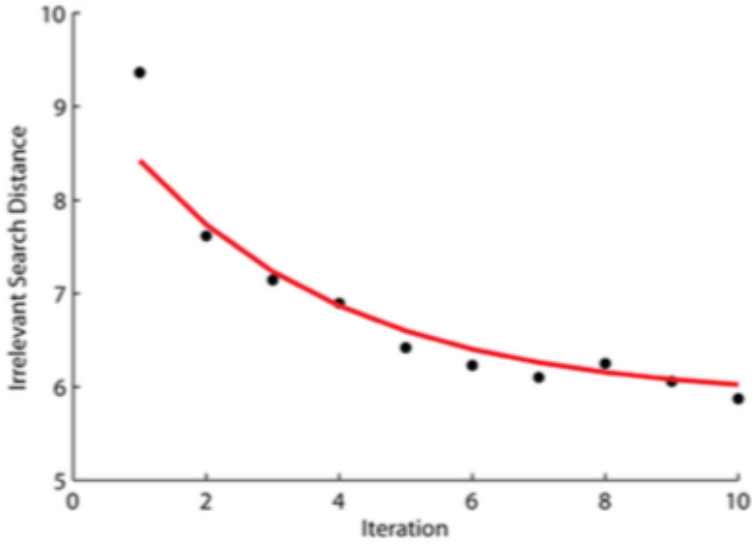


Figure 4.4: Power law of learning: *Exponential plot of performance of a behavioural learning task showing improvement in performance from first trial to last. From* (Stafford *et al.*, 2012)

4.2.3 Data Analysis

Data were analysed using MATLAB (MathWorks, Cambridge) where custom scripts were designed. Time taken to find the target for each of 15 trials were calculated for the ML and CC conditions and mean curves were plotted for each trial. In addition, the difference between ML and CC trials was averaged over each of trials 1-5,6-10 and 11-15 and used as the primary outcome measure of goal-directed control and early motor learning. Comparisons were made in order to examine the following:

- Effect of PD: Difference between PwP and healthy controls

- Effect of hand dominance: Difference between dominant and non-dominant hands (self-reported)
- Effect of severity of parkinsonism: Difference between most-affected side and least affected side (as determined by UPDRS III scores)
- Effect of FOG: Difference between freezers and non-freezers
- Effect of left-side affected parkinsonism: Difference between predominantly left-side affected PwP and right-side affected PwP

The learning curves for these groups were averaged and ML-CC differences were analysed using two-way ANOVAs and follow-up paired or unpaired t-tests as appropriate.

To test the hypothesis that predominantly left-side affected PwP experience a spatial neglect-type syndrome, average time spent in the left and right search space for all trials was calculated for left-side predominant and right-side predominant PwP and subjected to a two-way ANOVA.

4.3 Results

4.3.1 Demographics

The demographic and neurocognitive data for the Parkinson's disease cohort (divided by FOG status, and by right- and left-side predominant disease) is given in Table 4.1 below.

	All PwP	FOG	Non-FOG	RHS-PD	LHS-PD
N	21	7	14	14	7
Age	64.0 (12.5)	65.1 (8.7)	63.4 (9.0)	64.7 (8.8)	63.0 (8.7)
Gender (M:F)	12:9	4:3	8:6	8:5	6:4
H&Y stage	2.33 (0.69)	2.69 (0.95)	2.21 (0.56)	2.23 (0.99)	2.6 (1.51)
Disease Duration (years)	10.1 (8.7)	**15.1 (10.1)***	**6.6 (4.2)***	9.9 (9.1)	11.1 (8.2)
UPDRS	32 (12.9)	**37.1 (12.3)***	**29 (9.1)***	33.1 (12.6)	30.9 (10.4)
MOCA	27 (2.0)	25.2 (3.3)	26.5 (1.5)	26.9 (1.8)	27.3 (1.2)
FAB	16.8 (1.0)	15.8 (2.9)	17.1 (2.5)	16.6 (1.4)	17.0 (0.8)
Levodopa Dose	388.1 (191.4)	**496.6 (291.0)***	**325.8 (208.6)***	366.9 (180.5)	399.0 (280.5)

Table 4.1 Patient Demographics by FOG status and predominant side of disease: *Means shown with standard deviation in parentheses. * indicates statistically significant difference between groups ($p<0.05$)*

The 10 age-matched healthy control participants (6 male) had a mean age of 61.5 +/- 9.7 years.

4.3.2 All PwP v. Healthy controls

The initial analysis performed pooled all trials for PwP and healthy controls (right and left hands). The results of this analysis are shown in Appendix I (Figure A.1 and Table A.1). These show no significant difference between groups. However, the effect of handedness and predominantly-affected side is not taken into account when right and left-hand trials are pooled. Further analyses are given in Appendix I for dominant and non-dominant hands of the control subjects (Figure A.2, Table A.2) and most-affected vs least-affected sides for the PwP group (Figure A.3, Table A.3). This reveals a significant effect of hand dominance over the first 5 trials ($p=0.010$) which would impact the results. There were no significant intrasubject differences between most and least affected side. There was no significant difference in number of discovered targets for either hand. For these reasons, only dominant hand trials were examined for comparison between PwP and healthy controls.

The average time taken to find the target for each group for trials 1-15 (over 3 blocks) is plotted in Figure 4.5. In Table 4.2, the mean difference between CC and ML trials for both groups are shown for each of trials 1-5, 6-10 and 11-15. In the CC trials, the curves for both patients and controls show no difference. When the ML trials are normalised with respect to the CC trials, the PD patients take longer to search for the hidden target initially and also take longer over the first 5 trials ($p=0.007$). This remained significant when the first (exploratory) trial was excluded ($p=0.022$) implying a true deficit in early refinement of goal-directed behaviour in PD patients. After this, however, there is no difference in performance between the two groups.

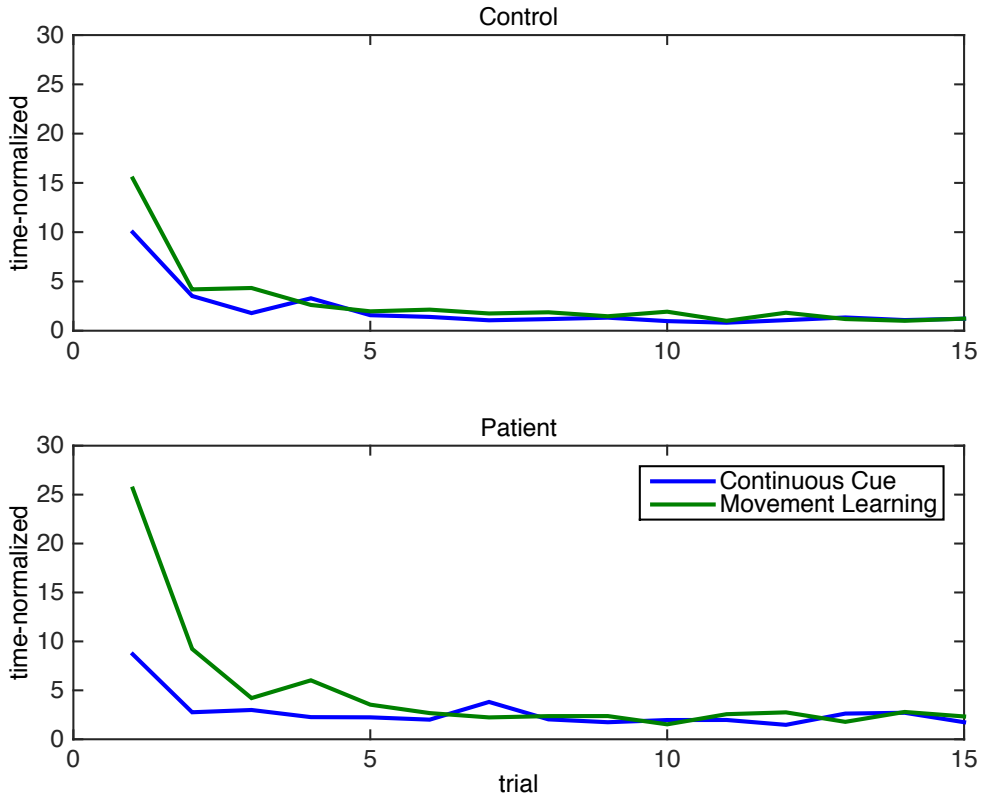


Figure 4.5. ***Motor learning for all PwP (N=21) vs Healthy Controls (N=10):*** *Mean learning curves (time taken to find target in secs vs trial number) of Movement Learning task (green) and Continuous Cue task (blue) for all trials (dominant hands) of healthy controls (upper plot) and PwP (lower plot).*

ML-CC	Controls		PwP		Difference
	Mean	SD	Mean	SD	p-value
Trials 1-5	1.68	11.33	5.94	17.59	**0.007****
Trials 6-10	0.64	2.85	-0.08	7.54	0.258
Trials 11-15	0.14	2.08	0.34	5.90	0.696

Table 4.2. Group differences by trial for all PwP vs Healthy Controls (dominant hands): *Mean and standard deviations of difference (in secs) between movement learning (ML) and continuous cue (CC) task for all trials (dominant hands) of healthy controls and PwP.*

4.3.3 Freezers v. Non-freezers

A similar analysis to that outlined above was performed for freezers and non-freezers and the results shown in Figure 4.6 and Table 4.3 below. As above, only dominant hand trials are included because of the significant effect of non-dominant hand performance in this study. Freezers had a longer disease duration, higher UPDRS scores and were taking higher doses of levodopa than the non-freezing group. Although on inspection of the learning curves, the freezers appear to take longer to find the target on early trials, there is no significant difference between groups on trials 1-5 or 6-10. As is shown in table 4.3, there was a statistically significant group difference in later trials (11-15) (p=0.008). Given the negative difference in means for the freezing group, however, this may be artefactual. It is notable however, that, in these later trials, the freezers find the target in the Movement Learning Condition on average 0.46 secs faster than they do in the Continuous Cue task.

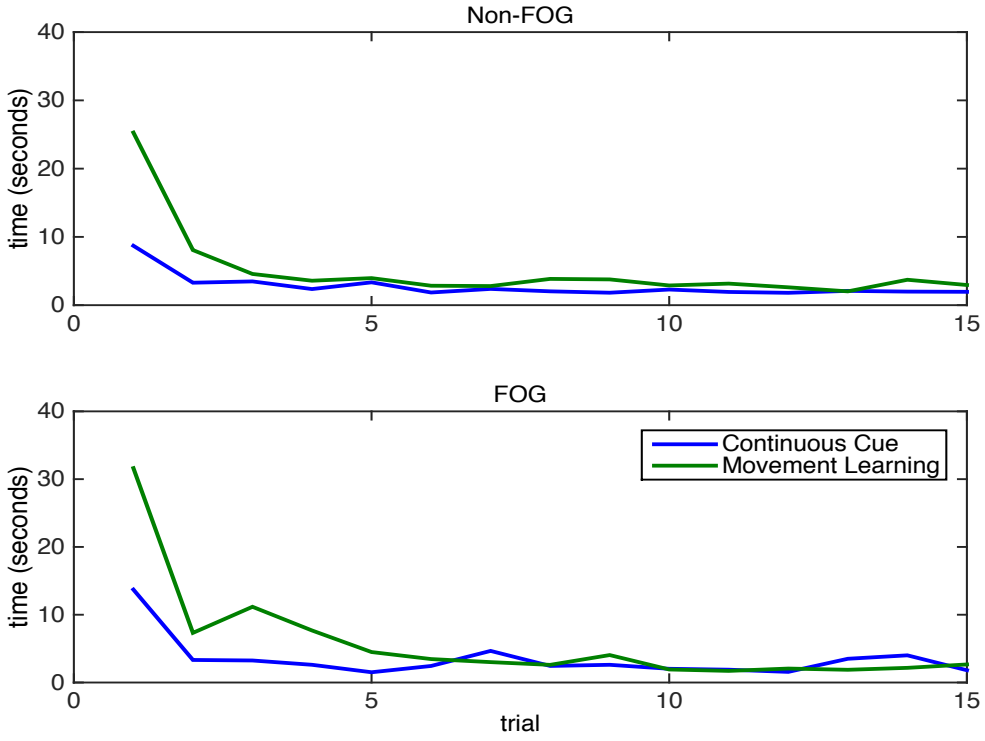


Figure 4.6. Motor learning for all non-freezers (N=14) vs freezers (N=7) (dominant hands): *Mean learning curves (time taken to find target in secs vs trial number) of Movement Learning task (green) and Continuous Cue task (blue) for all trials (dominant hands) of non-freezers (upper plot) and freezers (lower plot).*

ML-CC	*Non-FOG*		*FOG*		*Difference*
	Mean	*SD*	*Mean*	*SD*	*p-value*
Trials 1-5	4.86	18.72	7.58	25.16	0.139
Trials 6-10	1.16	6.18	0.17	10.48	0.151
Trials 11-15	0.94	5.70	**-0.46**	5.90	**0.008****

Table 4.3. Group differences by trial for non-freezers vs freezers (dominant hands): *Mean and standard deviations of difference (in secs) between movement learning (ML) and continuous cue (CC) task for all trials (dominant hands) of non-freezers and freezers.*

Although the sample size is very small, these results imply that refining goal-directed behaviour is ultimately better in freezers than in non-freezers. Superior final performance could be explained by increased exploration during the early trials, however there was no significant difference between groups during the first trial or trials 1-5, making this explanation unlikely.

4.3.4 RHS-affected PwP vs LHS-affected PwP

Given the importance of spatial attention and working memory on goal-directed behaviour and its lateralisation to the right frontoparietal cortex, a similar comparison of right side-affected patients and left-side affected patients was performed (Figure 4.7, Table 4.4). Again, only dominant hand trials were included. There was no difference in baseline clinical or neuropsychological characteristics between groups (Table 4.1). There was a significant difference between groups over the first 5 trials with the LHS-affected patients finding targets more slowly (p=0.046). This was largely driven by an increased exploratory phase during the first trial and, when this trial was removed, there was only a trend towards significance between groups (p=0.073). As the search is refined, there is no difference in performance between groups.

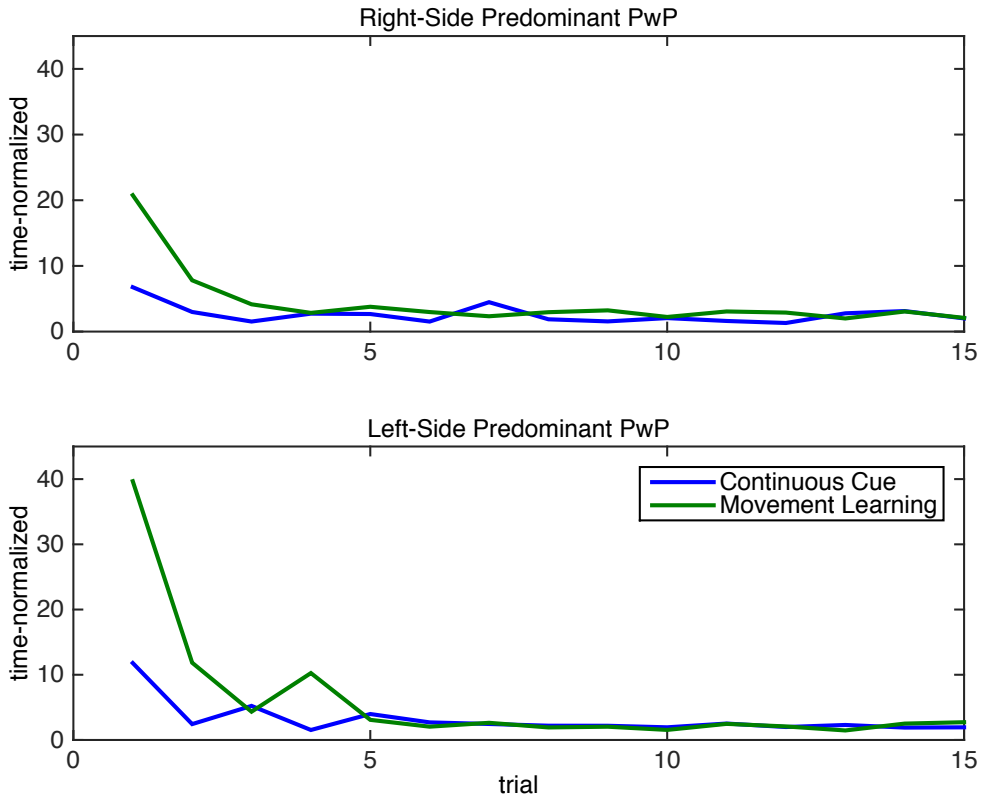


Figure 4.7. ***Motor learning for right-side predominant PwP (N=14) vs left-side predominant PwP (N=7) (dominant hands):*** *Mean learning curves (time taken to find target in secs vs trial number) of Movement Learning task (green) and Continuous Cue task (blue) for all trials (dominant hands) of right-side predominant PwP (upper plot) and left-side predominant PwP (lower plot).*

ML-CC	RHS-PD		LHS-PD		Difference
	Mean	SD	Mean	SD	p-value
Trials 1-5	4.54	13.03	8.85	26.70	**0.046***
Trials 6-10	0.45	8.71	-0.26	3.81	0.383
Trials 11-15	0.45	6.51	0.12	4.14	0.600

Table 4.4. Group differences by trial for right-side predominant PwP (RHS-PD) vs left-side predominant PwP (LHS-PD) (dominant hands): *Mean and standard deviations of difference (in secs) between movement learning (ML) and continuous cue (CC) task for all trials (dominant hands) of right-side predominant PwP and left-side predominant PwP.*

In order to test the hypothesis that a neglect-like syndrome or spatial attention deficit differences exist in those with predominantly left-sided disease, the total time spent searching in the right and left search space for Trial 1 were examined in both groups (Figure 4.8). It is in this initial exploratory phase that such spatial perceptual differences are most likely to be evident. Based on the literature, if a neglect exists in left-side predominant PwP one would expect this group to spend more time on average searching in the right hemispace. The difference between total time spent in right hemispace and left hemispace was calculated over all trials for both groups. Although the right-side predominant PwP spent more total time in the left-hemispace on Trial 1 (right space – left space = -66.88 ± 588.09 secs) and the left-side predominant PwP spent more total time in the right hemispace (right space – left space = 193.61 ± 1036.79 secs), this difference was not statistically significant (p=0.187).

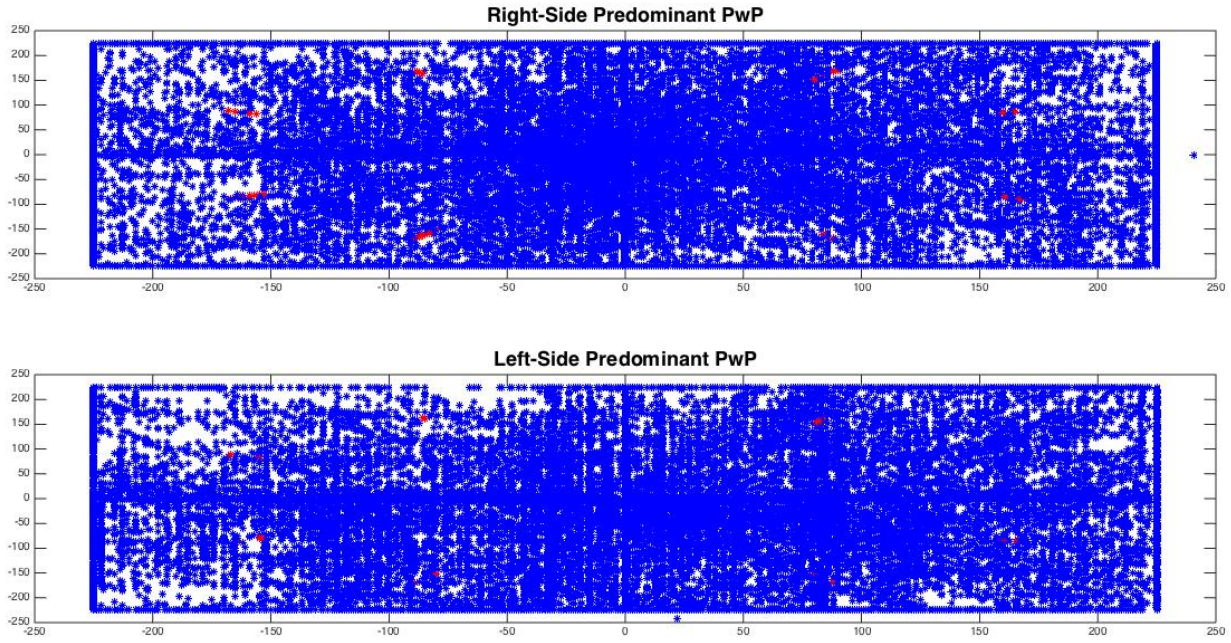


Figure 4.8. ***Sampled trajectories for all trials of right-side predominant PwP (N=14) vs left-side predominant PwP (N=7) (dominant hands):*** *Sampled trajectories (blue) of all trials (dominant hands) superimposed for all right-side predominant PwP (upper plot) and all left-side predominant PwP (lower plot). Locations of the targets for these trials are shown in red.*

4.4 Discussion

4.4.1 All PwP vs Healthy Controls

The primary aim of this study was to design a paradigm to quantitatively examine goal-directed motor control in PwP in a manner that takes into account sensory and perceptual abnormalities seen in this group as well as overall slowness of movement. In spite of bradykinesia, medicated PD patients performed goal-directed behaviour as well as healthy controls, provided no action selection is required by the basal ganglia (CC condition). Once action selection is required in the ML condition, however, early refinement of that goal directed behaviour becomes impaired compared with controls resulting in significant differences when normalized by baseline CC performance ($p=0.007$ for trials 1-5). However, ultimate performance of motor skill acquisition is comparable to controls implying that motor task learning, although slower, approaches that of controls over time. The improved final performance in PwP could be explained as a direct result of the increased early exploration(Mirolli and Baldassarre, 2013). However, the ability to refine goal-directed movement suggests that goal-directed networks remain intact in PwP, even in advanced disease. It has previously been suspected that progressive loss of dopaminergic innervation in the associative areas of the basal ganglia may lead to impairment of goal directed control in late PD. The equivalent final performance on this task suggests this is not the case, at least in the cohort studied herein.

4.4.2 Freezers vs Non-Freezers

A subgroup analysis was performed in the freezing and non-freezing cohort and, although the sample size was small, it showed that, in spite of no significant difference between freezers and non-freezers in exploration and early refinement, the final performance of freezers was similar to non-freezers, suggesting equivalent goal-directed motor control between groups. Note that, although freezers had a longer disease duration, they also had more severe motor impairment, as measured by the UPDRS. This finding validates (to a degree) that this paradigm examines motor learning in a way that is independent of overall motor performance. Importantly, the equivalent performance of freezers in this task suggests that action selection at the level of the basal ganglia is not impaired in FOG and therefore is unlikely to play a causative role the pathophysiology of freezing.

4.4.3 RHS-affected PwP vs LHS-affected PwP

A second subgroup analysis was performed comparing left-side predominant PwP and right-side predominant PwP. This was to assess the hypothesis that patients with left-sided disease display spatial attention and orientation deficits which might contribute to impairments in goal-directed control. There was no difference in performance of LHS-affected PD patients on the CC trial compared with RHS-affected. However, performance on the ML task was significantly worse over the first 5 trials for the LHS-affected group resulting in significant differences when normalized for baseline CC performance ($p=0.046$). Again, this was driven by greater exploration in the first trial (there is no significant difference over trials 2-5) suggesting an impairment in spatial attention or orientation in those with left-sided predominance rather than a true deficit in motor skill acquisition. This is consistent with the literature which has reported a visual neglect-like syndrome in those with left-sided onset, whereas those with right-sided onset generally do not (Ebersbach *et al.*, 1996; A. C. Lee *et al.*, 2001; Villardita *et al.*, 1983; W. G. Wright *et al.*, 2007). No visual stimulus was presented in the ML task so this phenomenon may represent a more extensive neglect syndrome which includes personal egocentric space. Refinement of goal directed movement in a learning fashion is similar in both groups implying intact goal-directed learning. Thus, a perceptual/spatial deficit contributes to the initial impairment in goal directed control seen rather than a primary deficit in motor control or learning. Although there is a suggestion that left-side predominant patients preferentially select movements to their right hemispace and right-side predominant patients select movements to their left, this was not statistically significant.

4.4.4 Impact, Limitations and Future Work

This paradigm allows consistent quantitative measurement of goal-directed movement and early motor skill acquisition, controlling for bradykinesia, and can therefore be used to answer specific questions about motor learning in PD (e.g. effect of medication, deep brain stimulation). The difficulty of the task can be altered by changing the size of the stimulus and the relationship between performance and timing/consistency of reward stimuli can be altered to gain a greater insight into the dynamics of reward and learning in PwP. Since this study examines action selection and behavior which involves the basal ganglia, the task could be used to examine goal-directed learning and reward physiology in other basal ganglia disorders such as Tourette syndrome, obsessive-compulsive disorder and Huntington's disease,

This was a preliminary study and the study sample size is small. In addition, a ceiling of performance was reached quickly by most participants (after 3-4 trials). Future work should be undertaken to make the

task more challenging (for example by making the target smaller, or by creating a delay between finding the target and presentation of the reward stimulus). This may create problems in a patient population where cognitive impairment is common and ultimately limits the utility of such a paradigm. The suggestion above that the outcome measure used in this study (time to find target in ML condition minus time to find target in CC condition) is exclusively a measure of action selection in the basal ganglia is a gross oversimplification. Differences in the relative contributions of cortex, hippocampus and cerebellum between the ML and CC may explain the differing results and warrants further exploration. Future work should examine the neural substrates of motor skill acquisition in PwP and FOG, either by performing this task with synchronous EEG or functional MRI. Finally, combining this task with a task of automatic habitual control would give greater insight into the relative deficiencies of each type of control in PwP and the ability to switch between goal-directed and habitual control in the setting of PD.

One research question from Chapter 2 was whether FOG could be caused by loss of goal-directed control in advanced/severe PD. The executive function deficits seen in FOG further suggest that these networks may be impaired. If loss of dopaminergic innervation to associative areas of the basal ganglia was responsible for the development of FOG, one would expect performance of freezers to be inferior to their non-freezing counterparts. This, however, was not found to be the case. This suggests preserved goal-directed control which would not explain the development of FOG. It is possible that the loss of habitual control from sensorimotor areas (as occurs progressively in PD) could lead to an overreliance on the systems that govern cognitive goal-directed control and this will be explored in the next chapter. The dramatic effect of dual tasking in FOG described in Chapter 1 suggests that overload on cognitive resources may be the primary problem. The current study supports intact associative pathways at the basal ganglia, both in PwP and particularly in freezers who perform comparably in spite of more severe disease. Increased reliance on goal-directed control could lead to an overload on the frontostriatal networks that govern goal-directed control as well as executive function. Chapter 5 will lend further support to this model of excessive activation at a cortical level in response to loss of habitual control in freezers.

4.5 Conclusions

In summary, this study validated a motor learning paradigm on a small cohort of PwP and healthy controls. This paradigm quantitatively assesses goal-directed motor control in PwP in a manner which is independent of sensory and perceptual abnormalities and bradykinesia. Although the initial refinement

of goal-directed movement is impaired in PwP, performance rapidly reaches that of healthy controls. Furthermore, final performance of this task is similar in freezers than in non-freezers which may be explained by a greater reliance on goal-directed control in this subgroup. This has significant implications for rehabilitation in PD and FOG. It is important to note, however, that the paradigm here studies only early refinement of goal-directed tasks rather than longer-term retention of motor tasks which is true motor learning. Further studies would be required to examine retention of learned motor skills which reflects conversion to more habitual control as well as tasks which combine both habitual and goal-directed control.

5. Getting Ready To Freeze: Motor Preparation Rather Than Decision-Making Differentiates Parkinson's Disease Patients With And Without Freezing of Gait

5.1 Introduction

The improvement in goal-directed motor control seen in freezers in Chapter 4 suggests that greater adaptive changes may be occurring in these patients to compensate for loss of habitual control. Freezing is closely associated with both motor deficits (stride time, gait asymmetry and rhythmicity) (Killane *et al.*, 2015) and cognitive impairment, in particular, executive dysfunction (Amboni *et al.*, 2008; Maruyama and Yanagisawa, 2006). The close interaction between cognitive and motor processes in FOG warrants a closer look at these, to greater understand these processes in freezers and non-freezers at a cortical level. Executive function is impaired in freezers compared to non-freezers, with specific deficits in divided attention (Spildooren *et al.*, 2010; Tard *et al.*, 2014), set-shifting (Shine, Naismith, *et al.*, 2013), response inhibition (Cohen *et al.*, 2014) and conflict resolution (Vandenbossche, *et al.*, 2012). Although cognitive dysfunction likely plays a significant role in its pathogenesis, objective quantitative measures of cognitive dysfunction in FOG are lacking. Neuroimaging studies in FOG cannot directly infer cognitive dysfunction and standard neurocognitive batteries remain an insensitive way to assess cognition. As mentioned previously, electroencephalography (EEG) has previously been used to explore freezing as its high temporal resolution allows accurate detection of brief neural responses such as those that likely occur during paroxysms of freezing (Handojoseno *et al.*, 2012; 2013; Shine *et al*., 2014; Singh *et al.*, 2013; Thevathasan *et al.*, 2012; Toledo *et al.*, 2014; Velu *et al.*, 2014). Few EEG-based studies in FOG exist to date (Shine *et al.,* 2014; Singh *et al.*, 2013; Toledo *et al.*, 2014; Velu *et al.*, 2014; Handojoseno *et al.*, 2012; 2013; Thevathasan *et al.*, 2012) but further support electrophysiological abnormalities at both cortical and subcortical levels in freezers. There remains some debate as to whether the cortical activations seen, for example, via BOLD responses or surface EEG in patients with FOG are merely a response to an established freezing episode (originating at a subcortical level) or whether they are involved in a causative manner. No EEG study in FOG has examined decision-making tasks which require motor output.

5.1.1 Cognitive Event-Related Potentials

Event related potentials (ERPs) are EEG surface potentials which are generated following occurrence of a psychophysiological event (usually a sensory stimulus). These low-amplitude signals cannot be readily detected from raw EEG data and therefore, must be averaged over many iterations of the same event. This averaging leads to summation of the stereotyped brain response which is locked in time to the stimulus (or response) with resultant cancellation of random noise. ERPs can reveal the time course of information processes with high temporal resolution. For this reason, they are often used as electrophysiological indices for cognitive function. The P300 (P3b) evoked potential is a particular event related potential associated with higher cognitive function (Figure 5.1). It is usually preceded by the N2 potential (N2b) which is a negative deflection associated with precognitive processing such as discrimination of sensory stimuli but also monitoring and control of motor responses such as response conflict and response inhibition. The "classical" P3b potential is a large-amplitude ERP detected over centroparietal scalp areas which has a positive peak around 300–600 msecs following a task-relevant stimulus (Polich, 2007). More recently (using different analysis methods), the equivalent term "centroparietal positive potential" (CPP) has been used to describe this potential (O'Connell *et al.*, 2012).

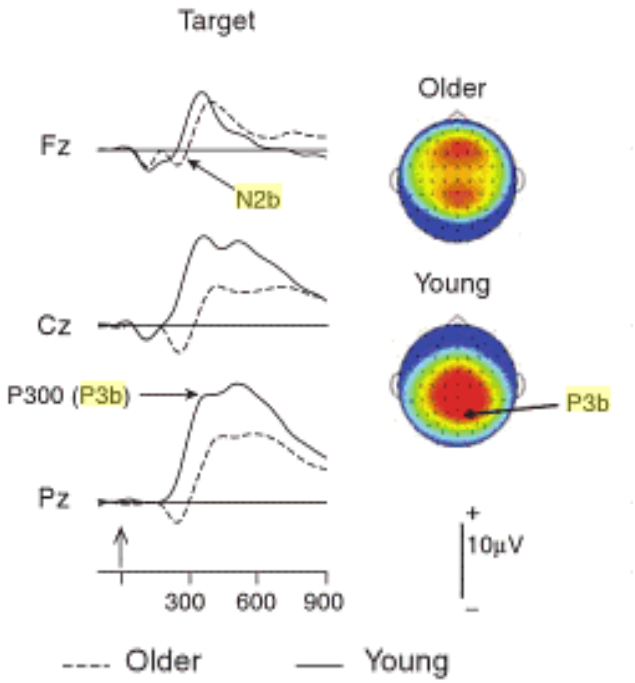


Figure 5.1 P3b potential in young and older subjects. *Event related potentials from an oddball task in young and older participants (shown on left) revealing differences in amplitude and latency of response with age. Topoplots showing the scalp distribution of potential amplitude are shown for both groups on right revealing differences in spatial distribution of response as well as amplitude and latency. From* (Friedman, 2008).

The P3b potential can be generated using a two stimulus oddball task wherein an infrequent target stimulus is presented randomly in a background of a frequent standard stimulus (Figure 5.2) (Polich, 2007). The participant is instructed to respond either mentally or physically to the target stimulus but not to the standard stimulus. Thus, when the participants are presented with a standard stimulus, a characteristic signal is detected which is detectable when a large number of trials are averaged. When the unexpected stimulus (target stimulus) is presented and the participant responds to this, the amplitude of the response is significantly greater (see figure 5.2). Any deficit in attentional/cognitive processing in these participants would lead to either attenuation or increased latency of the P3b or both.

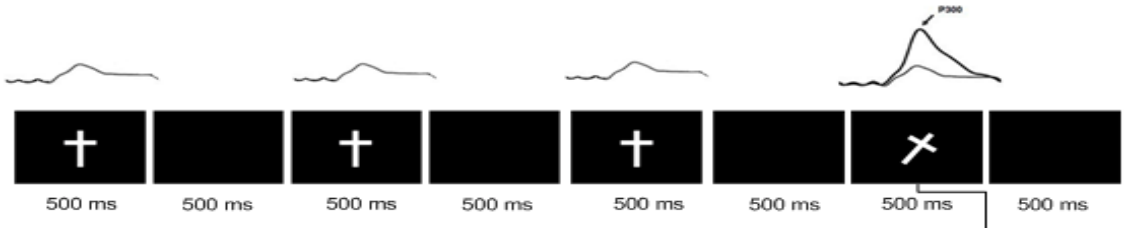


Figure 5.2 Generation of P3b from two-stimulus oddball task. *The presentation of a standard stimulus (upright cross) generates a small amplitude response detectable with averaged scalp responses (shown above stimulus). The unexpected target stimulus which requires a response generates a much larger amplitude signal (which also requires averaging to be detected).*

The precise neural substrates of the P3b/CPP are still not understood but P3b abnormalities correlate with markers of executive dysfunction (Kindermann *et al.*, 2001), response conflict and response inhibition (Groom and Cragg, 2015), all of which have been proposed to have a central role in FOG (Cohen *et al.*, 2014) (Vandenbossche, Deroost, *et al.*, 2012). Recently, the P3b has also been shown to be involved in decision making in response to sensory stimuli (Twomey *et al.*, 2015). Visual and auditory P3b studies in Parkinson's disease have shown that the latency is associated with disease severity (Silva Lopes *et al.*, 2014), cognitive dysfunction (Goodin and Aminoff, 1987; Katsarou *et al.*, 2004; Matsui *et al.*, 2007; O'Donnell *et al.*, 1987; Toda *et al.*, 1993) and impaired activities of daily living (Maeshima *et al.*, 2002). This latency reduces with dopaminergic therapy in newly diagnosed patients after 15 days on treatment but subsequently increases (Prabhakar *et al.*, 2000) and correlates with bilateral temporal cerebral blood flow on SPECT (Wang *et al.*, 2000). Reduced P3b amplitude along with delayed N2 have previously been shown during a visual oddball paradigm in PD patients suggesting that automatic information processing is impaired in PwP (Wang, Kuroiwa and Kamitani, 1999; Wang, Kuroiwa,

Kamitani, *et al.*, 1999). Furthermore, Bodis-Wollner et al. showed that a number of cognitive measures such as verbal fluency, memory, visual spatial perception, and abstract reasoning correlate with P3b and N2 abnormalities in response to visual and auditory oddball tasks in Parkinson's patients (Bodis-Wollner *et al.*, 1995). A table summarising the literature on ERP studies in PwP along with their main findings is given in Appendix II. The P3b potential has not been studied in FOG to date. Given the close relationship between cognitive dysfunction and FOG (especially deficits in executive function and attention), one expects that examination of P3b responses in freezers and non-freezers would reveal significant differences.

5.1.2 Movement-Related Cortical Potentials

In addition to the assessment of cognitive markers, ERP analysis can be used to study the electrical correlates of motor preparation. The readiness potential (also known as the bereitschaftpotential) is a movement-related cortical potential which precedes voluntary or goal-directed movement and reflects activity in the motor cortex, premotor area (PMA) and supplementary motor area (SMA) contralateral to the side of the body in which the movement occurs (Shibasaki and Hallett, 2006). This negative potential has to build to a certain threshold before movement or EMG activity is triggered (Figure 5.3). The negative potential begins as early as 1-2 seconds prior to generation of muscle activity but the greatest negative amplitude is seen in the last few hundred milliseconds prior to movement. This negative wave is thought to reflect activation of the motor cortex contralateral to the goal-directed intended response. Simultaneously, a positive potential occurs ipsilateral to the side of the intended goal-directed response. It is believed that this represents inhibition of unwanted responses in from the other cerebral cortex (van Wouwe *et al.*, 2014)..

Early readiness potentials for self-initiated (but not externally triggered) movements are attenuated in PwP and correlate with reduced regional blood flow in the SMA (Jahanshahi *et al.*, 1995). This SMA dysfunction may be compensated for by activation of lateral premotor areas (Cunnington *et al.*, 1995). As a result, there is no difference in amplitude of later component of the readiness potential (seen over contralateral frontocentral areas) (Dick *et al.*, 1989). Dysfunction of the SMA has previously been proposed to be central to the pathophysiology of FOG (Nutt *et al.*, 2011), however there has not been a study of readiness potentials in FOG previously. Freezing is characterized not only by the arrest of movement but also by the intention to move (Nieuwboer and Giladi, 2013). For this reason, it was hypothesized that the mechanisms of motor initiation in freezers and non-freezers will also be different.

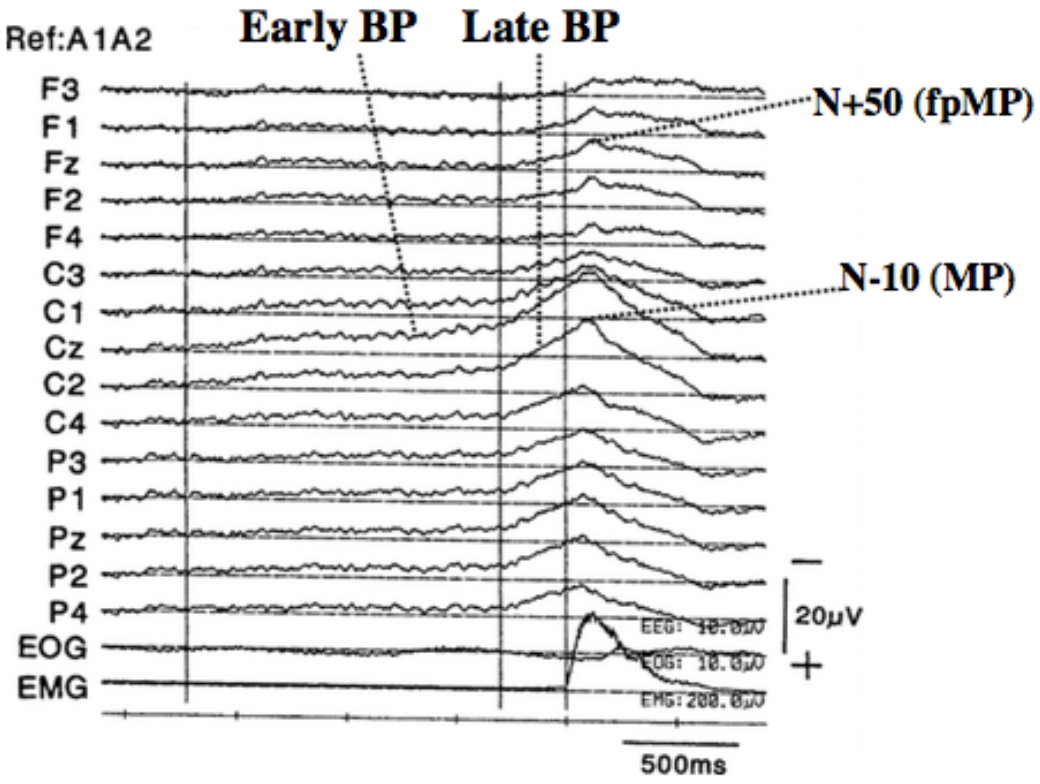


Figure 5.3. Readiness Potentials: *From* (Shibasaki and Hallett, 2006) *Movement-Related Cortical Potentials (Bereitschaft potentials, BP) related to self-initiated left wrist extension. The initial negative potential (early BP) starts 1.7 s before the onset of EMG activity maximal at the midline central electrode (Cz,) symmetric across both hemispheres. A later larger negative potential (late BP) starts 300 ms before EMG activity over the right frontocentral region (contralateral to the side of movement).*

Even simple motor tasks require both cognitive decision-making processes and motor preparation. Freezing is associated with both cognitive and motor deficits. An EEG-based analysis (employing a two-stimulus oddball task) was, therefore, performed in freezers and non-freezers in order to simultaneously analyse cognitive ERPs and motor readiness potentials to deduce whether impairments in cognitive processing or motor initiation (or both) differentiates freezers and non-freezers. In order to separate the motor preparation and decision making signals, a spatial filter known as the current source density (CSD) was used to increase the spatial resolution of the data. This has previously been shown to allow separation of these two signals in healthy participants (Kelly and O'Connell, 2013). These methods are described in detail below and the results lead to important insights into the cautious interpretation of ERP analysis in PwP.

The effects of dual-tasking on these processes is of particular interest in FOG. Therefore, participants performed the two-stimulus oddball task both sitting and stepping-in-place, to examine cognitive and motor potentials during a single and dual task conditions. This chapter will present the analysis of these cortical processes while sitting and Chapter 6 will present a similar analysis while stepping in place.

5.2 Methods

5.2.1 Participant Recruitment

Twenty patients with Idiopathic Parkinson's Disease (as defined by the UK Brain Bank Criteria (Hughes *et al.*, 1992), Hoehn and Yahr stage II-III) were recruited from the Movement Disorder Clinic in the Dublin Neurological Institute at the Mater Misericordiae University Hospital. Ethical approval was granted from the hospital ethics committee and informed consent was obtained from all participants. Each participant underwent clinical and neuropsychological testing with a neurologist including the New Freezing of Gait Questionnaire (NFOG-Q) Unified Parkinson's Disease Rating Scale III (UPDRS III), Montreal Cognitive Assessment (MoCA), Frontal Assessment Battery (FAB), Beck Depression Inventory II (BDI II). All participants were examined and tested in the "on" state. They patients were classified as "freezers" or "non-freezers" based on Question 1 of the New Freezing of Gait Questionnaire (Nieuwboer *et al.*, 2008):

1. **Did you experience "freezing episodes" over the past month?**

Freezing is the feeling that your feet are transiently glued to the floor while trying to initiate walking, making a turn or when walking through narrow spaces or in crowded places? Sometimes it can be accompanied with trembling of the legs and small shuffling steps.

0. I have not experienced such a feeling or episode over the past month
1. I have experienced such a feeling or episode over the past month

Patients who scored 1 on this question were classified as freezers (FOG). All others were classified as non-freezers (non-FOG). Participants were excluded if they a traumatic brain injury (or other neurological condition apart from idiopathic Parkinson's disease) or if they scored below 23/30 on the MoCA. All participants had normal corrected vision and were tested in the "on"-state.

For comparison, ten young healthy control participants were also recruited to validate the paradigm and protocol.

5.2.2 Experimental Test Setup

Participants were seated comfortably and performed a two-stimulus oddball task consisting of a flashing green cross presented randomly on a 42" LCD monitor. This visual stimulus consisted of either vertical (standard) or 45° rotated (target) green crosses presented for 500 msecs on a complex background (Figure 5.4). The complex background consisted of a movement through virtual reality corridor which consisted of alternating narrow doorways and corridors (which frequently trigger FOG in real environments). The purpose of the virtual environment was to create realistic visual flow for the stepping-in-place task in Chapter 6, providing an ecological environment consisting of complex sensory information which may contribute to freezing in real-life situations. The virtual environment was also presented during the sitting task so that the visual information presented during the sitting and stepping conditions was similar allowing direct comparison. The corridor design is presented in greater detail in the Chapter 7.

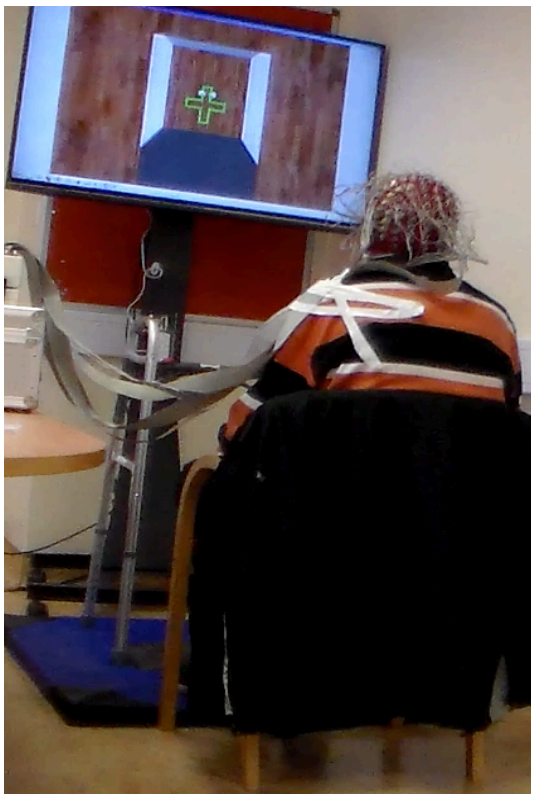


Figure 5.4. Experimental setup for two-stimulus oddball task: *Participant seated comfortably in front of LCD monitor with 128-channel EEG attached. Two-stimulus oddball task is presented superimposed on a complex background.*

This standard stimulus was presented with probability 0.8 in the upright position ('+') and the participant was instructed not to respond to this stimulus. For the remaining 20% of the time, the stimulus was rotated 45° (target stimulus, 'x') and participants were instructed to press a button on a remote control (Nintendo, Japan) as soon as the target stimulus was seen (Figure 5.5). Participants were instructed to focus their attention on the decision-making oddball task (rather then on the virtual environment) to optimise performance and were instructed to minimize head movements and during the trial. The standard and target stimuli were presented for 500 msecs randomly with a random interstimulus interval between 250 and 750 msecs. A single trial of 300 seconds was performed for each participant.

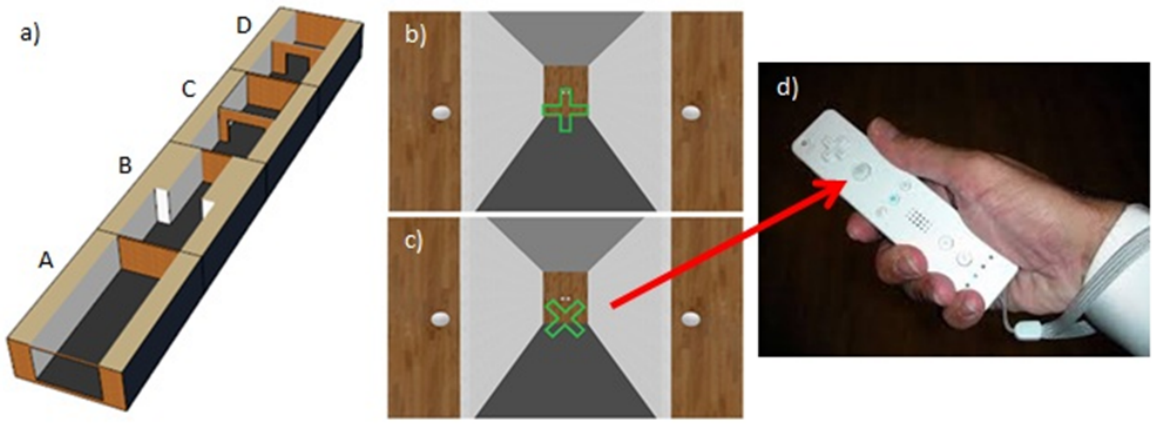
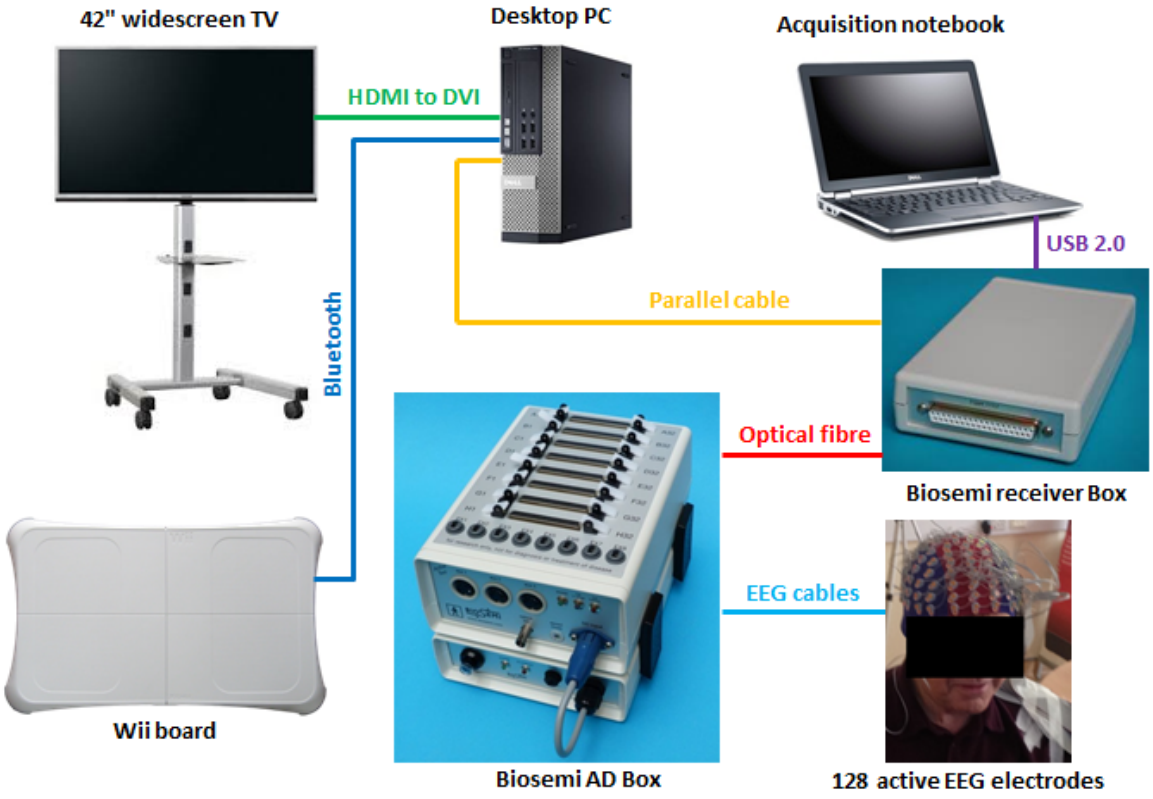
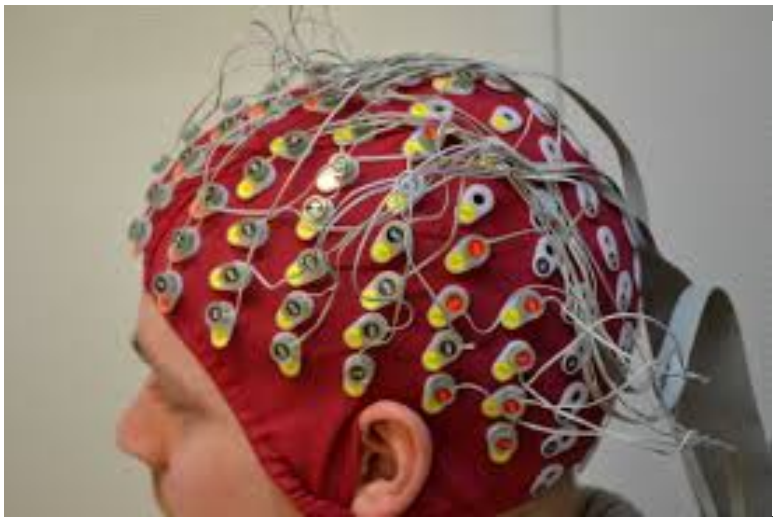


Figure 5.5 Two-Stimulus Oddball Task: *a) Virtual reality corridor with alternating narrow and wide corridors and doorways A-D; b) standard stimulus of visual oddball task superimposed on virtual reality corridor; c) target stimulus of visual oddball task superimposed on virtual reality corridor and d) Wii remote control used for detecting targets by pressing button "A".*

Synchronous EEG data were recorded in all subjects using a 128-channel ActiveTwo BioSemi EEG acquisition system (Fig 5.6). Active electrodes attached to a specialized cap were used to obtain continuous EEG signals from the subjects during the task. The caps were individually selected for best fit and the button holes were filled with an electrolyte gel (Signa Gel; Parker Laboratories, Inc.; Fairfield, NJ) to increase conductivity. Electrodes were placed using a "10-20" arrangement and amplified at source by the internal pre-amplifier. During the experiments, channel offsets were monitored to ensure a value less than 20mV. EEG data were recorded and digitized at a rate of 2048 Hz by the Biosemi analogue-to-digital converter with an open pass-band from DC to 150 Hz. During the experiment, the virtual reality

software was controlled via a desktop PC. The desktop PC sent triggers (to indicate when oddball paradigm stimuli were presented and when button presses occurred) to the receiver box via a parallel cable. The EEG data and triggers were then visualised with Actiview (Biosemi) software on a separate notebook. The details of the specific hardware connections are also shown in Fig 5.6.





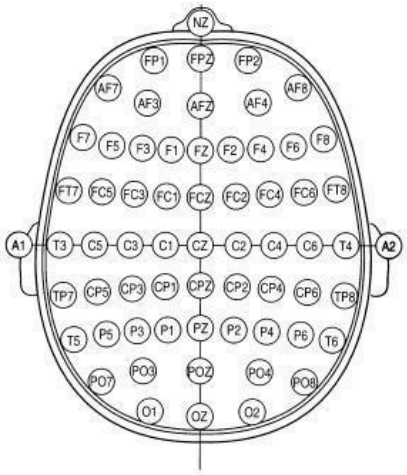


Figure 5.6 Hardware setup for EEG task: *(top) Hardware connections of measurement equipment (Wii board employed for stepping-in-place task only); (bottom, left) Connection of electrodes to scalp via cap; (bottom, right) The "10-20" system for electrode placement. Location of electrodes in the scalp for EEG recording of cortical activity of subjects. "10-20" refers to the electrode spacing, adjacent electrodes are either 10% or 20% of total front-back or right-left distance of the skull. From* (Nuwer *et al.*, 1999).

5.2.3 Data Analysis

5.2.3.1 Behavioural Data

Button press responses were acquired during the recording of the EEG and were processed offline using MATLAB (Mathworks, Natick, MA). Reaction times and number of correct responses to the two-stimulus oddball task were calculated as follows: Timings of button presses in response to stimulus were compared with automatically-generated triggers corresponding to the standard and target stimuli. From this data, reaction times and hit rates were calculated. Reaction time (RT) means and standard deviations were calculated for each participant. Only trials with reaction times falling within 200ms and 1000ms of target presentation were considered valid. Given that the data were collected from a clinical population with a hypokinetic movement disorder, significant inter- and intra-subject variability in reaction time was expected. The data were submitted to an unpaired t-test to assess group reaction time differences.

5.2.3.2 EEG Data

Using custom MATLAB scripts, the continuous data were downsampled to 512Hz (allowing for a more manageable file sizes for the frequencies of interest) and band-pass filtered offline between 0.1 and 30Hz (6 dB/octave). An additional notch filter was employed at 50 Hz for line noise removal. The filtered data was epoched to both standard and target stimuli as well as to button press responses. This allowed examination of both stimulus-locked and response-locked ERPs. Stimulus-locked epochs were taken from 200 msecs pre-stimulus to 800 msecs post-stimulus. Response-locked epochs were taken from 500 ms pre-response to 400 ms post-response. An automatic artifact rejection criterion of ±100μV was applied across all electrodes in the array, and channels with a standard deviation of <0.5μV were rejected. Trials with more than 5 artifact channels were rejected. In trials with less than 5 such channels, any remaining bad channels were interpolated using the nearest neighbor spline (Perrin *et al.*, 1989). The epochs were baseline corrected with respect to 200ms pre-stimulus period. Average responses were calculated for each participant and for each group to assess for the presence of between-group differences in amplitude of the components time-locked to the stimulus and to the response, separately.

The ERP data were also converted to current source density (CSD) to increase spatial selectivity and minimize volume conduction (Kayser and Tenke, 2006). This transformation performs a spatial high pass filter on the ERP data, reducing interference from remote sources and current diffusions through the skull. This step was introduced improve spatial resolution in order to better discriminate between frontocentral motor preparation signals (readiness potentials) and centroparietal decision-making signals (P3b). Only one CSD-based study has been performed in a PD cohort to date (van Wouwe *et al.*, 2014) and no study in FOG has utilized a CSD approach. Separate plots were generated for responses to the target stimulus, the standard stimulus and to the button press response for each group. The following regions of interest were considered:

1. To assess for differences in sensory processing of the stimulus, sensory responses were evaluated from the occipital electrodes (O_1, O_z, O_2; Figure 5.6).
2. To investigate the decision-making variable, activity over central parietal (CPz) area was chosen to represent the P3/CPP component.
3. To investigate unimanual motor preparation, a lateralised readiness potential (LRP) was calculated by subtracting the activity over the left frontocentral (FC4) area from the right frontocentral (FC3) area.

Given the dense recording montage for the planned comparisons and figures, each site of interest is represented by an average of the three nearest electrodes. This served to increase the signal-to-noise ratio. Average CSD responses were calculated for each participant and for each group in a similar manner to the ERP data.

For the stimulus-locked conditions, the average peak amplitude was encapsulated by a 200 msec time window around the mean group reaction time of 554ms. Group-related differences in the P3/CPP mean amplitude (suggesting group differences in decision making) were statistically assessed by two-way repeated measures ANOVA with factors of group (freezer, non-freezer) and condition (Target and Standard). Greenhouse–Geisser corrections were applied when appropriate. Group-related differences in the LRP amplitude were statistically assessed by pointwise unpaired t-tests. To test for differences in the LRP onset between groups (suggesting group differences in motor preparation) unpaired t-tests were conducted at each time point. To control for Type I errors, a period of statistical significance was only considered if an alpha criterion of 0.05 or less was obtained for at least 11 consecutive time points.

5.2.3.3 Regression Analysis

PwP progressively lose automatic (habitual) control of movement. This can be compensated for by recruiting frontal networks leading to an over-reliance on goal-directed motor control. It has previously been suggested that the apparent executive function deficits seen in PwP could be due to overloading these frontal networks in the setting of loss of automatic motor control (Redgrave *et al.*, 2010). To explore the relationship between the electrophysiological marker of motor preparation (the LRP) and the Frontal Executive Battery score, a regression analysis was performed on the PD group. An important confounder in many PD studies is disease duration. Therefore, the multiple linear regression was calculated to predict the LRP amplitude based on the patients total FAB score and years with symptoms.

5.3 Results

5.3.1 Demographics

The demographic and neurocognitive data for the PD cohort (divided by FOG status) is given in Table 5.1 below.

	Freezers	Non-freezers
N	10	10
Age (years)	65.3 (7.6)	62.5 (7.9)
Gender (M:F)	8:2	4:6
H&Y stage (median)	2.6 (0.37)	2.3 (0.35)
Disease Duration (years)*	13.5 (9.1)	7.0 (3.6)
UPDRS	28.3 (9.7)	29.1 (14)
MOCA	24.3 (2.9)	26.1 (2.9)
FAB*	15.2 (2.6)	17.3 (1.3)

***Table 5.1. Patient Demographics for sitting event related potential task by FOG status**. Means shown with standard deviation in parentheses. * indicates statistically significant difference between groups. H&Y stage = Modified Hoehn & Yahr stage; UPDRS III = Unified Parkinson's Disease Rating Scale III total; MOCA = Montreal Cognitive Assessment total; FAB = Frontal Assessment Battery total.*

There was no significant difference between groups in age, sex, Hoehn and Yahr stage, UPDRS III, Montreal cognitive assessment between freezers and non-freezers. Participants with FOG had significantly longer disease duration and Frontal Assessment Battery Scores.

5.3.2 Behavioural Data

There was no significant difference in mean reaction times between the non-freezers (M= 546.0, SD=72.95) and the freezers (M= 562.2, SD=57.02) conditions; ($t(18)=-0.5527$, $p = 0.58760$). Similarly, there was no significant difference in the standard deviation of reaction times for non-freezers (M=84.1, SD=28.6) and freezers (M=86.4, SD=24.53) conditions; ($t(18)=-0.1967$, $p = 0.84$).

5.3.3 EEG Data

5.3.3.1 Sensory Responses

The visual evoked potential for each group, calculated from left (O_1), central (O_z) and right (O_2) occipital electrodes are shown in Figure 5.7 below. The plots suggest a slight delay in sensory responses in the freezing group, particularly to the standard stimulus. This was not statistically significant, however.

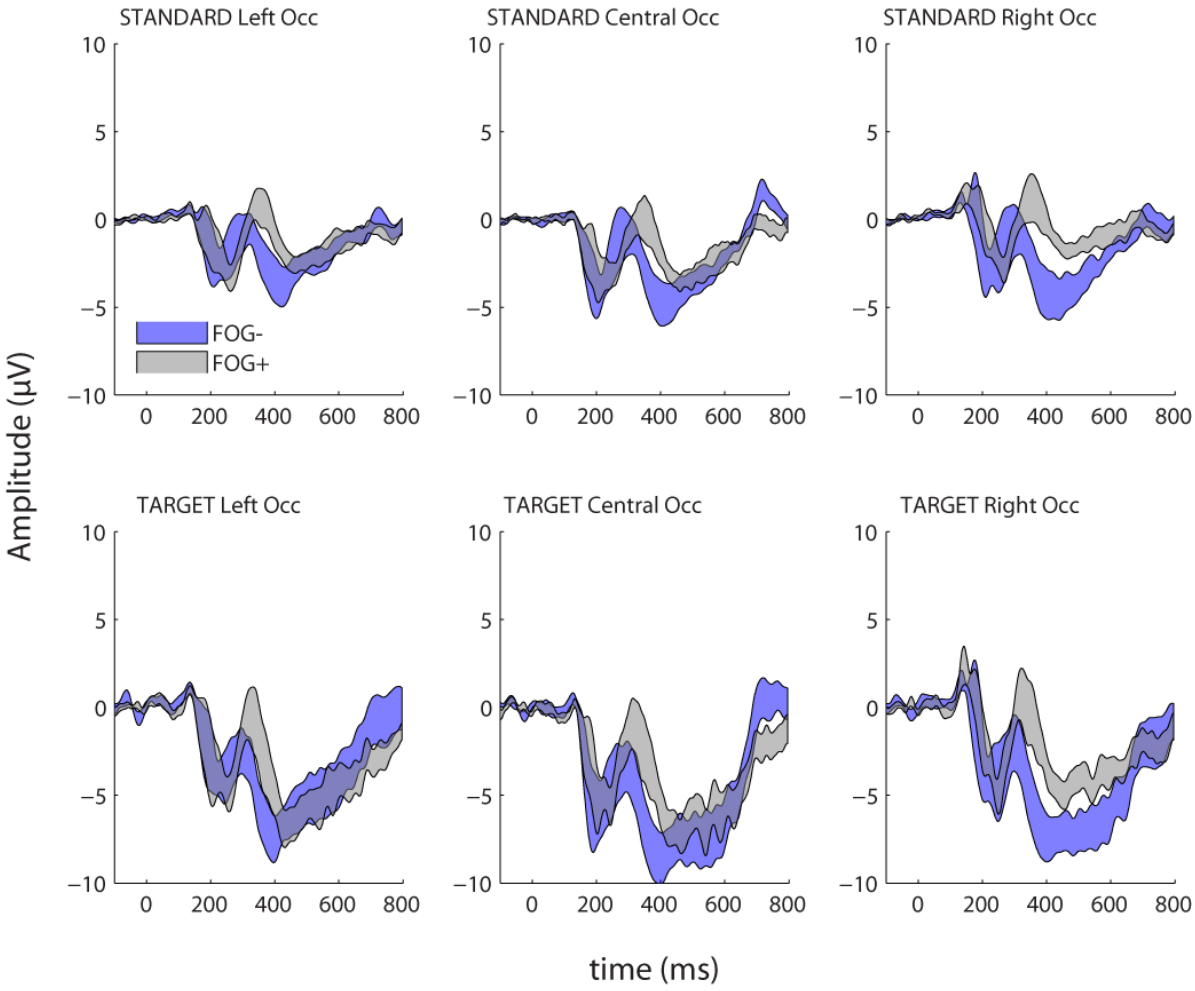


Figure 5.7 Visual Evoked Potentials. *Event related potentials over occipital region of interest to standard (upper plots) and target (lower plots) stimuli. Electrodes from left (O_1), central (O_z) and right (O_2) occipital electrodes (O_1, O_z, O_2) are shown.*

5.3.3.2 Cognitive Decision Making

Initially, an P3b analysis of the ERP data was undertaken. Figure 5.8 shows the mean and standard error of the mean (SEM) of the standard (green) and target (red) ERP response for both the freezing and non-freezing groups for electrodes over the central parietal scalp. To assess difference in the amplitude of the P3b response the mean amplitude responses were submitted to a mixed repeated measures with the factors group (non-freezer, freezer) and condition (target, standard). The analysis revealed a main effect of condition ($F(1,18)=38.565$, $p<0.001$) with no effect of group ($p=0.526$) but a trending interaction between group and condition ($F(1,18)=3.892$, $p=0.06$). The topoplots in Figure 5.8 show a clear difference in scalp distribution of energy for this signal between groups. Non-freezers have a well

defined signal focus over centroparietal area as is often seen in healthy subjects. The energy in freezers is more diffuse, with spread towards the right frontal area. This raises the possibility that the energy seen is a combination of two separate sources (a centroparietal P3b and a further signal in the right frontal area). The more spatially refined CSD approach below explores this further.

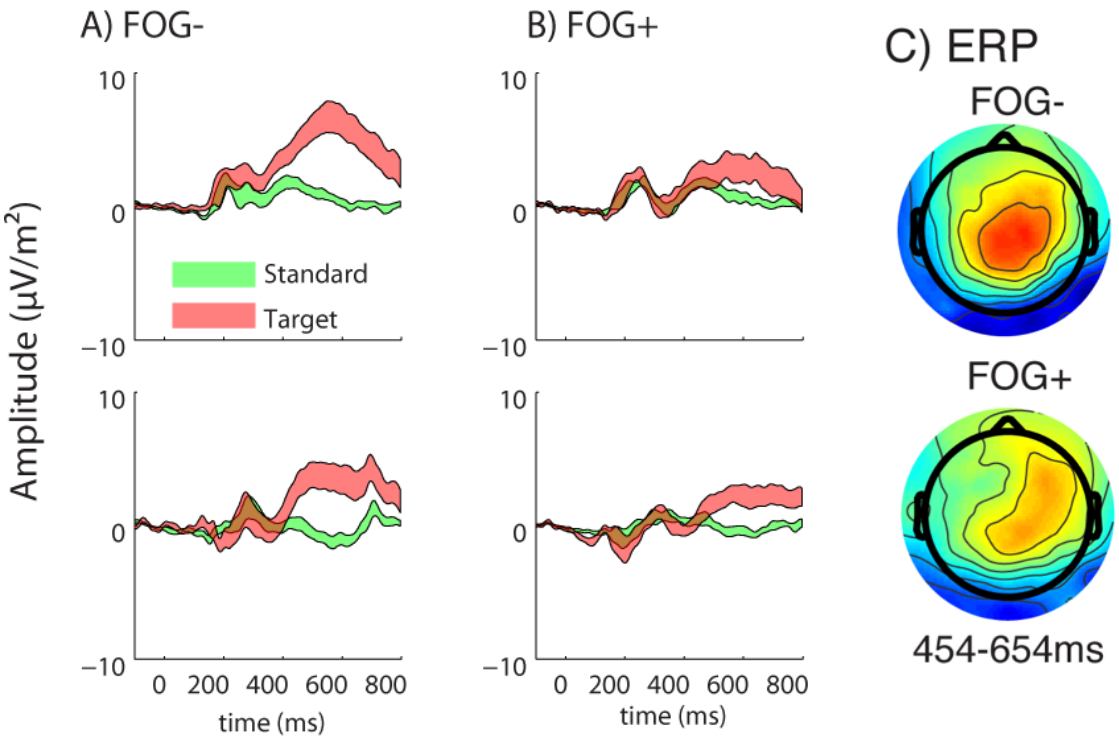


Figure 5.8. ERP Analysis for Decision Making. *The mean and standard error of the mean of the standard (green) and target (red) response over central (upper plots) and centroparietal (lower plots) scalp for A) the non-freezing (FOG-) group and B) the freezing (FOG+) group. C) The mean ERP scalp distribution for the non-freezing (top) group and the freezing (bottom) group) between 454 and 654 msecs*

The difference between target and standard responses for non-freezers (blue) and freezer (grey) are also shown in Figure 5.9 which shows the significant difference between groups described above. The black line shows the stimulus onset, the dashed vertical lines indicate the mean response time for the freezing (grey) group and non-freezing (blue) group.

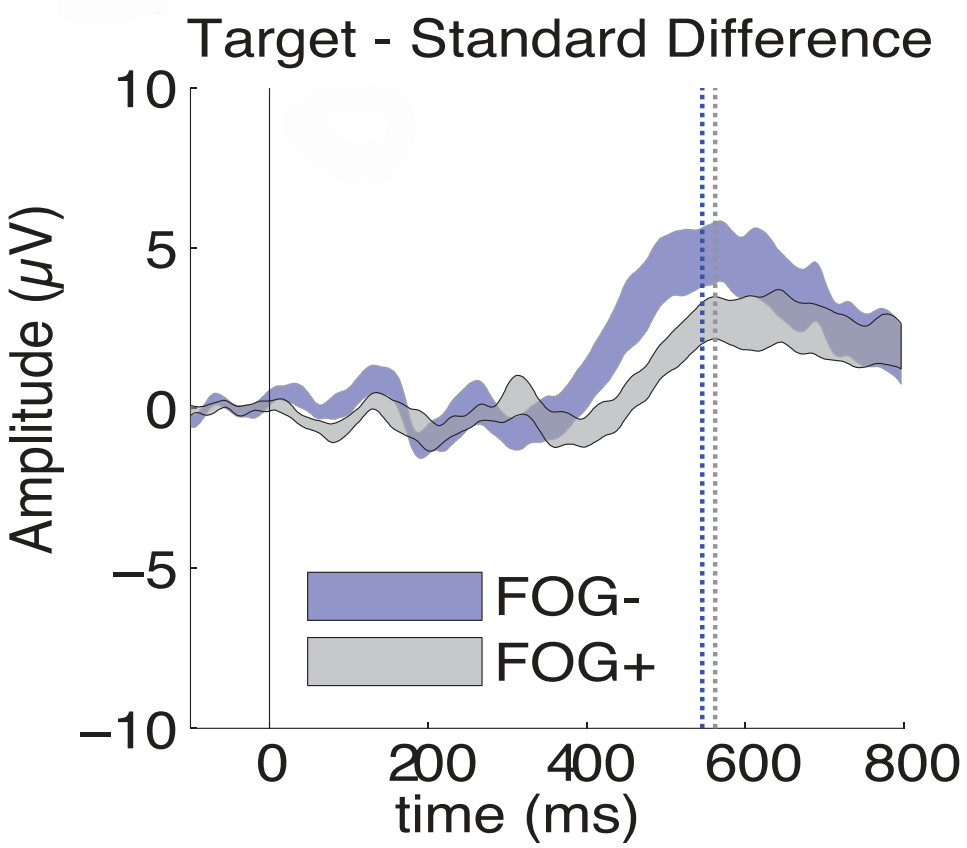


Figure 5.9. Group comparison of ERP response (Target-Standard): *ERP analysis showing the difference between target and standard responses for non-freezers (FOG-, blue) and freezers (FOG+, grey). Note apparent significant difference between groups. The solid black line indicates the stimulus onset, the dashed vertical lines indicate the mean response time for the FOG- (blue) group and FOG+ (grey) group.*

The topoplots for the ERP analysis above motivated re-analysis of the data using a CSD approach as outlined above. Figure 5.10 shows the mean and standard error of the mean (SEM) of the standard (green) and target (red) CSD response for both the freezing and non-freezing groups for electrodes over the central parietal scalp. To assess difference in the amplitude of the CPP (CSD-transformed equivalent of P3b) response the mean amplitude responses were submitted to a mixed repeated measures with the factors group (non-freezer, freezer) and condition (target, standard) The analysis revealed a main effect of condition ($F(1,18)=34.332$, $p<0.001$) with no effect of group ($p=0.55$) or interaction of group and condition ($p=0.486$) . The associated topoplots show that the non-freezing group maintain a localised response over centroparietal areas. The response freezing group on the other hand consists of two separate signal foci (as suspected from the ERP topoplots above). A centroparietal response, similar to

the non-freezers but also positive activity in the right frontal area (and negative activity in the left frontal area) which is distinct from this centroparietal positivity.

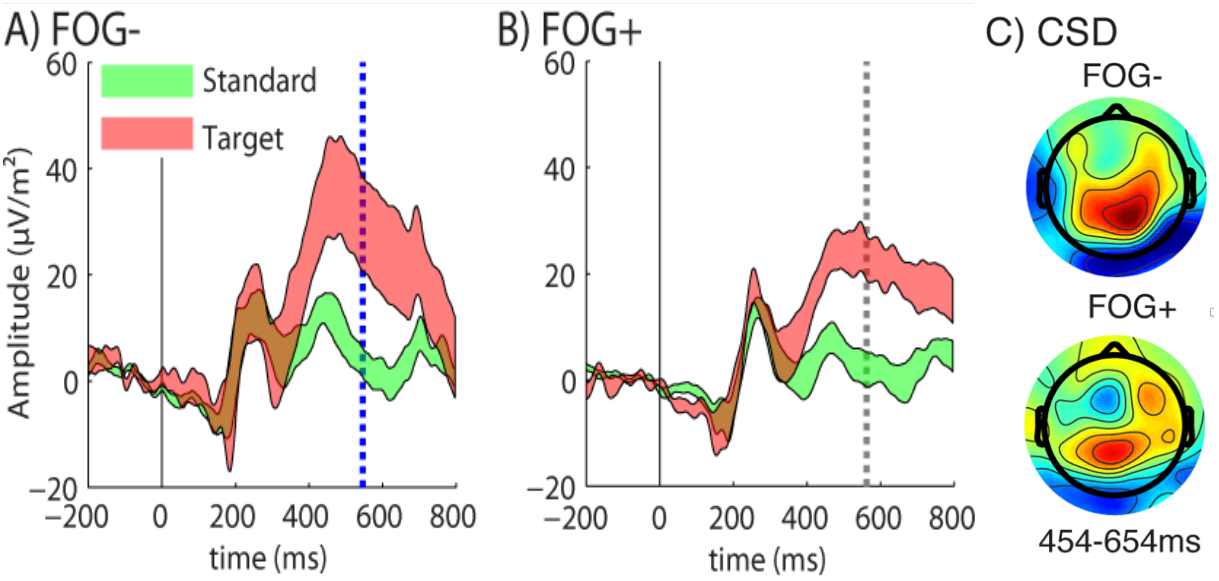


Figure 5.10 CSD analysis of decision making: *The mean and standard error of the mean of the standard (green) and target (red) response over central parietal scalp for the A. the non-freezing (FOG-) group and B. the freezing (FOG+) group.* ***The mean CSD scalp distribution for the non-freezer group (top) and freezer group (bottom) between 454 and 654 msecs.***

The difference between target and standard responses for non-freezers (blue) and freezer (grey) are also shown in Figure 5.11 which shows no significant difference between groups. The difference seen in the ERP analysis is probably due to interference from the frontal potentials. This interference is resolved using the CSD approach.

Individual participant waveforms using the ERP and CSD approaches are shown Appendix III.

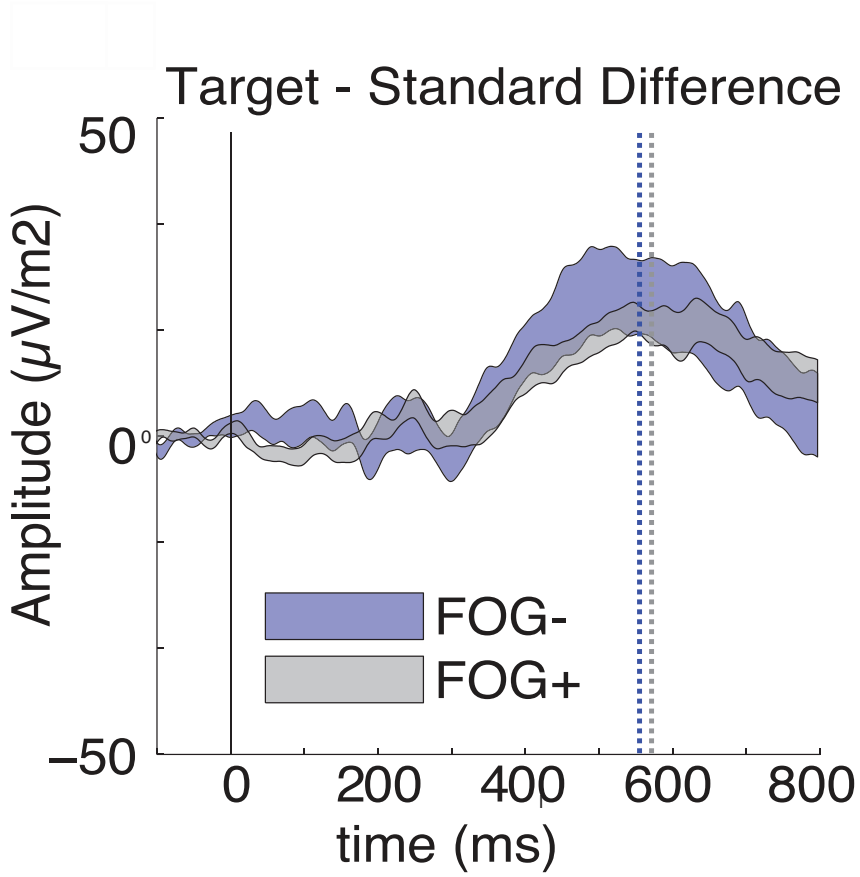


Figure 5.11. Group comparison of CSD response (Target-Standard): *CSD analysis showing the difference between target and standard responses for FOG- (blue) and FOG+ (grey). Note lack of significant difference between groups.*

5.3.3.3 Motor Preparation

To investigate motor preparation differences between the groups the LRP was calculated. Figure 5.12A shows the LRP CSD waveforms, the subtraction of the target response of left and right frontocentral areas, for the non-freezing group (FOG-, blue) and the freezing group (FOG+, grey). The LRP for each time point was submitted to a running unpaired t-test which showed significant amplitude differences between freezers (FOG+) and non freezers (FOG-) ($t(18)=2.388$; $p<0.05$). Time points of statistical differences in the LRP between the freezing and non-freezing groups are depicted as markers running along the bottom of the plot. The group differences onset just after 350msecs prior to the mean response time (indicated by the dashed vertical lines) and continue until just before the mean response time.

Figure 5.12B shows the CSD scalp distribution of the target response centered at 554ms over 200ms for the non-freezing (FOG-) group (top) and freezing (FOG+) group (bottom). The distributions show clear positive peaks over central parietal scalp for both groups consistent with the CPP response. Over frontal sites there were also left-right lateralized differences consistent with a lateralized readiness potential which was more prominent in the freezing group than the non-freezing group.

Figure 5.12C shows the ERP scalp distribution for the target response over 200ms centered at 554ms for the non-freezing group (top) and freezing group (bottom). The distributions show clear positive peaks over central scalp consistent with the P3b response which was more prominent in the non-freezing group than the freezing group. Importantly, the frontal lateralized differences are obscured by the diffuse nature of the signal with this method due to its lower spatial resolution.

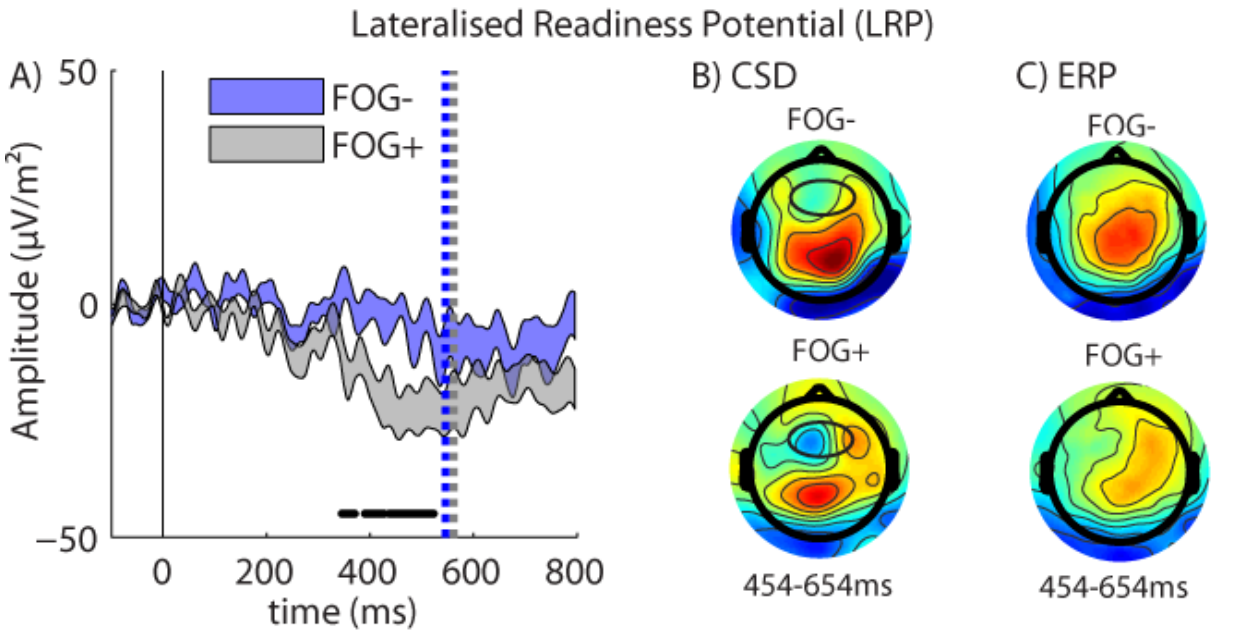


Figure 5.12 Group Comparison of Lateralised Readiness Potential: *A. Mean and standard error of the mean of the LRP CSD waveforms over frontal sites for the non-freezing (FOG-, blue) group and freezing (FOG+, grey) group. The solid black line indicates the stimulus onset, the dashed vertical lines indicate the mean response time for the non-freezing (blue) group and freezing (grey) group. The dots at the bottom of the graph indicate individual time points of statistically significant differences between the groups in the LRP waveform. B. The mean CSD scalp distribution for the freezing group (FOG-, top) and the non-freezing group (FOG+, bottom). C. The mean ERP scalp distribution for the freezing group (top) and non-freezing group (bottom).*

5.3.3.4 Regression Analysis

Statistically significant differences exist between the FOG+ and FOG- groups with respect to disease duration and FAB score (Table 1). To explore the relationship between the LRP and the FAB score taking disease duration into account, a regression analysis was performed on the entire PD cohort (Figure 5.13). The multiple linear regression was calculated to predict the LRP amplitude based on the patients total FAB score and years with symptoms. A significant regression equation was found ($F(2, 17) = 6.12$, $p < .01$), with an R^2 of 0.419. The LRP predicted amplitude is equal to -79.958 + 4.155 (FAB) -0.178(Years with symptoms). Total FAB score was a significant predictor of LRP amplitude ($t(19)=3.329$, $p<0.005$). Disease duration, however, was not a significant predictor ($p=0.644$). Separate regression analyses showed no correlation between LRP amplitude and markers of disease severity (Hoehn & Yahr stage and Unified Parkinson's Disease Rating Scale score). Thus LRP amplitude is not associated with overall motor performance in PD.

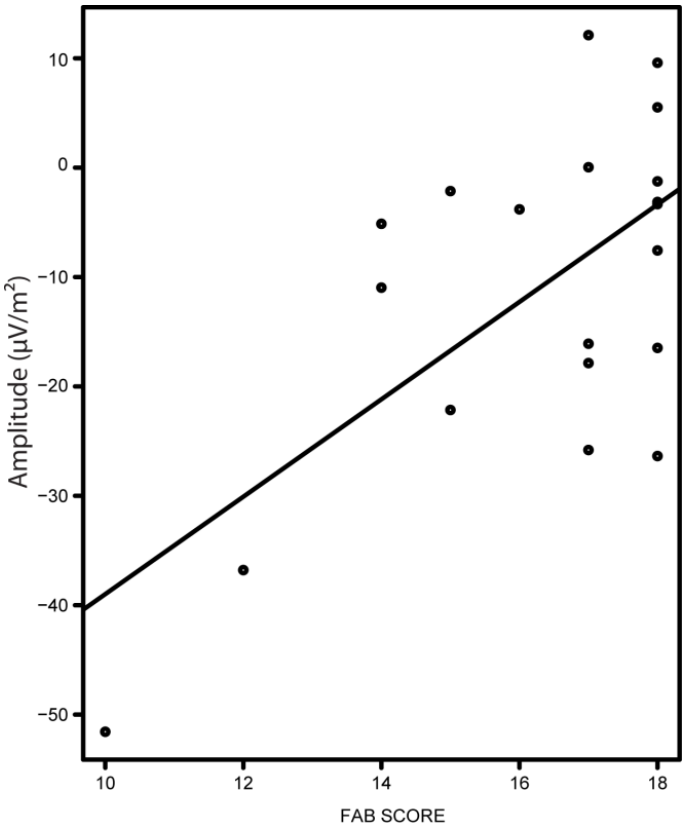


Figure 5.13 Relationship of LRP and FAB score. *Scatterplot displays total FAB score on the x-axis and the mean amplitude of the LRP from 454ms to 654ms on the y-axis. Each circle represents a person with Parkinson's disease, the solid line indicates the significant regression fit for the data.*

5.4 Discussion

Freezing of gait is associated with deficits in perceptual, motor and executive dysfunction. The standard ERP analysis shown in Figures 5.8 and 5.9 suggest significant differences in P3b morphology between freezers and non-freezers. However, the more spatially refined approach of CSD analysis reveals two distinct signals: a centroparietal positivity (equivalent to P3b) which is unaffected by FOG status; and a motor readiness potential (LRP) which has an earlier onset and greater (more negative) amplitude in freezers than in non-freezers. These results will be discussed separately. The findings above highlight the importance of cautious interpretation and analysis of ERP data in PD and show that motor preparation rather than cognitive dysfunction may be the primary deficit in FOG. These motor preparation differences occur even in the absence of any difference in motor performance (UPDRS III score and reaction time).

5.4.1 Event-Related Potential Analysis

The most common method of analyzing neurophysiological responses to stimuli is ERP analysis. The primary objective of this study was to examine differences in cortical markers of cognitive function between PwP with and without FOG. Given the close association between cognitive impairment and, in particular, executive dysfunction and freezing, one would expect significant differences in such markers between groups. The P3b is intricately linked to cognitive performance (Pelosi *et al.*, 1992), especially with respect to rapid allocation of attentional resources (Reinvang, 1999). The P3b has also been associated with context updating, stimulus classification and recently, decision making in response to sensory stimuli (Twomey *et al.*, 2015). These associations are important because FOG correlates with deficits in executive function and, in particular, divided attention and dual-tasking (Spildooren *et al.*, 2010; Tard *et al.*, 2015). The P3b has been studied extensively in PD and P3b latency is increased in PwP, correlating with cognitive dysfunction (Bodis-Wollner *et al.*, 1995; Goodin and Aminoff, 1987; Katsarou *et al.*, 2004; Matsui *et al.*, 2007; O'Donnell *et al.*, 1987; Toda *et al.*, 1993), disease severity (Silva Lopes *et al.*, 2014) and impairments of activities of daily living (Maeshima *et al.*, 2002). The ERP analysis in Figures 5.8 and 5.9, which suggests that P3b amplitude is smaller in freezers than in non-freezers, would support differences in cognitive processing between these two groups. However, the subsequent CSD analysis suggests that there is, in fact, no difference in this response between freezers and non-freezers, implying equivalent cognitive processing with respect to decision-making in both

groups. Whereas the freezing group had lower frontal executive (FAB) scores, there was no difference in overall Montreal Cognitive Assessment (MoCA) scores between the groups.

5.4.2 Current Source Density Analysis

The ERP topoplots in Figure 5.8 suggested there is also a difference in the spatial distribution of P3b between groups, with a more localized signal over the centroparietal area in non-freezers and a more diffuse amplitude distribution in freezers extending into the right frontal area. This motivated re-analysis of the data using a more spatially refined approach, the current source density (CSD), leading to increased spatial resolution. This CSD analysis (shown in Figures 5.10 and 5.11) shows that the P3b signal in freezers seen previously is, in fact, composed of two separate signals: a centroparietal positivity (the CSD-transformed equivalent of the P3b); and a slowly-rising negative potential in the frontal lobes. It is clear that interference from this second negative signal led to the underestimation of the amplitude of the P3b in the ERP analysis. This highlights the advantage of CSD analysis over standard ERP analysis in PwP where interference from frontal cortical activity is could lead to misinterpretation of results. It has previously been noted that motor potentials from button press tasks interfere with P3b morphology and lead to misinterpretation (Salisbury *et al.*, 2001). These findings raise questions regarding the results of the numerous ERP studies performed in PwP to date (Appendix II) as interference from the LRP may have led to falsely attenuated and delayed P3b responses. Only one previous study in PwP has employed CSD analysis in PwP (van Wouwe *et al.*, 2014). In our results, this method has allowed separation of these two distinct signals allowing a greater understanding of their roles.

As can be seen in Figure 5.11, there is no difference in amplitude or latency of the CPP between groups, implying equivalent cognitive processing with respect to decision-making in freezers and non-freezers. The second signal, the frontal negativity, is a readiness potential (or Bereitschaftpotential) which is defined as that cortical activity which precedes voluntary self-initiated movement (Shibasaki and Hallett, 2006). In the current study, the readiness potential has been analysed as a lateralised readiness potential by subtracting the CSD signal contralateral to the side of the response from the ipsilateral signal at a pair of standard frontocentral sites. The lateralised readiness potential is, therefore, a measure of unimanual motor readiness. These results show motor readiness differences between freezers and non-freezers. The onset of the lateralised readiness potential is earlier and the resultant amplitude is greater in the freezing group (Figure 5.12), yet there is no difference in reaction times. This suggests that motor preparation occurs earlier and to a greater degree in patients with FOG need to

recruit more resources (probably from lateral premotor areas, as discussed below) in order to achieve the same reaction time as those without FOG. It is important to note that these differences in motor preparation are seen in the absence of any difference in reaction times or difference in overall baseline motor performance (UPDRS III score) between groups. This suggests that motor preparation occurs earlier and to a greater degree in FOG+ than FOG-, even when the task is not challenging. Furthermore, the amplitude of the lateralised readiness potential correlates strongly with total FAB scores (Figure 5.13), providing a link between impairments in motor preparation and executive dysfunction in FOG.

5.4.3 Motor Preparation in Parkinson's Disease

Initiation of movement is crucially dependent on the supplementary motor area (SMA). Given that the SMA receives significant dopaminergic input from the basal ganglia (via the thalamus), it is often postulated that motor preparation deficits in PD arise primarily from SMA dysfunction (D'Ostilio *et al.*, 2013). Motor readiness potentials have been studied in PD previously (Dick *et al.*, 1989; Shibasaki and Hallett, 2006). Dick et al. recorded motor readiness potentials in PD patients off-medication during a simple motor task and showed that the very early component of the readiness potential (not recorded in this study) was reduced in the PD group but a later component (corresponding to the readiness potential discussed herein) was larger than in healthy controls resulting in a similar overall peak potential amplitude (Dick *et al.*, 1989). It was proposed that the reduced early component corresponded to SMA underactivity in the PD group and that the compensatory augmentation of the later potential was due to overactivity in lateral premotor areas (Praamstra *et al.*, 1996). PwP initiate movement earlier in response to a visual cue than an internally generated volitional movement (Praamstra *et al.*, 1996). This is achieved by initiating motor preparation in response to partial sensory information. As a result, lateralized readiness potentials in response to visual stimuli begin earlier in PwP than in healthy controls (Praamstra *et al.*, 1998). This allows PwP to achieve reaction times comparable to healthy controls for cued motor tasks, but at the expense of a greater number of errors as they commit to a response earlier. Thus, altered sensorimotor integration occurs in PwP via a compensatory shift from SMA activation to lateral premotor areas in order to facilitate movement. It is likely that the degree of dopamine loss and levodopa replacement therapy significantly modulate these effects as the deficient coupling between the lateral premotor areas, SMA and the primary motor cortex which occurs in PwP is reinstated by levodopa (Herz *et al.*, 2014).

5.4.4 Motor Preparation in Freezing of Gait

Motor readiness potentials have not been studied in FOG to date. However, motor preparation has been postulated to be central in the pathophysiology of FOG. Freezing commonly occurs at initiation of gait and rhythmic knee trembling is often seen during freezing episodes. This may represent excessive anticipatory postural adjustments (fine adjustments in lower limb muscle groups which are crucial for maintaining balance during movement preparation) due to compensation via altered SMA-mesencephalic connections (Jacobs *et al.*, 2009). This forms the basis for the decoupling model of FOG where a dissociation between a pre-planned motor program and motor initiation leads to a breakdown of controlled movement. Hence, SMA dysfunction has previously been proposed be central to the pathophysiology of FOG (Nutt *et al.*, 2011). Functional MRI studies have shown reduced activation in the SMA in FOG+ while turning (Gilat *et al.*, 2015) and structural and functional connectivity studies have also confirmed altered connectivity between the SMA and motor cortex (Canu *et al.*, 2015) and between the SMA and the subthalamic nucleus in FOG (Fling *et al.*, 2014). The differences in lateralised readiness potentials seen in the freezing group in the current study may reflect excessive recruitment of lateral premotor areas to compensate for SMA dysfunction. Furthermore, Vandenbossche et al. showed that freezers rely more on automatic response activations and hence, are less able to suppress automatic responses than non-freezers (Vandenbossche, Deroost, Soetens, Zeischka, *et al.*, 2012). Impairments in attentional set-shifting (Naismith *et al.*, 2010; Shine, Naismith, *et al.*, 2013) and dual-tasking (Peterson, Fling, *et al.*, 2014; Spildooren *et al.*, 2010) have also been frequently noted in FOG. Clearly, the excessive recruitment required to perform a simple task, not only makes inhibition of a response difficult, but also hinders the ability to quickly shift between tasks or undertake two tasks concurrently. This excessive cortical activation during movement in FOG is supported by imaging studies (Fasano *et al.*, 2015). fMRI studies reveal increased activation within frontoparietal cortical regions during freezing of gait (Shine, Matar, Ward, Bolitho, Gilat, *et al.*, 2013) as well as freezing of upper limb movements (Vercruysse, Spildooren, *et al.*, 2014). However, complex or bimanual motor tasks are required to reveal these changes (Peterson, Pickett, *et al.*, 2014). Our results show that these responses occur even with simple motor tasks such as a button press.

5.4.5 Information Overload

As mentioned above, Twomey et al. have recently proposed that the P3b (and by extension, the CPP) represents a decision variable in response to accumulation of information from sensory stimuli which

builds to a threshold above which a response is executed (Twomey *et al.*, 2015). Moreover, the time of onset of this signal and the rate of this build-to-threshold determines the speed of response. However, it has also been shown that the LRP displays similar build-to-threshold dynamics and interacts with the CPP (Kelly and O'Connell, 2013). Thus, both CPP and LRP build in response to presented sensory information in order for a motor response (such as a button press) to be triggered. Such a threshold concept is an attractive model for PD and FOG given that a sequence effect is often observed (Chee *et al.*, 2009; Iansek *et al.*, 2006) whereby gradual scaling of motor output is observed until a threshold is reached below which freezing occurs. This threshold model of FOG (Plotnik *et al.*, 2012) can be demonstrated in upper limb movements of freezers (Vercruysse *et al.*, 2012) and can be used to trigger freezing with rapid small steps or stepping in place (Snijders *et al.*, 2008).

It is important to note that we see motor processing differences between freezers and non-freezers in spite of equivalent reaction times between groups. The motor task used here is a simple one. During more complex tasks such as locomotion it is likely that excessive recruitment, which requires constant adaptation on-line would require extensive attentional resources in order to perform a seemingly simple task such as walking through a doorway and could lead to breakdown of motor function. Increased (and possibly disorganized) compensatory motor readiness could lead to significant interference, especially in the face of a competing cognitive/motor task or a complex sensory environment. The interference model of FOG, proposed by Lewis and Barker, formulates FOG as a breakdown of processing of concurrent motor, cognitive and limbic inputs through a deficient basal ganglia with a smaller capacity for parallel processing (Lewis and Barker, 2009). Recently, Beck et al. examined sensory and cognitive contributions to FOG while walking towards a doorway and concluded that FOG may be the result of an overload of cognitive and sensory information (Beck *et al.*, 2015). Our findings show explicitly that excessive motor processing occurs upstream at the level of the cortex, leading also to a greater amount of information to be processed. This effect is likely to be exaggerated in the setting of multiple cognitive tasks or complex sensory inputs.

Although executive dysfunction and motor preparation have both been proposed to be central to FOG pathophysiology, few studies have linked these two entities. We have shown that, as executive function worsens, the lateralized readiness potential becomes larger. Thus, aberrant motor preparation in FOG may require both loss of basal ganglia-SMA connectivity and frontal executive dysfunction. Alternatively, the loss of automaticity in PD and the resultant constant reliance on goal-directed control may lead to a persistent overload on frontal processing mechanisms, resulting in an apparent impairment in executive

function (rather than a primary deficit in executive function). Either way, the strong correlation between the lateralized readiness potential amplitude and FAB scores suggest that altered cortical motor preparation coincides with the appearance of executive dysfunction in FOG, (although a causative association cannot be demonstrated in the current study). However, it is likely that any superimposed executive dysfunction in FOG+ would stress these limited resources further, increasing the likelihood of motor breakdown in conflict or dual-task situations, resulting in FOG.

5.4.6 Limitations and Future Work

The sample size in the current study is small and the gender imbalance between group may have contributed significantly to the results. Future work should include examining the effect of dopaminergic therapy on the above findings. All patients were tested in the "on"-medication state. Although there were no differences in medication doses or timings between groups, it would be necessary to confirm these findings can be replicated off medication. In additions, future work should consider the effect of deep brain stimulation on these parameters as this may shed light on why stimulation can relieve FOG in some cases and induce it in others. Finally, this paradigm could be used to explore other disease cohorts such as patients with progressive supranuclear palsy and vascular parkinsonism in whom FOG and cognitive dysfunction are common.

5.5 Conclusions

In summary, these results suggest that no difference in CPP morphology exists between freezers and non-freezers, implying that decision making in response to sensory information is equivalent in both groups. However, motor preparation occurs earlier and requires greater recruitment in freezers suggesting that this may be the primary deficit in FOG. These motor preparation differences occur even when overall motor performance is equivalent but probably overload frontal networks in more complex tasks. There is a significant difference in FAB scores between groups, which correlates strongly with the amplitude of the lateralized readiness potential, highlighting the important interaction of executive dysfunction and motor preparation in the evolution of FOG.

6. Neurophysiological Correlates of Decision Making and Motor Preparation While Stepping in Place

6.1 Introduction

The study outlined in Chapter 5 above has show that freezers display motor preparation differences while seated and performing a cognitive task requiring a simple motor response. Motor preparation occurs earlier and greater recruitment of premotor cortical networks are required to execute a response. The cognitive cortical responses measured during this study were not affected by this simple task. The motor preparation differences are evident even though performance (reaction time) is unaffected suggesting that these adaptive changes are compensatory but what happens when we stress this system with a second, more complex, motor task? If such dual-tasking leads to impairment of task performance, does this impairment arise from deficits in motor preparation or decision-making or both? Ideally, one would like to observe the effect of locomotion on both of these processes. In this chapter, we consider the effect of dual-tasking on these event related potentials by performing the two-stimulus oddball task both seated and stepping in place.

6.1.1 Ambulatory EEG and Event-Related Potentials

Ambulatory electroencephalography (EEG) has only been employed in human studies in the last 5 years. Cortical involvement in gait had been shown using positron emission tomography (PET) (la Fougère *et al.*, 2010) and functional near-infrared spectroscopy (fNIRS) (Beurskens *et al.*, 2014; Harada *et al.*, 2009). However, most functional imaging techniques lack the temporal resolution required to accurately discern areas of activation during locomotion or paroxysmal gait disorders such as freezing. Gwin et al. were the first to employ high-density EEG to record cortical potentials associated with cognitive tasks during locomotion (Gwin *et al.*, 2010; 2011). Using a paradigm of a two-stimulus oddball task while walking on a treadmill, they found that visual evoked responses did not differ in young healthy subjects when walking compared with standing (Gramann *et al.*, 2010). Similarly response times did not change during dual-tasking. This implies that "dual-task interference" (i.e. simultaneous performance of two tasks results in deterioration of performance in one or both of those tasks) did not occur in these healthy subjects. Thus the task load created by walking does not affect the perceptual processing stages, cognitive decision making and motor output of a simple task in young healthy individuals.

However, similar paradigms using more complex tasks have shown that when task load is increased, although behavioural measures may be unchanged during dual-tasking (implying no dual task effect or cost), event related potentials differences can been noticed when walking is compared to sitting (amplitude and latency of P3b and N2b) (De Sanctis *et al.*, 2014). The P3b occurs earlier when walking compared to sitting in young healthy subjects. In addition, changes in P3b topography suggests compensatory recruitment of prefrontal resources during the more cognitively demanding dual task. Importantly, the N2b response became attenuated in this study when walking. As mentioned in the introduction to Chapter 5, the N2 potential (N2b) is a negative deflection which precedes the positive P3b and is associated with precognitive processing such as discrimination of sensory stimuli but also monitoring and control of motor responses such as response conflict and response inhibition (Folstein and Van Petten, 2007). Changes in these event-related potentials imply an adaptive difference in cognitive processing where more frontal regions are recruited to undertake more demanding tasks. This "anteriorisation" is also seen with normal ageing (Friedman, 2008). The effect that walking has on these cognitive processes (generation of N2b and P3b) indirectly implies that these functions compete with locomotion for resources. If capacity to perform these tasks simultaneously is large and the tasks are not so complex that they require significant resources to be recruited, the two tasks can be processed in parallel without interference. If dual-task interference occurs during walking, it suggests that locomotion is, at least partly, under cortical cognitive control. If resources (e.g frontal executive capacity) becomes limited, as may occur with ageing or in neurodegenerative conditions, dual-task interference is more likely to occur, even with simple tasks.

Recently, ambulatory event-related potential studies have been undertaken in heathy older adults (Malcolm *et al.*, 2015). Walking while performing a cognitive task (particularly one which recruits executive function) is more likely to lead to impairment in performance in older subjects compared with younger participants (Srygley *et al.*, 2009). The adaptive N2b/P3b changes seen in younger participants described in de Sanctis et al. above, were notably absent in older participants. No N2b amplitude variation occurred when older subjects walked compared to sitting and there was an overall delay in cognitive processing compared with young healthy controls. Older adults show later processing changes while walking (increase in P3b amplitude) whereas young controls show early (reduced N2b amplitude) and late (reduced P3b latency) adaptations. This suggests that older participants have less flexibility to allocate and recruit frontal resources during dual-tasking than young controls. This lack of flexibility is associated with impaired behavioural performance during the dual-task (a drop in response accuracy). Other studies have found that P3b latency is increased in older people when performing a dual-task

suggesting a generalised cognitive or decision-making processing delay when dual-tasking occurs (Fujiyama *et al.*, 2010; Matthews *et al.*, 2006).

The interaction between the P3b/centroparietal positivity and the lateralised readiness potential in the accumulation of information for response generation has been discussed in Chapter 5 above. The role that the N2b plays in this process has recently been considered (Loughnane *et al.*, 2016). The N2b has a role in monitoring sensory information and selecting relevant information and also determines reaction time (partially via influencing centroparietal positivity build-up). Inability to select relevant stimuli (and by extension suppress irrelevant stimuli) would be associated with loss or attentuation of the N2b.

Only one study to date has examined the lateralised readiness potential in dual-task paradigms (Sangals *et al.*, 2007). This study showed that, with practice of a dual-task involving visual and auditory stimuli, healthy controls display reduced dual task interference. In addition, the time between stimulus and onset of lateralised readiness potential is shortened. Since onset of the lateralised readiness potential marks the point of response selection, the authors suggest the response decision bottleneck is the source of improvement with practice. No study has explored the effect of dual tasking on the lateralised potential, however, in healthy or pathological states.

6.1.2 Ambulatory EEG and Parkinson's Disease

Surprisingly few ambulatory EEG studies have been undertaken in PwP to date. The only existing studies have examined FOG (Handojoseno *et al.*, 2012; 2013; Shine *et al.*, 2014; Singh *et al.*, 2013; Thevathasan, Pogosyan, *et al.*, 2012; Toledo *et al.*, 2014; Velu *et al.*, 2014). Wavelet-based methods were the first employed to investigate whether spectral energy is different during freezing episodes and normal walking (Handojoseno *et al.*, 2012; 2013). Delta, theta and alpha energies were all found to be different during onset of freezing and freezing conditions that during normal walking. Furthermore, total wavelet energy was shown to be a marker for changes between onset of a freezing episode and the freezing episode itself. Shine et al. showed that freezing episodes and the transition into FOG are associated with increased theta power in central and frontal regions compared with normal locomotion and thus could be an alternative signature of FOG (Shine *et al.*, 2014). Velu et al. investigated the effect of visual cues on spectral connectivity in two patients with Parkinson's disease and FOG (one who responded to visual cues and one who did not) (Velu *et al.*, 2014). The method employed virtual reality based visual cues generated by VR glasses while the subject walked a set path with synchronous 64-channel EEG. They found that the patient who responded to visual cues had increased information flow in occipital to

central electrodes and occipital to parietal electrodes in the beta range along with a reduction in delta and alpha-band powers. These results suggest that visual feedback cues affect activity and information flow in the occipital-parietal-motor network, although the sample size was small.

Local field potential studies have found signatures of FOG in deep brain nuclei also. Toledo et al. showed higher beta power in the subthalamic nucleus in freezers when in "off"-state compared with non-freezers. This increased beta power decreased when they switched to "on"-state with associated reduction in FOG (Toledo *et al.*, 2014). In addition, low beta frequencies were enhanced in freezers while walking/standing, but especially so during the swing phase of walking (Singh *et al.*, 2013). Hence, there are electrophysiological abnormalities at both cortical and subcortical levels in freezers. There remains some debate as to whether the cortical activations seen, for example, via BOLD responses or surface EEG in patients with FOG are merely a response to an established freezing episode (originating at a subcortical level) or whether they are involved in a causative manner.

6.1.3 Freezing, Walking and Stepping

The association between FOG and sensory/perceptual, premotor, motor and cognitive deficits have been described in detail in previous chapters. There is a close association between dual-tasking and freezing of gait (Spildooren *et al.*, 2010) which will be discussed in greater detail in Chapter 7. FOG has also been associated with deficits in response inhibition and conflict resolution (Vandenbossche *et al.*, 2011; Vandenbossche, Deroost, Soetens, Zeischka, *et al.*, 2012). These processes are fundamental to locomotion and although freezing is not limited to gait (freezing of upper limb, speech and swallowing have all been previously reported), freezing occurs predominantly while walking. The results outlined in Chapter 5 above support differences in the way motor responses are generated in patients with FOG. One is more interested, however, in studying these processes during locomotion. Ambulatory EEG allows examination of these neural processes while walking, in a more ecological manner. However, ambulatory EEG is problematic. Noise, interference and artefact can degrade recordings, primarily as a result of head movements during locomotion. This artefact can have an amplitude that is an order of magnitude larger than the EEG signals of interest (Gwin *et al.*, 2010). This problem becomes even more prevalent in PwP where motor impairment and tremor are prominent.

Stepping-in-place is likely to reduce some of this artefact as participants remain relatively stationary while stepping. Stepping has previously been validated as a surrogate to examine gait parameters in FOG (Chomiak *et al.*, 2015; Nantel *et al.*, 2011). Stepping-in-place has also been used to examine local

field potentials in PwP (Fraix *et al.*, 2013) but has never been employed in ambulatory surface EEG studies in PD or FOG. In this chapter, the experiments of Chapter 5 were repeated on a cohort of PwP with and without FOG while sitting and stepping-in-place. Participants stepped in place through a virtual reality corridor projected on a monitor and the two-stimulus oddball task is superimposed on this environment. The experiment was, therefore, designed to approximate the complex processes involved in walking which might trigger FOG in PwP. By combining two motor tasks (locomotion, button press) with a cognitive test (oddball task) presented on a complex sensory background (virtual reality corridor), conflict and dual-task interference is expected to occur. By examining cognitive and motor event-related potentials during this task, the effect of simultaneous locomotion on these decision making signals and motor preparation signals introduced in Chapter 5 can be studied.

Although some of the ambulatory EEG studies in FOG above examined oscillatory changes *during* freezing episodes, none have examined motor and cognitive processes in situations which provoke freezing episodes (i.e. cognitive-motor dual-tasking). Ambulatory event related potentials have never been examined in FOG. Indeed, event related potentials while walking have never even been studied in PwP previously. The cognitive load added by performing the task during locomotion increases the likelihood of freezing occurring, and it is precisely that cognitive task which generates the evoked potential. In this way, we gain an insight into the neural processes occurring at the exact moment that a freezing episode is likely to occur (i.e. dual tasking), creating a window into the processes that may underlie FOG.

Similar analysis methods are employed to those outlined in Chapter 5. We have seen that a current source density (CSD) approach improves spatial resolution and allows separation of the signals of interest. Hence, only the CSD method was employed herein. In addition, however, another technique was used to improve data quality. When differences in reaction times arise and variability in individual reaction times is present, spreading of the averaged cortical response can occur. This could lead to misinterpretation of event-related potential amplitudes and latencies. For this reason, the waveforms are time-locked to the button press response, rather than the stimulus. In Chapter 5, there was no difference in reaction times between groups and and therefore stimulus-locking was sufficient to produce robust results.

The aims of this chapter are:

1) To attempt to replicate the experiment outlined in Chapter 5 while participants step in place. Given the noise associated with ambulatory EEG in healthy subjects, as well as motor difficulty and tremor present in PwP this is an ambitious task and has not been attempted previously.
2) To examine the effect of locomotion on reaction time, centroparietal positivity and lateralised readiness potential in freezers and non-freezers and hence, gain insight into the mechanisms of dual task impairment in FOG.

6.2 Methods

6.2.1 Participant Recruitment

Twenty patients with Idiopathic Parkinson's Disease (as defined by the UK Brain Bank Criteria (Hughes *et al.*, 1992), Hoehn and Yahr stage II-III) were recruited from the Movement Disorder Clinic in the Dublin Neurological Institute at the Mater Misericordiae University Hospital. Ethical approval was granted from the hospital ethics committee and informed consent was obtained from all participants. Each participant underwent clinical and neuropsychological testing with a neurologist including the New Freezing of Gait Questionnaire (NFOG-Q) Unified Parkinson's Disease Rating Scale III (UPDRS III), Montreal Cognitive Assessment (MOCA), Frontal Assessment Battery (FAB), Beck Depression Inventory II (BDI II). All participants were examined and tested in the "on" state. They patients were classified as "freezers" or "non-freezers" based on Question 1 of the New Freezing of Gait Questionnaire (Nieuwboer *et al.*, 2008):

1. Did you experience "freezing episodes" over the past month?

Freezing is the feeling that your feet are transiently glued to the floor while trying to initiate walking, making a turn or when walking through narrow spaces or in crowded places? Sometimes it can be accompanied with trembling of the legs and small shuffling steps.

i. I have not experienced such a feeling or episode over the past month
ii. I have experienced such a feeling or episode over the past month

Patients who scored 1 on this question were classified as freezers (FOG). All others were classified as non-freezers (non-FOG). Participants were excluded if they had a traumatic brain injury (or other neurological condition apart from idiopathic Parkinson's disease) or if they scored below 23/30 on the MOCA. All participants had normal corrected vision and were tested in the "on"-state.

6.2.2. Experimental Test Setup

A two-stimulus oddball task was combined with the virtual reality corridor task as described in Chapter 5 above. The task was performed sitting and stepping-in-place. Participants were seated comfortably with automatic visual flow through the corridor or progressed through the virtual environment by stepping in place on the Nintendo Wii Balance board. A Nintendo Wiimote was also used to record responses to the oddball paradigm.

The two-stimulus oddball task was superimposed on the environment in the form of flashing green cross (Figure 6.1). This standard stimulus was presented 80% of the time in the upright position ('+') and the participant was instructed not to respond to this stimulus. For the remaining 20%, the stimulus was rotated 45° (target stimulus, 'x') and participants were instructed to press a button as soon as the target stimulus was seen. Participants were instructed to focus their attention on the oddball task (rather then on gait or visual flow) to optimise performance. The standard and target stimuli were presented randomly with a random interstimulus interval between 250 and 750 msecs. This ensured that the oddball task did not serve as a rhythmical sensory cue, which could have facilitated gait in this cohort.

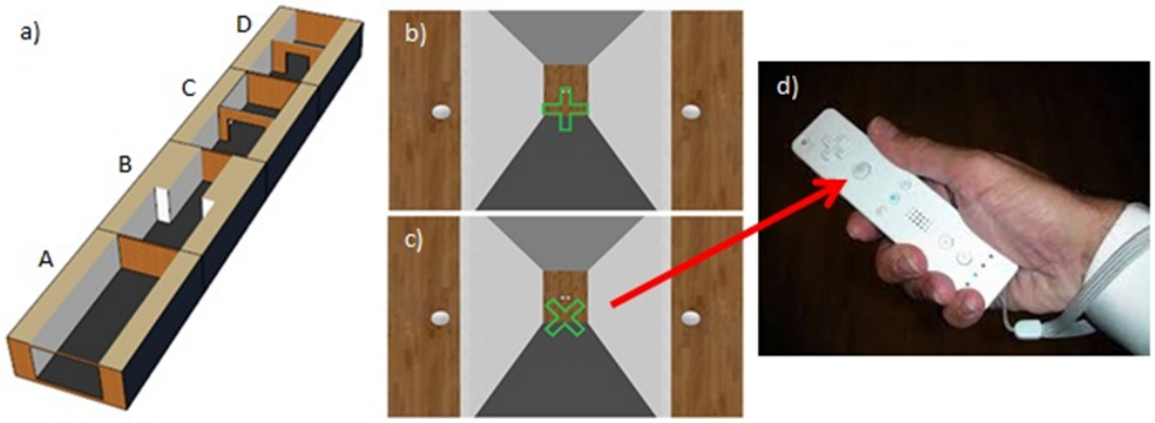
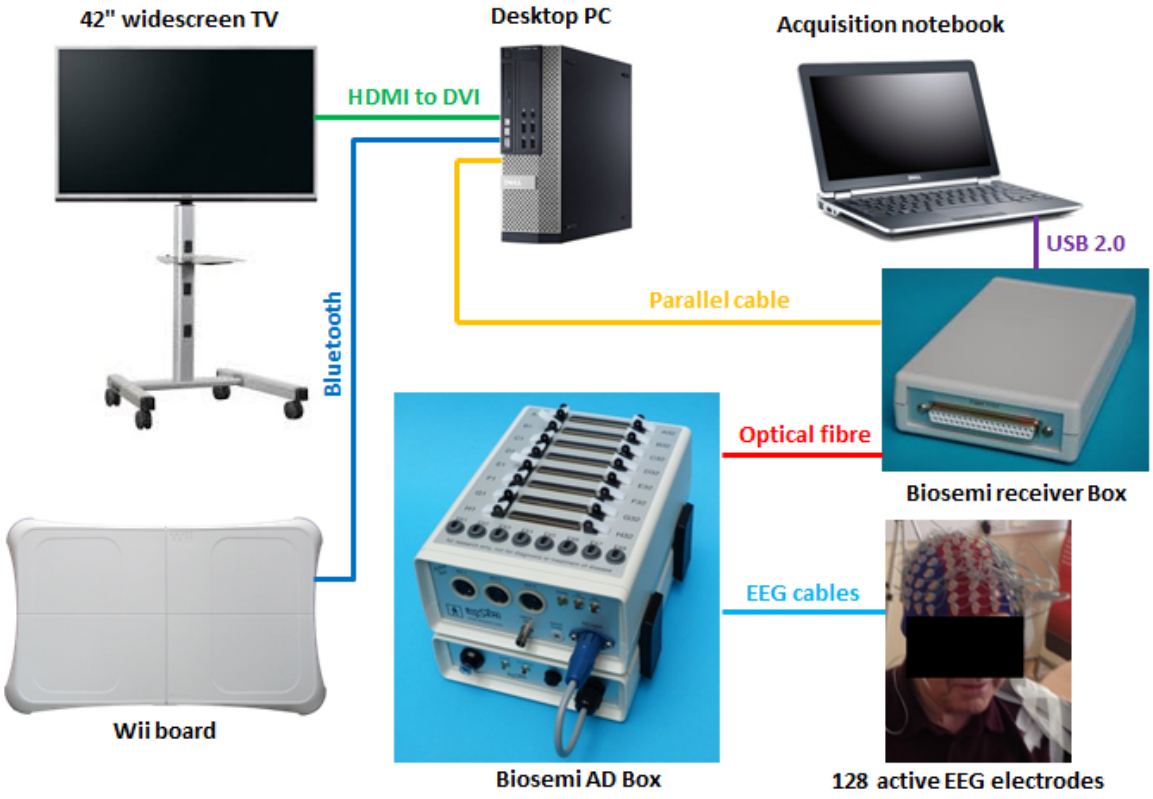
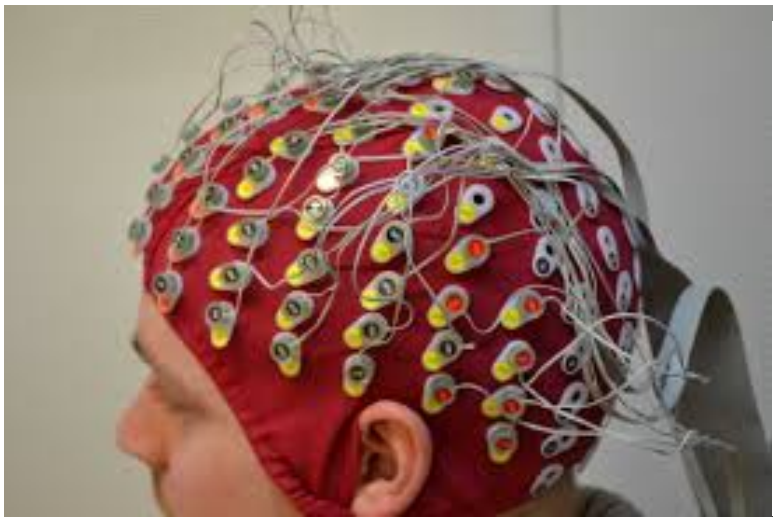


Figure 6.1 Two-Stimulus Oddball Task: *a) Virtual reality corridor with alternating narrow and wide corridors and doorways A-D; b) standard stimulus of visual oddball task superimposed on virtual reality corridor; c) target stimulus of visual oddball task superimposed on virtual reality corridor and d) Wii remote control used for detecting targets by pressing button "A".*

Synchronous EEG was recorded in all subjects using a 128-channel ActiveTwo BioSemi EEG acquisition system (Figure 6.2). Active electrodes attached to a specialized cap were used to obtain continuous EEG

signals from the subjects during sitting and walking tasks as described in the next section. The caps were individually selected for best fit and the button holes were filled with an electrolyte gel (Signa Gel; Parker Laboratories, Inc.; Fairfield, NJ) to increase conductivity. Electrodes were placed using a "10-20" arrangement and amplified at source by the internal pre-amplifier. During the experiments, channel offsets were monitored to ensure a value less than 20mV. EEG data were recorded and digitized at a rate of 512 Hz by the Biosemi analogue-to-digital converter. After digitization, the signal is sent via fibreoptic cable to a receiver box. During the experiment, the virtual reality software was controlled via a desktop PC and the environment was presented on a 42" widescreen TV to maximise immersion in the VR environment. The desktop PC sent triggers (to indicate when oddball paradigm stimuli were presented) to the receiver box via a parallel cable. The EEG data and triggers were then visualised with Actiview (Biosemi) software on a separate notebook. The details of the specific hardware connections are also shown in Figure 6.2.





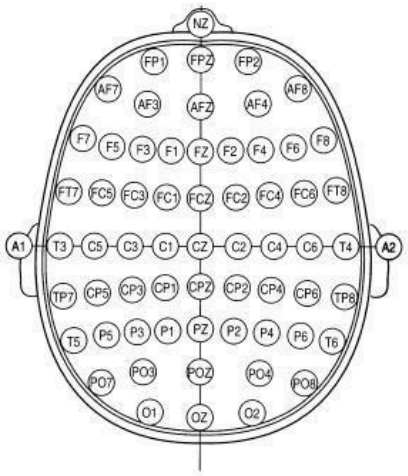


Figure 6.2 Experimental setup for stepping task: (a:top) Hardware connections of measurement equipment; (b: bottom, left) Connection of electrodes to scalp via cap; (c: bottom, right) The "10-20" system for electrode placement: Location of electrodes in the scalp for EEG recording of cortical activity of subjects. "10-20" refers to the electrode spacing, adjacent electrodes are either 10% or 20% of total front-back or right-left distance of the skull. Reproduced from Nuwer et al. (1998)(Nuwer *et al.*, 1999).

6.2.3 Experimental Protocol

Participants performed the VR experiment above under 2 conditions as outlined in the table below:

Condition	Paradigm	Participant	Visual input	Duration
1	Stepping with Visual Flow and Oddball Task	Stepping	Moving corridor	3x100s
2	Sitting with Visual Flow and Oddball Task	Sitting	Moving corridor	1x300s

This task was performed in the sitting position as in Chapter 5 for a single trial of 300msecs and while stepping in place on the balance board as 3 separate trials of 100 msecs (Figure 6.3).

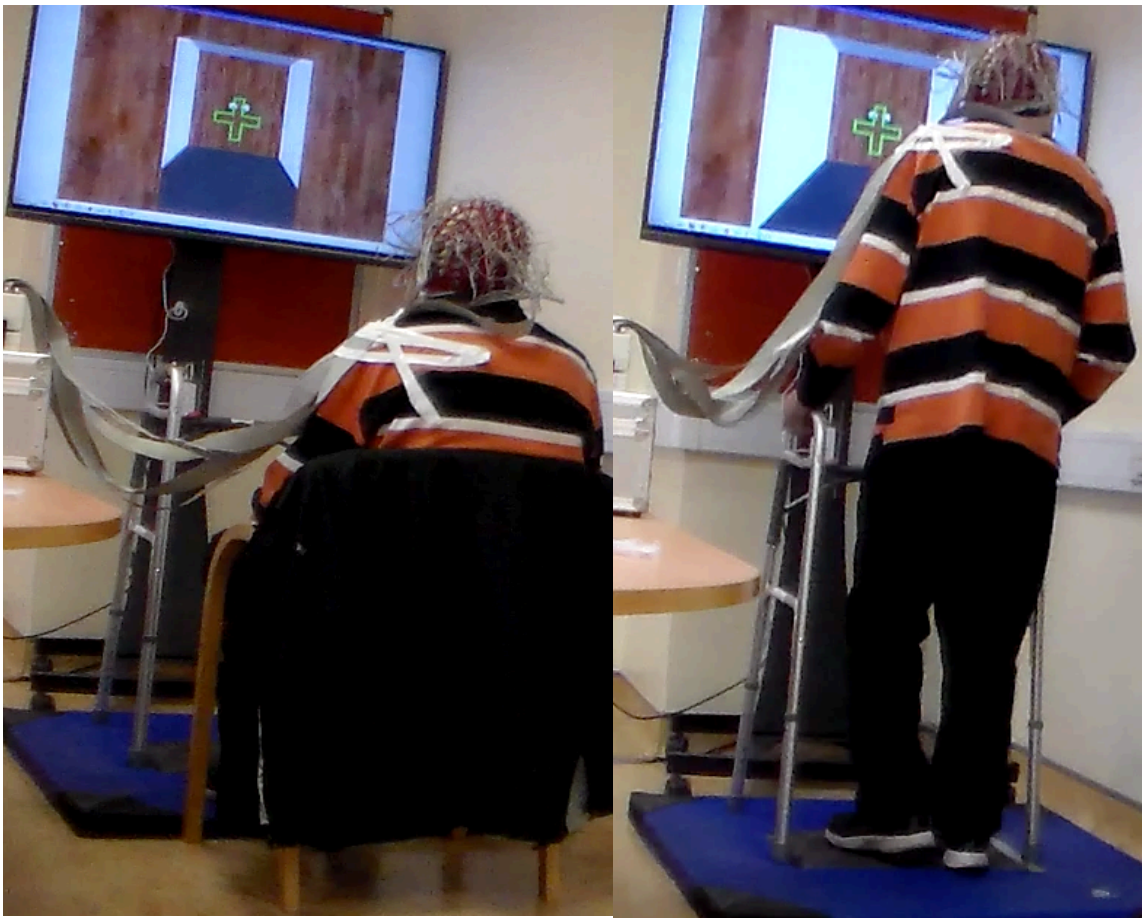


Figure 6.3. Two-stimulus oddball task sitting and stepping: *Participant seated comfortably (left) and stepping in place (right) in front of LCD monitor with 128-channel EEG attached. Two-stimulus oddball task is presented superimposed on the virtual reality corridor.*

6.2.4 Data Analysis

6.2.4.1 Behavioural Data

Button press responses were acquired during the recording of the EEG and were processed offline using MATLAB (Mathworks, Natick, MA). Reaction times during the two-stimulus oddball task were calculated for both conditions as follows: Timings of button presses in response to stimulus were compared with automatically-generated triggers corresponding to the standard and target stimuli. From this data, reaction times were calculated. Reaction time (RT) means and standard deviations were calculated for each participant. Only trials with reaction times falling within 200ms and 1000ms of target presentation were considered valid. Given that the data were collected from a clinical population with a hypokinetic

movement disorder, significant inter- and intra-subject variability in reaction time was expected. A mixed-groups factorial ANOVA was performed to examine the effects of FOG group (freezer, non-freezer) and condition (sitting, stepping) on reaction time.

6.2.4.2 EEG Data

Using custom MATLAB scripts, the continuous data were downsampled to 512Hz (allowing for a more manageable file sizes for the frequencies of interest) and band-pass filtered offline between 0.1 and 30Hz (6 dB/octave). An additional notch filter was employed at 50 Hz for line noise removal. The filtered data was epoched to both standard and target stimuli as well as to button press responses. This allowed examination of both stimulus-locked and response-locked ERPs.

Stimulus-locked epochs were taken from 200 msecs pre-stimulus to 800 msecs post-stimulus. Response-locked epochs were taken from 500 ms pre-response to 400 ms post-response. An automatic artifact rejection criterion of ±100µV was applied across all electrodes in the array, and channels with a standard deviation of <0.5µV were rejected. Trials with more than 5 artifact channels were rejected. In trials with less than 5 such channels, any remaining bad channels were interpolated using the nearest neighbor spline. The epochs were baseline-corrected with respect to the 200ms pre-stimulus period. Average responses were calculated for each participant and for each group to assess for the presence of between-group differences in amplitude of the components time-locked to the stimulus and to the response, separately.

The data were converted to current source density (CSD) to increase spatial selectivity and minimize volume conduction (Kayser and Tenke, 2006). As mentioned in Chapter 5, this transformation performs a spatial high pass filter on the ERP data, reducing interference from remote sources and current diffusions through the skull. This step was introduced improve spatial resolution in order to better discriminate between frontocentral motor preparation signals (readiness potentials) and centroparietal decision-making signals (P3b/CPP). Because of the variability in responses in patients while walking, plots were generated time-locked to both stimulus (target, standard) and button press (response) for each group (freezers and non-freezers) and for each condition (sitting and stepping). As mentioned in the introduction, response-locking was undertaken to avoid spreading of the averaged cortical responses which would occur for stimulus-locked waveforms due to variable motor responses patients with a hypokinetic movement disorder. Time-locking the waveforms to the button press response overcomes this problem. This was not required in Chapter 5 as there was no difference in reaction times

between groups or variability. The regions of interest were the same as those used in Chapter 5, specifically:

1. To investigate the decision-making variable, activity over central parietal (CPz) area was chosen to represent the P3/CPP component.
2. To investigate unimanual motor preparation, a lateralised readiness potential (LRP) was calculated by subtracting the activity over the left frontocentral (FC4) area from the right frontocentral (FC3) area.

Given the dense recording montage for the planned comparisons and figures, each site of interest is represented by an average of the three nearest electrodes. This served to increase the signal-to-noise ratio. Average CSD responses were calculated for each participant and for each group in the sitting and stepping conditions.

The analysis outlined in Chapter 5 was performed on the data to check that it could be validated on ambulatory EEG data. In addition, the effect of simultaneous stepping (dual-tasking) on these potentials was investigated. A mixed-groups factorial ANOVA was performed to examine the effects of FOG group (freezer, non-freezer) and condition (sitting, stepping) on the stimulus-locked and response-locked P3b amplitude (encapsulated by a 200 msec time window around the mean group reaction times for each group in each condition). Given the previously reported N2b abnormalities reported in the literature, a similar analysis was employed for the stimulus-locked N2b amplitude between 250 and 350ms. Finally, a similar mixed-groups factorial ANOVA investigated effects of FOG group (freezer, non-freezer) and condition (sitting, stepping) on the LRP amplitude in the stimulus-locked (450 to 600 msecs) and response-locked (-150 to 0 msecs).

To test for differences in the LRP onset between groups (suggesting group differences in motor preparation) unpaired t-test were conducted at each time point. To control for Type I errors a period of statistical significance was only considered if an alpha criteria of 0.05 or less was obtained for at least 11 consecutive time points.

6.3 Results

6.3.1 Demographics

The demographic and neurocognitive data for the PD cohort (divided by FOG status) is given in Table 6.1 below. The EEG data of 3 patients (1 non-freezers, 2 freezers) on the basis of the number of rejected

trials. There was no significant difference between groups in age, sex, Hoehn and Yahr stage, UPDRS III, Montreal Cognitive Assessment, Frontal Assessment Battery scores or disease duration between freezers and non-freezers.

	Freezers	Non-freezers
N	8	9
Age	65.0 (6.8)	62.6 (8.3)
Gender (M:F)	6:2	3:6
H&Y stage (median)	2.6 (0.3)	2.3 (0.4)
Disease Duration (years)	13.4 (9.8)	6.5 (3.5)
Total Levodopa Dose (mg)	412 (305)	270 (255)
UPDRS	27.6 (10.3)	28.1 (14.7)
MOCA	24.8 (2.8)	26.1 (3.1)
FAB	15.9 (2.1)	17.2 (1.3)

Table 6.1. Patient Demographics by FOG status for sitting and stepping tasks*. Means shown with standard deviation in parentheses. * indicates statistically significant difference between groups by unpaired t-test. H&Y stage = Modified Hoehn & Yahr stage; UPDRS III = Unified Parkinson's Disease Rating Scale III total; MOCA = Montreal Cognitive Assessment total; FAB = Frontal Assessment Battery total.*

6.3.2 Behavioural Data

The behavioural results of the oddball task are shown in Table 6.2 and Figure 6.4 below. These show reaction times for each group in both sitting and stepping in place conditions. There was an interaction between FOG group and condition (stepping vs sitting): $F(1,15)= 15.427$, $MSE= 29620$, $p < 0.005$. The freezing group had slower reaction times in the stepping condition compared with the sitting condition ($t(7)=2.909$, $p<0.05$), but the non-freezing group had faster reaction times in the stepping condition compared with the sitting condition ($t(8)=-2.486$, $p<0.05$). In addition, there was a main effect of FOG group ($F(1,15)= 4.919$, $MSE= 54146$, $p<0.05$), with slower reaction times for the freezing group. This difference was only statistically significant in the walking condition ($t(15)=3.277$, $p<0.01$). There was a main effect of condition ($F(1,15)= 4.698$, $MSE= 9020$, $p<0.05$).

GROUP	condition	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
FOG+	STEPPING	673.800	33.012	602.996	744.604
	SITTING	578.625	27.516	519.609	637.642
FOG-	STEPPING	529.550	29.114	467.106	591.993
	SITTING	557.035	24.267	504.988	609.083

Table 6.2. Reaction Times for Sitting and Stepping Tasks*. Mean and standard deviations for reaction times are shown for the freezing (FOG+) and non-freezing (FOG-) group for both stepping and sitting conditions. 95% confidence intervals are also shown.*

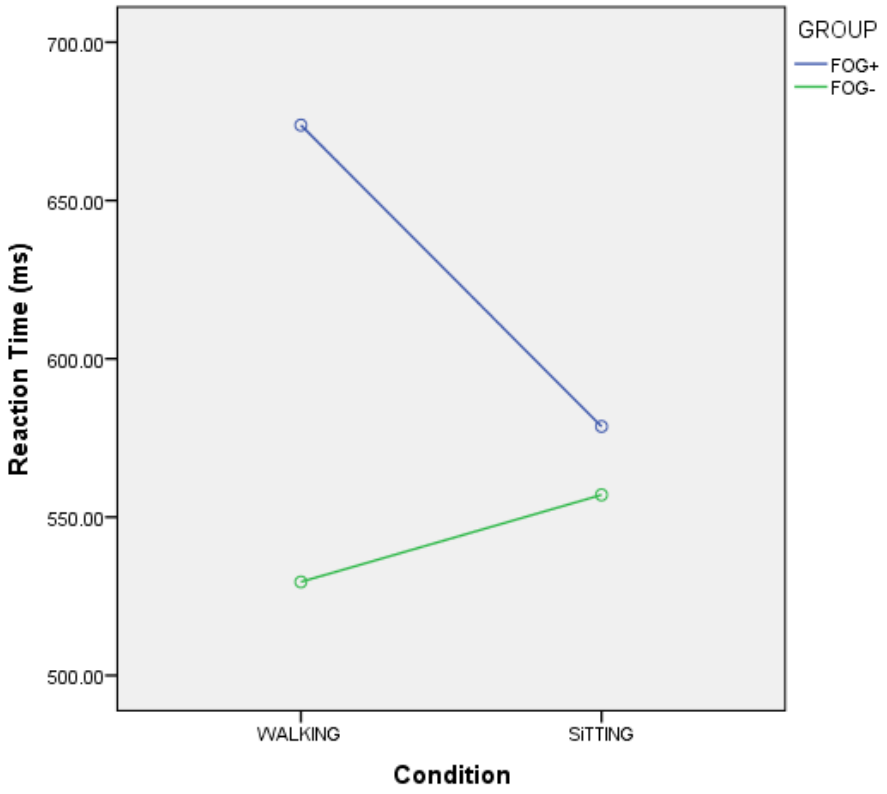


Figure 6.4: Group Mean Reaction Times For Stepping and Sitting by Group. *Reaction times during two-stimulus oddball task for freezers (FOG+), Non-freezers (FOG-) during walking (stepping in place) and sitting conditions.*

We turn our attention first to replicating the experiments of Chapter 5 while stepping in place.

6.3.3 EEG Data I: Comparison of Freezers and Non-Freezers While Stepping

To investigate cognitive decision-making in freezers and non-freezers while stepping in place and sitting, the centroparietal positivity was examined, in a similar manner to the methods employed in Chapter 5. Figure 6.5 shows the mean and standard error of the mean (SEM) of the standard (green) and target (red) ERP response for both the freezing and non-freezing groups while sitting and stepping in place for electrodes over the centroparietal scalp.

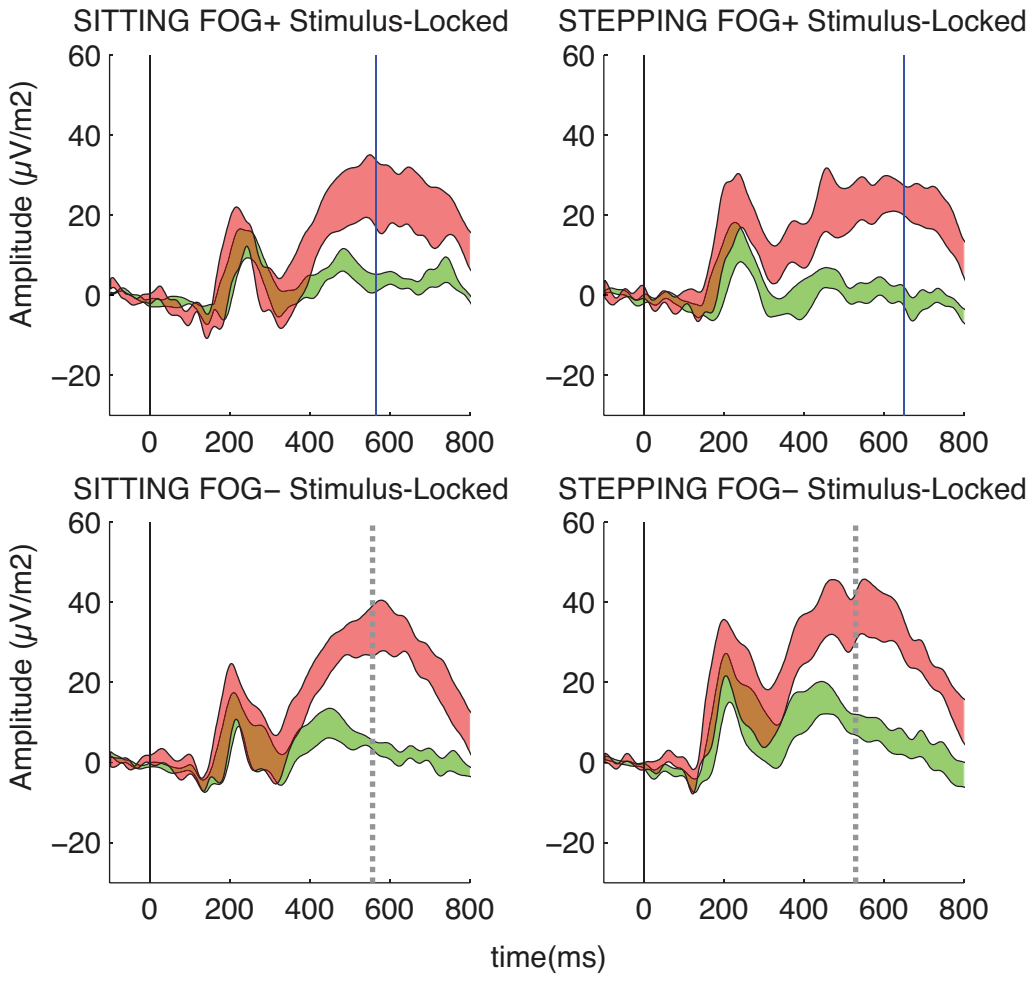


Figure 6.5. CSD Analysis of Centroparietal Positivity (CPP) Waveforms in Sitting and Stepping Conditions. *The mean and standard error of the mean of the standard (green) and target (red) current source density (CSD) response over centroparietal region of interest freezing (FOG+) group and non-freezing (FOG-) group in sitting (left) and stepping-in-place (right) conditions. The solid black line indicates the stimulus onset, the vertical lines indicate the mean response time for the FOG- (blue, solid) group and FOG+ (grey, dashed) group.*

The more usual way to assess stimulus-locked P3b/CPP responses is to compare the group differences between the average target-locked response and standard-locked response. Because of the variability of motor responses and the differences in reaction times between groups shown above, direct comparisons of the stimulus-locked waveforms (i.e. between groups) is problematic due to variable reaction times. Time-locking the waveforms to the button-press response, however, allows comparison between freezers and non-freezers. The mean and standard error for the response-locked CPP amplitudes for each group and condition is shown in Table 6.3 below. Figure 6.6 shows the group CPP comparisons for the sitting and standing conditions employing both stimulus-locked (target-standard) and response-locked methods. For the stimulus-locked condition, the mixed-groups factorial ANOVA revealed no interaction between FOG group and condition (p=0.776), no main effect for condition (p=0.781) and no main effect of FOG group (p=0.473). Similarly, for the reponse-locked condition, there was no interaction between FOG group and condition (p=0.664), no main effect for condition (p=0.433) and no main effect of FOG group (p=0.827). This suggests no difference in CPP morphology and supports equivalent cognitive decision making both sitting and stepping conditions.

			Std.	95% Confidence Interval	
GROUP	condition	Mean	Error	Lower Bound	Upper Bound
FOG+	STEPPING	15.344	4.038	6.684	24.005
	SITTING	13.986	5.169	2.900	25.071
FOG-	STEPPING	15.901	3.561	8.263	23.540
	SITTING	11.230	4.558	1.453	21.006

Table 6.3. Centroparietal Positivity Amplitudes for sitting and stepping tasks*. Mean and standard errors for centroparietal positivity amplitudes (microvolts) in response-locked waveforms the freezing (FOG+) and non-freezing (FOG-) group for both stepping and sitting conditions. 95% confidence intervals are also shown.*

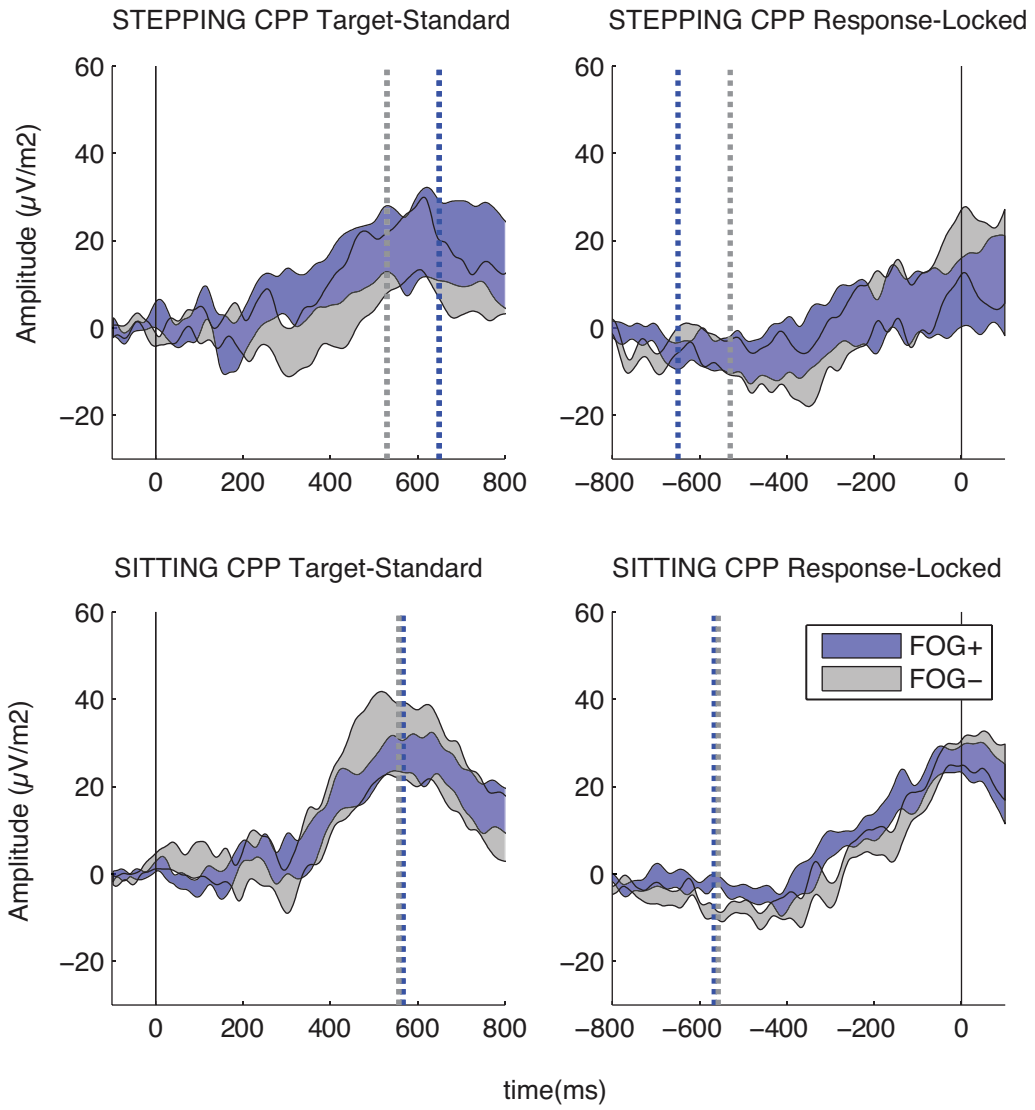


Figure 6.6. Group Comparison of Centroparietal Positivity while Stepping and Sitting: *Current source density (CSD) analysis showing: (left plots) Mean and standard error of the mean of the difference between target and standard centroparietal positivity responses for non-freezers (FOG-, blue) and freezers (FOG+, grey) in the stepping and sitting conditions; (right plots) mean and standard error of the mean of the response-locked centroparietal positivity for freezers and non-freezers. In the target-standard plots, the solid black line indicates the stimulus onset and the dashed vertical lines indicate the mean response time for the FOG- (blue) group and FOG+ (grey) group. In the response-locked plots, the solid black line indicates the response time for both groups and the dashed vertical lines indicate the mean stimulus onset for the FOG- (blue) group and FOG+ (grey) group.*

To investigate motor preparation differences between the groups while stepping and sitting, the lateralized readiness potential (LRP) was calculated for both conditions. Figure 6.7 shows the LRP CSD waveforms (i.e. the subtraction of the target response of left and right frontocentral areas) for the non-freezing group (FOG-, blue) and the freezing group (FOG+, grey) in both stepping-in-place and sitting conditions. The target-locked and response-locked waveforms are shown. In each case, the LRP was submitted to a running unpaired t-test. Time points of statistical differences in the LRP between the freezing and non-freezing groups are depicted for each condition (stepping, sitting) as markers running along the bottom of the plot. For the response-locked waveforms in the stepping condition, the group differences onset just after 200 msecs prior to the mean response time (indicated by the solid vertical lines for the response-locked plots and the the dashed vertical lines for the target-locked plots) and continue until approximately 100 msecs before the mean response time. A similar pattern is seen for the sitting waveforms. Note that the standard error is significantly greater for the stepping waveforms due to poor signal to noise ratio but the differences remain statistically significant between groups.

For the stimulus-locked condition there was no interaction between FOG group and condition ($p=0.1$) and no main effect for condition ($p=0.294$) on LRP amplitude. There was a trending main effect of FOG group ($p=0.076$). Similarly, for the response-locked condition, there was no interaction between FOG group and condition ($p=0.3$) and no main effect for condition ($p=0.289$). There was a trending main effect of FOG group ($p=0.071$).

These results confirm that the findings of chapter 5 can be replicated while stepping in place. In particular, the CPP and LRP waveforms can be accurately detected in PwP both with and without FOG while stepping in place, there is no difference in CPP morphology between freezers while stepping but there remains a significant difference in LRP onset while stepping and a trending group-condition interaction in amplitude. The mean and standard error for the response locked CPP amplitudes for each group and condition is shown in Table 6.4 below.

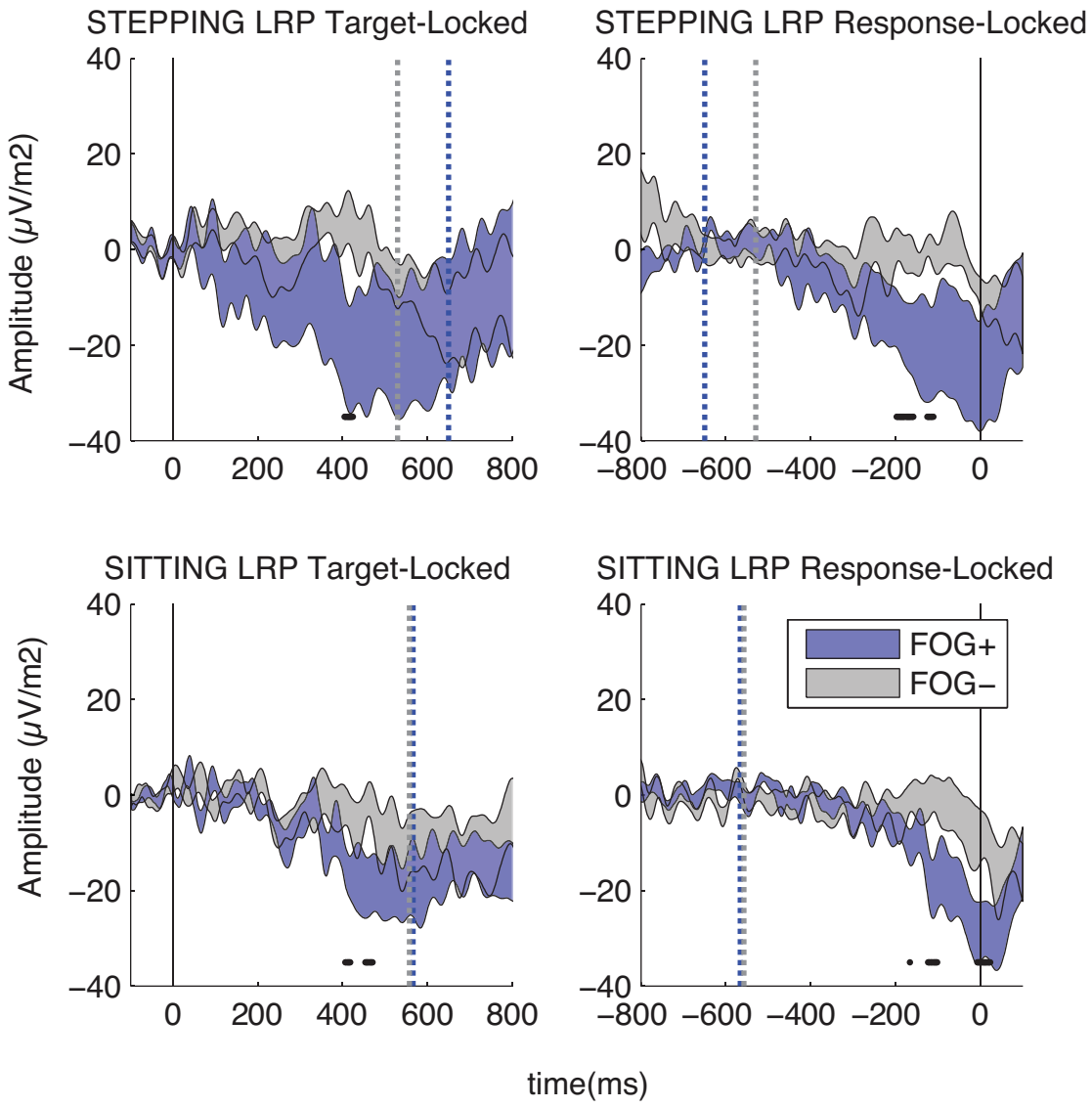


Figure 6.7 Group Comparison of Lateralised Readiness Potential while Stepping and Sitting: *Mean and standard error of the mean of the lateralized readiness potential (LRP) current source density (CSD) waveforms over frontal sites for the non-freezing (FOG-, blue) group and freezing (FOG+, grey) group in stepping (upper) and sitting (lower) conditions. The waveforms locked to the target stimulus (left) and response (right) are shown. In the target-locked plots, the solid black line indicates the stimulus onset, the dashed vertical lines indicate the mean response time for the non-freezing (blue) group and freezing (grey) group. In the response-locked plots, the solid black line indicates the button press response, the dashed vertical lines indicate the mean stimulus onset for the non-freezing (blue) group and freezing (grey) group. The dots at the bottom of the graph indicate individual time points of statistically significant differences between the groups in the LRP waveform.*

GROUP	condition	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
FOG+	STEPPING	-27.515	7.599	-43.813	-11.216
	SITTING	-17.202	7.147	-32.530	-1.873
FOG-	STEPPING	-5.863	6.702	-20.237	8.511
	SITTING	-5.423	6.303	-18.942	8.095

Table 6.4. Lateralised Readiness Potential Amplitudes for Sitting and Stepping tasks*. Mean and standard errors for centroparietal positivity amplitudes (microvolts) in response-locked waveforms the freezing (FOG+) and non-freezing (FOG-) group for both stepping and sitting conditions. 95% confidence intervals are also shown.*

Now that these results can be reproduced with confidence, the effect of stepping on CPP and LRP morphology of freezers and non-freezers will be explored.

6.3.4 EEG Data II: Effect on Stepping on CPP and LRP

Figure 6.8 shows the group CPP comparisons for the freezers and non-freezers employing both stimulus-locked (target-standard) and response-locked methods. Waveforms for sitting and stepping conditions were compared using a running unpaired t-test between points. Time points of statistical differences in the LRP between the stepping and sitting conditions are depicted for each group as markers running along the bottom of the plot. As mentioned above, there was no effect of condition or interaction between group and condition for CPP amplitude for either stimulus- or response-locked conditions. This suggests that stepping (i.e. dual-tasking) did not have an effect on CPP amplitude for either group.

Although the CPP amplitude was unchanged, there was a significant difference between the sitting (green) and stepping (yellow) waveforms in the stimulus-locked (target-standard) plots for freezers only. While seated, the freezers display a negative deflection between 200 and 400 msecs. This negative deflection is lost when the task is performed while stepping. Note that this negative deflection is preserved in non-freezers in *both* sitting and stepping conditions. The negative deflection corresponds to the N2b potential described above. The condition difference onsets approximately 250 msecs after stimulus onset and ends approximately 310 msecs after the stimulus.

When this potential (between 250 and 350ms) was submitted to a mixed-groups factorial ANOVA, there was no interaction between FOG group and condition ($p=0.12$). There was no main effect for FOG group

(p = 0.9). There was a main effect of condition (F(1,15)= 10.488, MSE= 657.8, p < .01), with the N2b being attenuated in the walking condition compared with the sitting condition. In the freezing group, the N2b amplitude was significantly smaller in the walking condition compared with the sitting condition (t(7)=3.196, p<0.025), but the difference between condition was only descriptive in the non-freezing group (t(8)=1.215, p=0.259). These amplitudes are shown in Table 6.5 below.

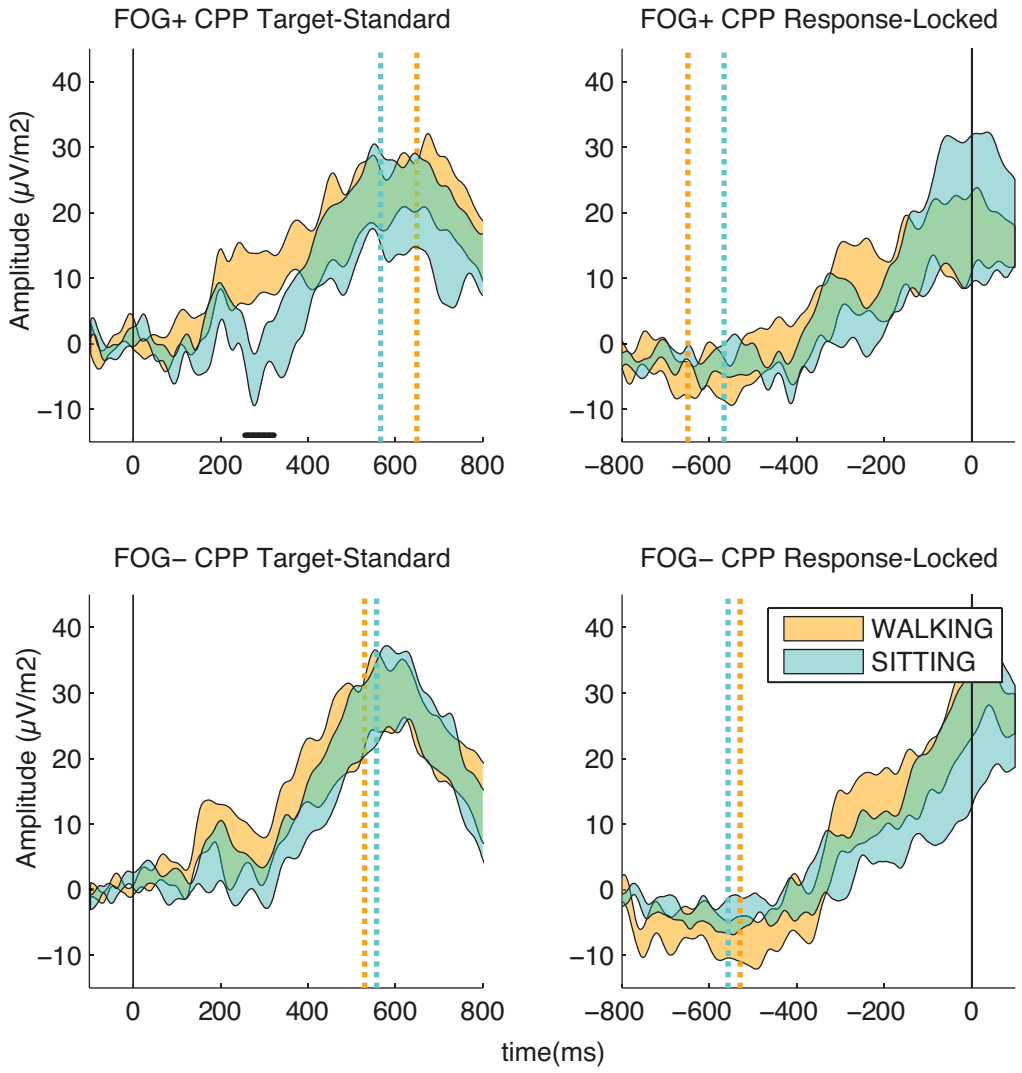


Figure 6.8. Comparison Across Conditions of Centroparietal Positivity for Freezers and Non-freezers: *Current source density (CSD) analysis showing: (left plots) Mean and standard error of the mean of the difference between target and standard centroparietal positivity responses for the sitting (green) and stepping in place (yellow) conditions for freezers (FOG+, upper) and non-freezers (FOG-, lower); (right plots) mean and standard error of the mean of the response-locked centroparietal positivity for the sitting and stepping condition in both groups. In the*

target-standard plots, the solid black line indicates the stimulus onset and the dashed vertical lines indicate the mean response time for the sitting (green) and stepping (yellow) condition. In the response-locked plots, the solid black line indicates the response time for both groups and the dashed vertical lines indicate the mean stimulus onset for the sitting (green) and stepping (yellow) conditions. The dots at the bottom of the graph indicate individual time points of statistically significant differences between the groups in the CPP waveform.

GROUP	condition	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
FOG+	STEPPING	11.521	3.383	4.265	18.777
	SITTING	-2.295	3.247	-9.259	4.669
FOG-	STEPPING	7.252	2.984	.853	13.651
	SITTING	2.790	2.864	-3.352	8.931

Table 6.5. N2b Potential Amplitudes for sitting and stepping tasks*. Mean and standard errors for N2b amplitudes (microvolts) in stimulus-locked waveforms the freezing (FOG+) and non-freezing (FOG-) group for both stepping and sitting conditions. 95% confidence intervals are also shown.*

To investigate the effect that locomotion has on motor preparation, the lateralized readiness potential (LRP) was calculated for both groups in sitting and stepping conditions. Figure 6.9 shows the LRP CSD waveforms (i.e. the subtraction of the target response of left and right frontocentral areas) for the sitting (green) and stepping (yellow) conditions in freezers and non-freezers. The target-locked and response-locked waveforms are shown. In each case, the LRP was submitted to a running unpaired t-test. Time points of statistical differences in the LRP between the stepping and sitting conditions are depicted for each group as markers running along the bottom of the plot. This revealed significant amplitude differences between stepping and sitting conditions in freezers between 180 msecs and 160 msecs prior to the button press. Stimulus onset and button press response are depicted as before. There is a small inconsistent difference in the target-locked plots for non-freezers around the time of button press. The significance of this is uncertain but the significant difference is not continuous over that period. As mentioned above statistical analysis of peak amplitude showed no interaction between FOG group and condition ($p=0.3$) and no main effect for condition. Thus, although there is a small period where the LRP amplitudes separate between the sitting and stepping conditions for freezers, stepping does not impact peak LRP amplitude in either group.

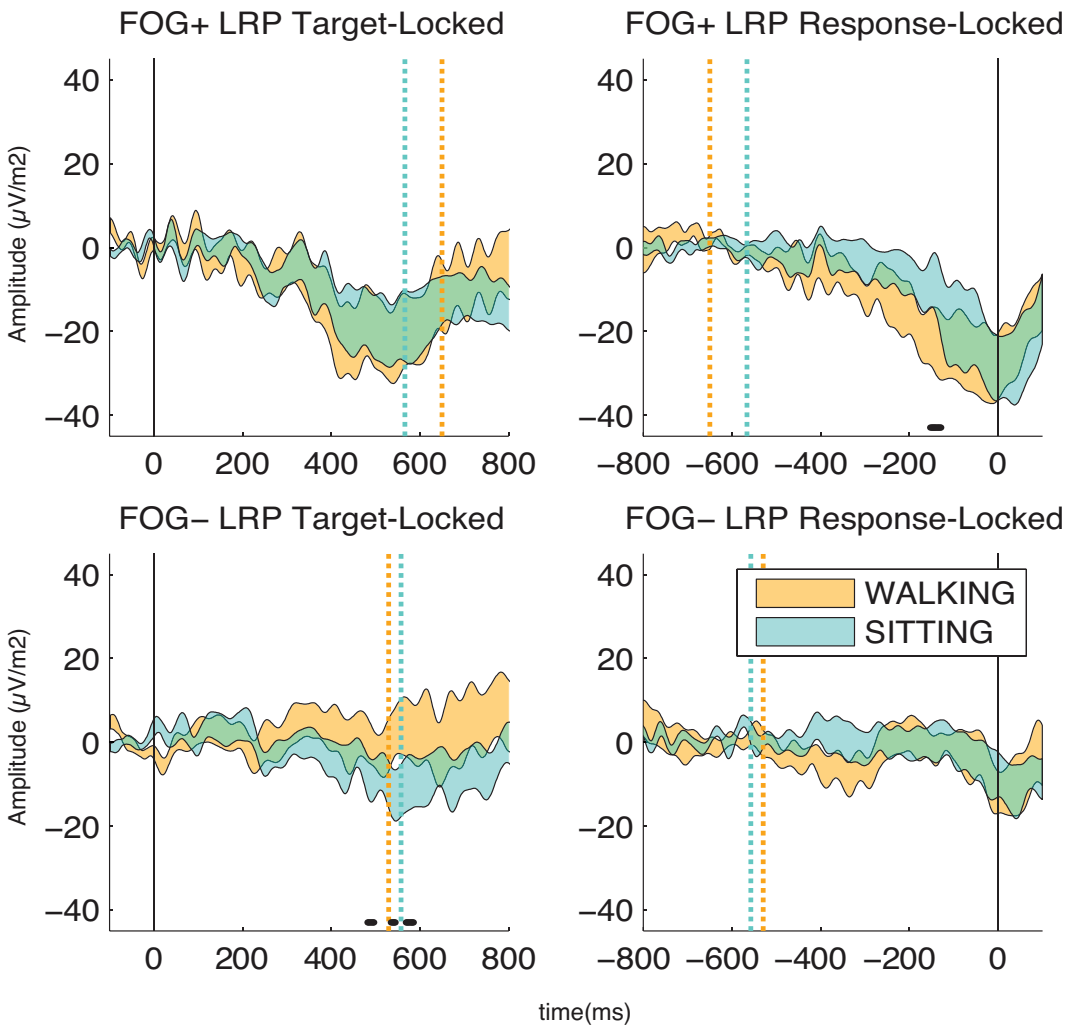


Figure 6.9 Comparison Across Conditions of Lateralised Readiness Potential for Freezers and Non-freezers: *Mean and standard error of the mean of the lateralized readiness potential (LRP) current source density (CSD) waveforms over frontal sites for the sitting (green) and stepping (yellow) for the freezing (FOG+) and non-freezing (FOG-) groups. The waveforms locked to the target stimulus (left) and response (right) are shown. In the target-standard plots, the solid black line indicates the stimulus onset and the dashed vertical lines indicate the mean response time for the sitting (green) and stepping (yellow) condition. In the response-locked plots, the solid black line indicates the response time for both groups and the dashed vertical lines indicate the mean stimulus onset for the sitting (green) and stepping (yellow) conditions. The dots at the bottom of the graph indicate individual time points of statistically significant differences between the groups in the LRP waveform.*

6.4 Discussion

The primary aim in this chapter was to conduct an ambulatory event-related potential (ERP) analysis in a patient cohort, in particular PwP. Few studies have attempted ambulatory ERP analysis in healthy controls but it has never been attempted in pathological conditions such as Parkinson's disease. Intuitively, ambulatory electroencephalography (EEG) is difficult in patients with movement disorders where interference from abnormal body movements can cause significant artefact.

Secondly, the study presented here attempted to replicate the findings presented in Chapter 5 while stepping in place (specifically, that there is no difference in centroparietal positivity (CPP) morphology, but that onset and amplitude of the lateralised readiness potential (LRP) is significantly different in freezers compared with non-freezers). Stepping provides a more ecological environment to study gait disorders while limiting the degree of EEG artefact created by locomotion.

Finally, these data were used to examine the effect of locomotion on decision making (CPP morphology) and motor preparation (LRP morphology) in freezers and non-freezers. The second task (stepping) introduces dual-task interference in an effort to stress the frontal networks suspected to be dysfunctional in freezing of gait. Reaction times are expected to be greater in this situation than while seated (i.e. single task) with a resultant change in the cortical evoked potentials described above.

The findings were as follows:

1) Reaction times while stepping were significantly slower than while seated for the freezers. However, reaction times were significantly *faster* in the stepping condition for the non-freezers. Whereas there is no significant difference in reaction times between groups while seated, simultaneous stepping forces the reaction times of the freezing group to be significantly slower than the non-freezers. This implies greater dual-task interference in the freezing group.
2) Event-related cognitive (CPP) and motor (LRP) potentials can be readily detected in PwP while stepping in place.
3) These potentials display the same differences between freezers and non-freezers while stepping as were seen during the same task seated in Chapter 5. No significant difference in CPP onset or amplitude is seen between freezers and non-freezers. However, the onset of the LRP is earlier in freezers and there was a trending difference in amplitude with a greater amplitude in freezers. This suggests that the addition of a second complex motor task does not alter the detectability

of group differences in these potentials, nor does ambulation create significant interference rendering these signals undetectable. This both validates the results of Chapter 5 and also suggests that these signals can be explored in a more ecological setting (i.e. stepping in place).

4) When sitting is compared to stepping, freezers display a loss of the N2b potential while stepping only. Non-freezers preserve this potential during dual-tasking. There is no difference in CPP amplitude or onset between conditions in either group. However, the LRP onset is slightly earlier only in freezers while stepping in place compared with sitting.

There are no differences in demographic or neuropsychological profiles of the groups at baseline. In particular, age, gender, motor scores (UPDRS III), disease duration, or executive function testing (FAB) were not statisctically significantly different between freezers and non-freezers. However, there is a statistically significant behavioural difference between groups in task performance (as measured by reaction times) while stepping in place only, with slower reaction times for the freezers. This suggests responses are more impaired in the freezing group when a dual-task is performed. This implies a greater dual task cost incurred by locomotion in the freezing group compared with the non-freezers. The faster reaction times in the stepping condition for the non-freezers was a surprising result. This may be a result of an ability to recruit additional attentional resources to perform the dual task. One can therefore infer that the freezing group also has less cognitive reserve for dual-tasking from the behavioural data alone.

The earlier onset of the LRP while stepping in freezers suggest that, as the frontal networks become overloaded, freezers compensate by initiating movement even earlier (as mentioned previously, this probably occurs via recruitment of lateral premotor areas). This is likely to exacerbate the overload problem described in Chapter 5 whereby, as the motor/cognitive task becomes more complex, even more premotor resources are recruited at an early stage. This overload could lead to significant dual-task interference, difficulty with shifting sets and response inhibition errors. These three phenomena all have close associations with FOG. In the setting of multiple complex tasks or cognitive impairment, severe motor dysfunction could result, leading to freezing.

Although there was no significant difference between CPP amplitudes or onsets between groups or across conditions, the findings were remarkable for the loss of the N2b component in the freezing group during stepping in place. The clear presence of this component in the sitting condition for the freezing group implies that this loss is specific to the dual-task condition. There is, furthermore, no significant difference in N2b amplitude or onset in the non-freezing group across conditions. This finding is

therefore specific to patients with freezing of gait.

The N2b response is well studied in the neuropsychology literature and has specific roles in monitoring and control of motor responses. A primary association is in monitoring of response conflict - comparing ongoing motor actions and detecting errors with respect to planned actions. For example, it compares “how fast I responding?" with "how fast should I be responding?" Monitoring of motor responses is crucially important in specific gait tasks (e.g. manoeuvring one's way through a doorway). It is important to note that this component occurs before the P3b/CPP and is, therefore, pre-cognitive. It plays a role in automatic motor preparation and monitoring rather than conscious cognitive control of motor tasks. The effect of interference with such processes is unknown but could give rise to episodic motor disturbances (e.g. cessation of motor output as in freezing of gait).

Another association of the N2b potential is in response inhibition (i.e. cancelling of a planned response during its execution). This concept is very closely linked with dual tasking as, when one needs to decide which task to prioritise and which task to suppress, the unwanted response will need to be inhibited. Areas associated with response inhibition in functional imaging studies include the right inferior frontal gyrus (an area central to resolution of dual task interference (Herath *et al.*, 2001)), the premotor area and the primary motor cortex. Involvement of the right inferior frontal gyrus is notable as this area is selectively atrophied in volumetric MRI studies in patients with freezing of gait (Kostic *et al.*, 2012).

The mechanism by which the right inferior frontal gyrus inhibits responses is by the hyperdirect pathway to the subthalamic nucleus (Figure 6.10). Recent structural and functional neuroimaging has shown that this hyperdirect pathway is deficient in all patients with PD comparing healthy controls (Fling *et al.*, 2014). Moreover, those patients with freezing of gait appeared to develop increased functional connectivity with other movement centres such as the mesencephalic locomotor region (including the pedunculopontine nucleus) and the cerebellar locomotor region. This raises the question of whether freezing of gait is an adaptive (or rather maladaptive) response to the loss of this pathway. The loss of the N2b component in the current study gives further support to the hypothesis that dysfunction in this pathway is associated with freezing of gait.

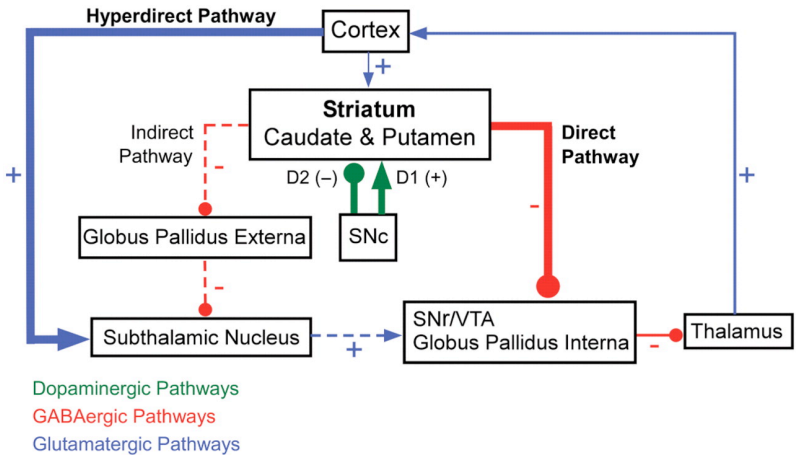


Figure 6.10. Anatomy of hyperdirect, indirect and direct pathways. From Fling et al 2014.

Indeed, inhibitory control has previously been suggested as being central to freezing of gait. Vandenbossche et al showed a generalized impairment in conflict resolution and response inhibition using two behavioural tasks: an Attentional Network Task and Stroop Test (Vandenbossche *et al.*, 2011; Vandenbossche, Deroost, Soetens, Zeischka, *et al.*, 2012). These tasks require suppression of irrelevant information which interferes with a relevant stimulus and thus imply that control of incorrect reflex-like behavioural responses is impaired in patients with FOG. The authors further suggest that freezers rely more on automatic response activation than on the deliberate controlled route as a result of impaired executive function. This concept will be dealt with in the next chapter. However, the findings of the current study provide electrophysiological evidence of impairment in response control in FOG. It should be noted however, that the optimal test for response is a Go No-Go Task rather than the two-stimulus oddball task employed in the current study. This task has been used to show attenuation of P3b and N2b components in PD patients compared with controls during the No-Go task, which correlates with the number of response inhibition errors on the task. The reason the oddball task was chosen was to test for differences in cognitive decision-making (which were not found to be present). The N2b attenuation was an unexpected finding. Future work should aim at reproducing the above finding in freezers and non-freezers using a Go No-Go task.

An alternative explanation to the current findings would be the presence of multiple interfering cortical signals obscuring the N2b potential during locomotion. This could arise from excessive cortical responses to these subjects' inability to resolve dual task interference. Thus, when the epochs are averaged, there is no detectable N2b component. Indeed, there is some evidence to support this idea:

functional MRI studies have shown extensive cortical activation both during freezing episodes and normal locomotion in patients with freezing of gait (Shine, Matar, Ward, Bolitho, Gilat, *et al.*, 2013). Alternatively, in a similar fashion to the stimulus-locked central responses, variation in latencies of these N2b components could also lead to it being obscured in group averages.

Either way, this study shows neurophysiological evidence of premotor cortical dysfunction (loss of N2b and earlier onset of LRP) in freezers while performing a dual-task which is associated with dual tasking and conflict resolution. In contrast, the CPP response appears to be robust in the face of dual-task interference. This is further evidence that the behavioural differences seen in reaction times between freezers and non-freezers are not driven by decision-making impairment in freezers.

6.4.1 Limitations and Future Work

The sample size in the current study is small. Future work should include examining the effect of dopaminergic therapy on the above findings. All patients were tested in the "on"-medication state. Although there were no differences in medication doses or timings between groups, it would be necessary to confirm these findings can be replicated off medication. In additions, future work should consider the effect of deep brain stimulation on these parameters. Finally, this paradigm could be used to explore other disease cohorts such as patients with progressive supranuclear palsy and vascular parkinsonism in whom FOG and cognitive dysfunction are common.

6.5 Conclusions

In summary, this is the first study to record and analyse ambulatory event-related potentials in PwP. The analysis confirmed, in an ecological setting, that motor preparation, rather than cognitive decision-making differentiates freezers and non-freezers. This study has shown significant differences in oddball task performance in patients with freezing of gait in both sitting and stepping in place conditions and a greater dual-task cost associated with simultaneous locomotion in freezers only. This implies differences in cognitive reserve with respect to dual tasking between freezers and non-freezers. Furthermore, the more pressure that is place on the patient (in terms of cognitive and motor loads), the more marked

these premotor differences become. This suggests a maladaptive system which is prone to overload in stressful situations, which could result in motor breakdown and freezing of gait.

7. Combined Perceptual, Motor and Cognitive Intervention for Freezing of Gait

7.1 Introduction

Chapters 3-6 above have demonstrated abnormalities in sensory processing, motor preparation and dual tasking, in addition to improvements in goal-directed control in patients with FOG compared to those without. These changes may be compensatory adaptations to the parkinsonian state (wherein automatic habitual control is lost). Rather than a single unifying causative process underlying FOG, it is likely that all of these contribute in some way to freezing. Most existing interventions targeting FOG have focused on ameliorating freezing by triggering movements using external cues employing alternative pathways to bypass the deficits that cause FOG. These effects are short-lived as the cueing strategy can be forgotten after a number of weeks (especially in the setting of cognitive dysfunction). The questions asked in this chapter is: can PwP be trained to increase their capacity to simultaneously process sensory, motor and cognitive information and would this lead to improvements in FOG. If so, instituting such training early in PD may prevent or delay the onset of FOG.

As mentioned previously, there is a close association between cognitive deficits (and in particular executive function deficits (Amboni *et al.*, 2008; 2013; Yogev *et al.*, 2005; Yogev-Seligmann *et al.*, 2008) and freezing of gait, specifically divided attention (as assessed by dual-tasking paradigms) (Spildooren *et al.*, 2010), response inhibition (Cohen *et al.*, 2014), conflict resolution (Vandenbossche, Deroost, Soetens, Zeischka, *et al.*, 2012) and implicit sequence learning (Vandenbossche *et al.*, 2013) and set-shifting (Shine, Naismith, *et al.*, 2013). Furthermore, those with FOG display specific impairments in divided attention compared with those without FOG (Tard *et al.*, 2015). These effects are accentuated under time pressure. Examining these relationships may enhance our understanding of FOG.

Freezing can be provoked by dividing one's attention while walking, e.g. by asking them to perform a second task at the same time (dual-tasking). Dual-tasking deficits play a significant role in gait impairment in Parkinson's disease (Hausdorff, Balash, *et al.*, 2003; Yogev *et al.*, 2005; Yogev-Seligmann, Rotem-Galili, *et al.*, 2012) and in particular, in FOG (Pieruccini-Faria *et al.*, 2014; Spildooren *et al.*, 2010). One explanation for the dual-task effect seen in PwP is loss of habitual motor control, requiring increased cognitive control of motor tasks which were previously performed automatically. Hence, locomotion, which was once a habitual movement, now competes with secondary cognitive tasks for

cognitive resources resulting in dual-task interference (i.e. simultaneous performance of two tasks results in deterioration of performance in one or both of those tasks). Dual-task paradigms are a powerful way to evaluate the effect of divided attention on individual tasks (e.g. gait, cognitive tasks). By comparing the performance of a dual-task (e.g. combined motor and cognitive tasks) with the individual component tasks, one can estimate the capacity of the individual to divide attention between these tasks. Dual-task performance can be assessed quantitatively as a percentage change in performance between a single-task and dual-task condition (the dual-task effect). Dual-task paradigms place a cognitive load on the participant while walking and thus increase the attention required to perform a task. These dual-task paradigms have been shown to affect gait in those with FOG (Pieruccini-Faria *et al.*, 2014; Spildooren *et al.*, 2010). Difficulty with dual-tasking in Parkinson's disease is not limited to patients with FOG and impact overall gait in Parkinson's disease (Hausdorff, Balash, *et al.*, 2003; Yogev *et al.*, 2005; Yogev-Seligmann, Rotem-Galili, *et al.*, 2012).

Gait impairments in Parkinson's disease are manifested while walking during both single- and dual-task conditions. During single gait tasks PwP have greater deficits in gait speed, stride length, double support time and stride time variability compared to healthy controls (Kelly *et al.*, 2012). The additional stress exerted during dual gait tasks manifests in a further reduction in gait speed, stride length, decreased symmetry and stride time variability. In addition, these dual-task gait impairments (as well as some single-task gait impairments such as stride-time variability) do not improve with dopamine replacement therapy. Greater deficits in stride length (Chee *et al.*, 2009), stride time, gait symmetry (Frazzitta *et al.*, 2012), rhythmicity (Hausdorff, Schaafsma, *et al.*, 2003; Plotnik and Hausdorff, 2008) and dual-tasking also exist freezers compared with non-freezers.

Dual-task training for PD in general can lead to improvements in step length while dual-tasking, even when limited to a single 20-minute session (Brauer and Morris, 2010). The positive effects of dual-task training on gait parameters in PwP suggest that this training enhances divided attention abilities during locomotion (Santos Mendes *et al.*, 2012; Yogev-Seligmann, Giladi, *et al.*, 2012). These findings are consistent with studies in healthy older adults where interventions incorporating executive function strategies and dual-task training improve postural control, dual-tasking ability and falls risk (Hiyamizu *et al.*, 2012; Li *et al.*, 2010). Whereas these interventions clearly have an impact in Parkinson's disease in general, less is known about their specific benefit in FOG. In particular, the effects of combined perceptual, motor and cognitive training has never been considered.

Combinations of perceptual and motor training have previously been undertaken in both PD and FOG cohorts. Since the response of FOG to dopaminergic therapy is limited, physiotherapy-based approaches have remained the mainstay of treatment (Tomlinson *et al.*, 2013). The RESCUE trial showed benefit of physiotherapy in treatment of FOG which incorporated rhythmical sensory cueing techniques suggesting that the addition of a cueing strategy may augment the effect of physiotherapy (Nieuwboer et al., 2007). Interestingly, a subgroup analysis in this trial showed beneficial effects only while walking with a dual task. Single task walking deteriorated with cueing. This suggests that cueing improves the ability to dual-task possible by reducing the attention required to walk, freeing cognitive resources to perform the cognitive task unimpeded. The positive effect of cueing training in PwP has been confirmed in a recent systematic review and meta-analysis (Lim *et al.*, 2005; Spaulding *et al.*, 2013). The precise mechanism by which cueing improves freezing is not known but attentional and sensory mechanisms have been implicated. Cueing appears to shift attentional focus, prioritising gait at the expense of the secondary (e.g. cognitive task) (Peterson and Smulder, 2015). Thus, although cueing may improve gait while dual-tasking in PD, it does not improve overall capacity to perform dual tasks.

The close association between cognitive deficits and FOG has directed recent attention towards considering the use of cognitive-based therapeutic approaches for treat freezing of gait (Walton *et al.*, 2014). Early cognitive interventions can impact cognitive decline and improve posture and gait in healthy older adults (Li *et al.*, 2010; Mowszowski *et al.*, 2010; Segev-Jacubovski *et al.*, 2011; Verghese *et al.*, 2010) but there are limited available cognitive therapies for PwP (Hindle *et al.*, 2013). A number of small studies have investigated cognitive interventions in PD and have suggested a potential benefit on cognition (Hindle *et al.*, 2013; Naismith *et al.*, 2013; París *et al.*, 2011). One study to date has specifically investigated the effect of cognitive training on gait in PD and showed that home-based computerized cognitive training can improve global cognitive scores as well as mobility(Milman *et al.*, 2014).

Virtual reality (VR)-based interventions allow such training to be employed in a home-based setting. VR has been employed to examine FOG in PwP previously (Shine, Matar, Bolitho, *et al.*, 2013); however, VR environments have not been considered to date as a specific therapeutic strategy for FOG. Interventions incorporating VR for PwP improve obstacle crossing performance and dynamic balance (Liao *et al.*, 2014). Furthermore, interventions incorporating VR motor training on a treadmill reduce gait variability and increase gait speed during dual-tasking. The experimental setup of interventions is typically immobile and expensive. A recent review by Hindle et al. found little evidence existing on the use of balance boards, an inexpensive mobile solution, for cognitive training in Parkinson's disease but these

cheap and portable platforms have shown benefits in cognitive function and gait when compared with balance exercises alone (Hindle *et al.*, 2013). These technologies, which allow stepping-in-place have previously been employed as a surrogate to examine gait parameters in FOG (Nantel *et al.*, 2011).

No study to date has investigated the effects of a combined perceptual, cognitive and physical training for FOG. Given the close link between gait and cognition as well as the impairments in dual-tasking seen in patients with FOG, it was hypothesised that training specifically targeting dual-tasking while walking could improve FOG as well as overall gait in Parkinson's disease. As suggested in Chapter 5, if the apparent executive function seen in FOG is the result of overloading frontostriatal networks, such an intervention could also lead to improvements in cognitive function. This chapter presents results on a virtual reality-based dual-task intervention which combines perceptual, motor and cognitive tasks, in a cohort of participants with and without FOG, in an effort to improve dual-task ability in these patients. The specific aims of this chapter are as follows:

i. To assess baseline differences in temporal gait parameters (while stepping in place) and cognitive performance between freezers and non-freezers during single-task (stepping task alone, cognitive task alone) and dual task (stepping with cognitive task) conditions.
ii. To measure differences in these parameters before and after a combined perceptual, motor, cognitive intervention in order to assess improvements in dual task capacity.
iii. To compare frequency and duration of self-reported freezing pre- and post-intervention via the New Freezing of Gait Questionnaire (Nieuwboer, Rochester, Herman, *et al.*, 2009).
iv. To compare gait performance and freezing in a real environment pre- and post-intervention.
v. To compare performance on standardised cognitive assessments pre-and post-intervention.

7.2 Methods

7.2.1 Participant Recruitment

20 community-dwelling patients with Idiopathic Parkinson's Disease (13 with FOG and 7 without FOG) were recruited from the Movement Disorder Clinic in the Dublin Neurological Institute at the Mater Misericordiae University Hospital to participate in the intervention. They were classified as "freezers" or "non-freezers" based on Question 1 of the New Freezing of Gait Questionnaire (Nieuwboer *et al.*, 2008) as above. Ethical approval was granted from the Ethics Committee at the Hospital and informed consent

was obtained from all participants. All patients underwent a clinical assessment by a movement disorder specialist, a pre-intervention assessment, an intervention, and a post-intervention assessment, as described in detail below. The two groups were age and disease stage matched. Intervention Design

The intervention was conducted in a hospital environment but was designed to be as mobile as possible to allow future translation to a home-based intervention given the reduced mobility of this patient group. Participants were invited to attend the hospital for twenty-minute intervention sessions on eight occasions over a two-week period. Two participants attended simultaneously for each session and performed the intervention at either end of the same room. All participants were on their regular medications for all sessions and no adjustments to medication regimens took place during the intervention period.

7.2.2 Intervention Design

The intervention design consisted of a virtual reality maze game combining perceptual, cognitive and motor training elements through which the participant navigated with the assistance of a map legend in the top left-hand corner of the screen (see figure 7.1). The primary concept behind the design of this maze was to replicate an environment in which perceptual, cognitive and motor information has to be processed simultaneously. In this way the environment replicates situations in which FOG is likely to occur and hence, trains the participant's ability to process these complex inputs simultaneously (rather than facilitating motor output by bypassing the apparent bottleneck of information processing which is proposed to cause FOG (Lewis and Barker, 2009).

Optic flow (the apparent motion of a visual scene due to relative motion between the participant and the environment) contributes to freezing while walking through doorways (Martens, Pieruccini-Faria, *et al.*, 2013) and FOG occurs most commonly during gait initiation, in narrow spaces, while walking through doorways and during turning(Rahman *et al.*, 2008). The maze, therefore, incorporated turns, narrow corridors and doorways as well as optic flow as participants navigated through it (at a constant velocity of 1 m/s). In order to create a valid ecological environment the VR included objects on the wall (framed paintings, radiators), creating a complex sensory environment. The maze was displayed on a fifty-five inch LCD screen placed at a height of 2 metres, 1 metre in front of the participant.

Figure 7.1: Virtual Reality Maze: *Screenshot of virtual reality maze intervention showing complex sensory environment through which participants found their way with the aid of a map legend (top left) under pressure from a countdown timer (top right).*

Navigation was achieved by stepping in place on a balance board (Nintendo Wii, Kyoto, Japan) and pressing remote control buttons (Nintendo Wii, Kyoto, Japan) to turn left and right (Figure 7.2). The participants had to find their way out of the maze within a time limit indicated by a countdown timer in the top right hand corner of the screen. This temporal pressure was included to further recreate a situation in which a FOG episode might occur. To ensure safety, a walking frame and a non-slip mat were modified to incorporate the balance board and remotes. However, participants' movements were not restricted.

Figure 7.2. Experimental setup for Virtual Reality Maze: *Virtual reality maze projected on 55-inch LCD screen with balance board (black) embedded in non-slip mat (blue). Walking frame provides safety support as well as housing remote controls.*

The intervention also included a simultaneous cognitive task, a modified Stroop test, which exerted an additional cognitive load on participants whilst stepping in place (Stroop, 1935). The Stroop test has previously been employed in dual-task studies in Parkinson's disease (Wild *et al.*, 2013) and requires participants to match word-colour pairs, probing executive control and selective attention. During navigation through the maze, participants were repeatedly presented with word-colour pairs at the bottom of the screen. If the word and colour matched, they were asked to press the left remote button, or the right button if a mismatch was presented. Thus, the modified Stroop test added an additional cognitive load (by dividing attention) for participants to that of navigating their way through the maze under time pressure. The Stroop Test has also been closely linked with conflict resolution and response inhibition, as irrelevant information needs to be suppressed when selecting the correct response. Since response inhibition has previously been shown to be impaired in FOG, training with such a cognitive task that links both attention and response inhibition may allow improvements in these deficits. On-screen feedback of Stroop test performance was presented to the participants on completion of each maze to

encourage them to navigate through the maze and perform the cognitive task with equal priority. In order to maintain a training burden during each intervention session, the maze increased in complexity (e.g. increased number of turns, shorter time to complete maze, more route options and dead-ends) as participants' performance improved.

7.2.3 Pre- and Post-Intervention Assessment

7.2.3.1 Clinical Assessment

The testing protocol included an assessment before (pre-assessment) and after (post-assessment) the intervention. All assessments took place on regular medications but there were no differences in medication doses or timings between the assessments. All participants underwent clinical and neuropsychological testing including Montreal Cognitive Assessment (MOCA), Frontal Assessment Battery (FAB) and Unified Parkinson's Disease Rating Scale III (UPDRS III) as well as the New Freezing of Gait Questionnaire (Nieuwboer *et al.*, 2008) and Parkinson's Disease Questionnaire (PDQ-39). An alternative version of the MOCA was used in the post-intervention assessment to minimize any learning effect.

7.2.3.2 Virtual Reality-Based Assessment

Different cognitive tasks and virtual environments were employed in the intervention and the assessments to ensure that results would not reflect learning during the intervention. The VR environment is the same as that employed in Chapters 5 and 6 consisting of a single long corridor with doorways and narrowings (Figure 7.3). The cognitive task involved the two-stimulus visual oddball task described in detail in Chapter 5. This task has been utilized previously when investigating cognitive function during locomotion (Gwin *et al.*, 2011). To reiterate, this visual stimulus consisted of either vertical (standard) or 45° rotated (target) green crosses and was superimposed in the VR environment. It was displayed pseudo-randomly (with an inter-stimulus interval between 250 and 750msecs). Participants were asked to press a button every time that a target stimulus was presented (oddball probability 0.2) but to ignore the standard stimulus.

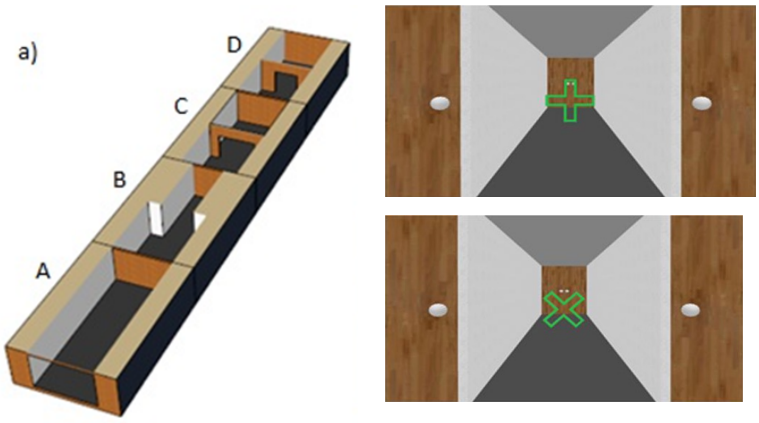


Figure 7.3. Virtual Reality Corridor and Visual Oddball Task: Left: Virtual reality (VR) corridor schematic for pre- and post- assessment with wide (A) and narrow (B) corridor and wide (C) and narrow (D) doorway. Right upper: standard stimulus of the two-stimulus oddball task; superimposed on VR. Right lower: target stimulus of the two-stimulus oddball task superimposed on VR

Participants were asked to perform three different tasks in the virtual environment:

1. A single motor task: stepping in place on the balance board through the VR corridor with no additional cognitive task

2. A single cognitive task: performance of the two-stimulus visual oddball task while seated. The visual flow through the corridor took place automatically to ensure minimal change of visual input between tasks.

3. A dual motor-cognitive task: performance of the two-stimulus visual oddball task while stepping in place on the balance board through the virtual reality corridor.

Instructions were given to perform the tasks as quickly as possible and to perform all dual-tasks with equal priority. Participants undertook three 100-second trials of each task. Tasks were counterbalanced to remove any practice effect.

7.2.3.3 Real Walking Environment Assessment

Finally, a Modified Timed Up and Go (TUG) Test (Figure 7.4) was performed based on that described by Snijders et al. (Snijders *et al.*, 2012). This involves patients standing up from sitting and performing a 12-m self-selected speed gait trajectory including: a full 360° narrow turn in a self-selected direction,

walking through a narrow passage (two chairs placed 50 cm apart), a full 360° narrow turn in the opposite direction, a narrow 180° turn in a self-selected direction at the midpoint and, returning to the chair via the same narrow turns and passageway. Snijders et al. have suggested that such testing provokes FOG more effectively than standard walking tests (Snijders *et al.*, 2012). Patients performed the above test twice and average time taken to complete the test and number and duration of FOG episodes were recorded.

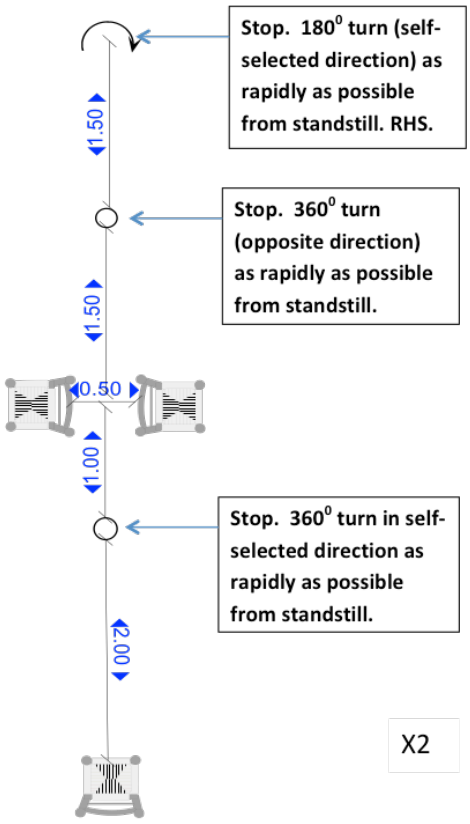


Figure 7.4: Modified Timed Up-and Go Test: Real walking circuit incorporating narrow, rapid 360⁰ turns & turning from standstill in both directions. Two iterations of the protocol were performed.

7.2.3.4 Assessment Measures

Motor and cognitive measures were acquired during both pre-assessment and post-assessment. In addition, standard clinical measures of motor and cognitive function were also acquired. Montreal Cognitive Assessment (MOCA), Frontal Assessment Battery (FAB) and Unified Parkinson's Disease Rating

Scale III (UPDRS III) as well as the New Freezing of Gait Questionnaire (Nieuwboer *et al.*, 2008) and Parkinson's Disease Questionnaire (PDQ-39) were compared pre- and post- the intervention.

The cognitive measures employed in the VR environment were mean reaction time (RT) and hit rate accuracy of oddball task acquired during both the single cognitive task and the dual motor-cognitive task. Only correct responses were included in analysis. Cognitive measures were also calculated during the intervention to assess performance: time to complete maze, reaction time and hit rate accuracy (correct answers).

The motor measures employed: stepping time (average peak-to-peak interval per stepping cycle); rhythmicity (stride time variability); and symmetry were acquired from vertical ground reaction force data from the balance board during both the single motor task and the dual motor-cognitive task. Symmetry is commonly calculated as a ratio of shorter to longer mean swing time (time during which one foot off the ground) but this was difficult to determine due to participants not fully lifting feet from the balance board. Instead, the loading and unloading times of both feet was used to assess whether the gait was symmetrical. Loading and unloading occurs during the double stance phase when the majority of weight shifts from one foot to the other and corresponds to the crossover points of the force signals for each foot on a synchronized plot of the data. The loading time of one foot corresponds to the unloading time of the other foot, and is 50% of a full gait cycle for a symmetrical gait(Nantel *et al.*, 2011). Coefficient of variation (CV) (ratio of standard deviation to the mean) was used to evaluate rhythmicity and symmetry. Analysis included all gait data for each trial, incorporating all FOG episodes. Thus any improvement or worsening in gait parameter results also incorporates increases and decreases in FOG. For stepping time and rhythmicity, left and right leg values were pooled together. All measures were calculated in MATLAB (MathWorks, Cambridge) and all results are presented as mean ± standard deviation.

Dual-task effect is a measure of the cost incurred by performing a dual task compared with baseline performance of the task alone. Dual-task effect was calculated as follows for the above cognitive and motor measures:

$$\text{Dual Task Effect, DTE} = \frac{(\text{Single Task Performance} - \text{Dual Task Performance})}{\text{Dual Task Performance}}$$

Therefore, a deterioration in performance of a task due to dual-tasking is represented by a positive value and an improvement by a negative value.

All trials were video recorded and analysed offline by a movement disorder specialist. The number and duration of freezing episodes on the balance board and percentage time spent frozen for each trial was also calculated. A freezing episode on the balance board was defined as foot rise failure from the balance board for two consecutive stepping cycles, ending when one full stepping cycle was complete. Finally, the average time taken to complete the modified Timed Up-and Go test was recorded along with average number of freezing episodes and average percentage time spent frozen during the test.

7.2.4 Statistical Analysis

Both within-participant (pre-to-post intervention) and between-participant (FOG, Non-FOG) variances were investigated using conservative F tests (Huynh-Feldt, Greenhouse-Geisser and Box's) included in a repeated measures ANOVA at significance $p<0.05$. Gait measures for the single motor task and the dual motor-cognitive tasks, in addition to reaction time for the single cognitive task and the dual motor-cognitive task were investigated. All ANOVA assumptions were met. Paired t-tests were employed to examine differences in FOG episodes ($p<0.05$). Two participants from the FOG group were excluded from the gait analyses, one due to running in place instead of stepping in place (no double-support stage for comparison), and one due to incomplete gait data.

7.3 Results

7.3.1 Demographics

The entire PD cohort had a mean age of 64.2 ± 7.3 years, with Hoehn & Yahr: 2.4 ± 0.74, UPDRS: 29 ± 10, MOCA, 26 ± 2, FAB: 16 ± 1.5. The two groups were age and disease stage matched but those with FOG had significantly worse scores on UPDRS III, MOCA and FAB. There were more males in the freezing group. Table 7.1 details the personal characteristics of each group.

	Freezers	Non-Freezers
N	13	7
Age (years)	64.2 ± 2.4	64.0 ± 1.6
Gender (M:F)	9:4	3:4
Hoehn & Yahr	2.6 ± 0.1	2.3 ± 0.1
UPDRS III	31.8 ± 2.8	22.3 ± 3.2*
Montreal Cognitive Assessment	25.1 ± 0.8	28.1 ± 0.5*
Frontal Assessment Battery	15.8 ± 0.6	17.7 ± 0.2*
New Freezing of Gait Questionnaire	17.3±7.5	-

Table 7.1 ***Patient Demographics by FOG status:*** *Means shown with standard deviation in parentheses. * indicates statistically significant difference between groups (p<0.05). H&Y stage = Modified Hoehn & Yahr stage; UPDRS III = Unified Parkinson's Disease Rating Scale III total; MOCA = Montreal Cognitive Assessment total; FAB =* Frontal Assessment Battery total.

7.3.2 Cognitive and Motor Assessment Measures

Differences between groups during the intervention (Figure 7.5) show improvements in all measures (time taken to complete maze, reaction time to modified Stroop test and number of correct answers on modified Stroop test). In particular, the improvements seen were greater for the freezing group.

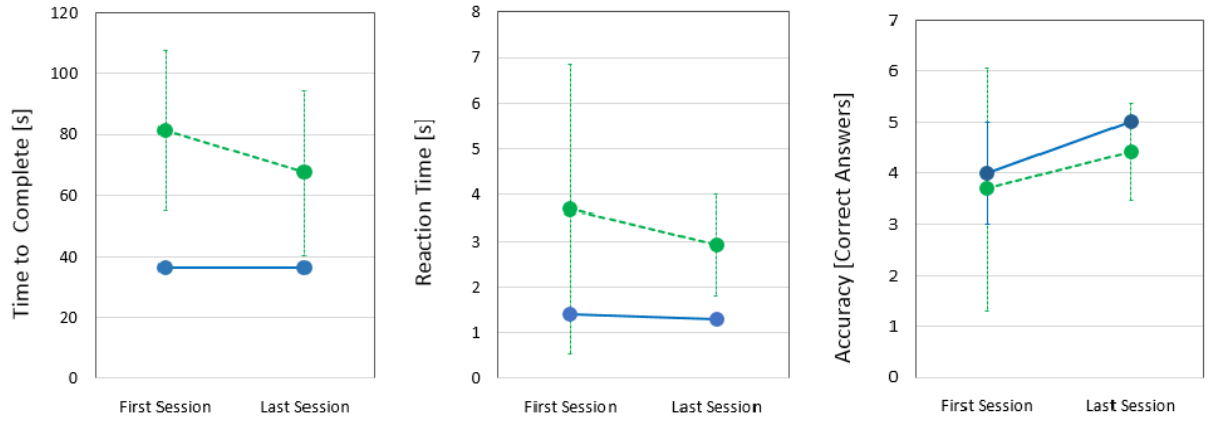


Figure 7.5 Intervention Outcome Measures: *Time taken to complete maze (left) Reaction Time to modified Stroop test (centre) and Number of Correct Answers on Modified Stroop test (right) for Freezers (Green) and Non-Freezers (Blue) in the first and last sessions of intervention.*

Pre- and post-intervention differences between groups are shown in Table 7.2. Comparing those with and without FOG at baseline, significant differences between groups were found for all three gait parameters for both single- and dual-tasks. Dual-task reaction time and reaction time dual-task effect were also significantly different between groups prior to the intervention. Following the intervention, gait parameter differences persisted between groups, but were less significant. Furthermore, the dual-task reaction time and reaction time dual-task effect differences demonstrated between groups prior to the intervention were no longer significant. There was no effect of gender on these results.

Within group intervention differences are also shown in Table 7.2. For the freezing group there was a significant post intervention improvement ($p<0.05$) in single-task stepping time and in dual task stepping time, rhythmicity and reaction time, as well as dual task effects for reaction time and accuracy. Single-task reaction time also improved but not significantly ($p=0.052$). For the non-FOG group, there was a significant post-intervention improvement in single-task stepping time, single-task reaction time and dual-task stepping time. There were noteworthy improvements in rhythmicity dual-task effect, $p = 0.06$ ($-15.1 \pm 46.0\%$) and reaction time during the dual task.

Post-intervention the number of FOG episodes on the balance board per trial decreased for the dual-task and were unchanged for the single-task. This was not found to be statistically significant (freezing episodes per trial (mean ± sd): Single-task: 2 ± 2.7 (pre) vs. 2 ± 2.9 (post): $p = 0.57$, Dual-task: 3 ± 3.9, 1 ± 2.0, $p=0.10$. The total time spent frozen per trial did not change following the intervention: Single-task: 13 ± 21.3 (pre), 14 ± 26.6 (post): $p=0.9$, Dual-task: 20 ± 29.1 (pre), 18 ± 37.3 (post), $p=0.9$.

COGNITIVE AND GAIT PERFORMANCE MEASURES BY GROUP (FOG VERSUS NON-FOG) FOR THE SINGLE MOTOR TASK (ST), DUAL MOTOR-COGNTIVE TASK (DT), AND DUAL-TASK EFFECT (DTE) PRE- AND POST-INTERVENTION

		Intervention Differences Within Group						Difference between Groups	
		FOG Group			Non-FOG Group				
Task	Gait measure	Pre (mean ± sd)	Post (mean ± sd)	*p*-value (Pre-Post)	Pre (mean ± sd)	Post (mean ± sd)	*p*-value (Pre-Post)	Pre (*p*-value)	Post (*p*-value)
ST	RT [s]	0.75 ± 0.37	0.7 ± 0.07	0.052	0.79 ± 0.27	0.62 ± 0.07	0.049*	0.380	0.060
	Accuracy [%]	77.1 ± 11.1	58.1 ± 23.9	0.159	63.1 ± 29.5	59.0 ± 18.7	0.694	0.526	0.973
	Stepping time (s)	0.96 ± 0.15	0.80 ± 0.23	< 0.001*	0.78 ± 0.12	0.62 ± 0.09	< 0.001*	< 0.001	< 0.001*
	Rhythmicity (CV)	31.73 ± 25.59	24.32 ± 18.04	0.080	19.66 ± 13.12	17.44 ± 11.66	0.438	0.009	0.040*
	Symmetry (CV)	34.38 ± 35.02	26.67 ± 28.46	0.373	7.46 ± 2.14	7.86 ± 2.64	0.614	0.002	0.006*
DT	RT [s]	0.92 ± 0.34	0.76 ± 0.14	0.03*	0.71 ± 0.19	0.62 ± 0.08	0.104	0.038	0.080
	Accuracy [%]	78.3 ± 24.6	74.1 ± 17.3	0.622	65.2 ± 20.2	61.9 ± 21.8	0.555	0.208	0.221
	Stepping time (s)	0.88 ± 0.19	0.76 ± 0.22	0.003*	0.77 ± 0.11	0.61 ± 0.09	0.005*	< 0.001	< 0.001*
	Rhythmicity (CV)	41.41 ± 38.6	28.85 ± 24.6	0.044*	18.95 ± 12.27	15.15 ± 11.89	0.182	0.001	0.001*
	Symmetry (CV)	53.13 ± 63.22	31.93 ± 36.36	0.132	7.28 ± 2.33	7.71 ± 2.45	0.571	0.002	0.004*
DTE	RT [s]	−27.5 ± 25.5	−8.2 ± 13.3	0.004*	−4.8 ± 21.4	−1.1 ± 16.2	0.533	0.034	0.400
	Accuracy [%]	−8.3 ± 17.3	−1.1 ± 17.8	0.009*	−11.5 ± 15.2	0.9 ± 17.6	0.159	0.328	0.839
	Stepping time (%)	17.54 ± 31.79	5.36 ± 11.40	0.269	2.44 ± 6.70	2.04 ± 3.47	0.891	0.239	0.471
	Rhythmicity (%)	−31.43 ± 50.49	−16.35 ± 31.62	0.434	−1.31 ± 21.77	19.17 ± 14.59	0.061	0.161	0.015*
	Symmetry (%)	−51.16 ± 77.77	−15.57 ± 30.93	0.195	−0.72 ± 12.86	0.00 ± 12.04	0.916	0.113	0.228

Table 7.2 Single- and Dual-task Cognitive and Motor Differences Before and After Intervention: *Single-task performance, dual-task performance and dual task effect for motor and cognitive tasks, pre- vs post-intervention by group. Statistically significant differences (between groups or pre- vs post-intervention) are given by p-values (*** = p<0.001 ** = p<0.01 * = p<0.05).*

The average time taken to complete the Modified Timed-Up and Go Test for the freezing group reduced significantly after the intervention (56.5 secs v 42.4 secs; p=0.015). Although there was small reduction in the time for the non-freezing group (41 secs v 35.5 secs), this was not statistically significant. This finding parallels the improvement in stepping-in-place parameters (stride time and rhythmicity) seen in those with FOG above. There was no significant difference in number of freezing episodes or percentage time spent frozen during the modified TUG test.

Total MOCA, FAB, NFOG-Q and PDQ-39 scores did not change significantly following the intervention. However, the trailmaking test within the MOCA improved following the intervention with a trend towards statistical significance (p=0.10). There was also a significant improvement in Question 6 of the New Freezing of Gait questionnaire ("How long is your longest freezing episode when initiating the first step?") (Nieuwboer *et al.*, 2008) suggesting that self-reported freezing of gait improved following the intervention.

7.4 Discussion

The main objective of this study was to investigate whether combined perceptual, motor and cognitive virtual reality (VR) training improves dual-tasking ability in patients with and without FOG. The secondary objectives were to determine whether any dual-tasking improvement could also improve frequency and severity of FOG, overall gait parameters and/or cognitive function. Few studies exist to date specifically targeting cognitive or combined motor-cognitive training in FOG or Parkinson's disease. This study found a significant improvement in single- and dual-task performance for those with FOG following this intervention as measured by improvements in dual-task stepping time, rhythmicity and reaction time during dual-tasking. In addition, this study highlighted improvements in a modified Timed Up-and-Go test, subjectively reported freezing and cognitive flexibility. Outcome measures were acquired during different experimental paradigms than those employed in the intervention. These results are novel due to the assessment measures utilized (dual-task cognitive and gait parameters while stepping) as well as the modality of the intervention (perceptual-motor-cognitive incorporating virtual reality) in this population (FOG).

At baseline, there were significant differences in balance board gait parameters between freezers and non-freezers. Those with FOG had significantly longer stepping time and less rhythmicity and symmetry than those without FOG. These findings are consistent with previously described gait disturbances in this

group. Dual task performance was also worse in the freezing group at baseline with a significant difference in dual-task reaction time and reaction time dual-task effect pre-intervention.

Post-intervention, there were improvements in both cognitive and motor performance during dual-tasking, (especially in those with FOG) as assessed by stepping time and rhythmicity (motor), and reaction time and reaction time dual-task effect (cognitive). There were also tendencies to improvement in the non-FOG group, with single-task and dual-task stepping time significantly improved post-intervention. Mean reaction time, accuracy and time to complete the maze also improved during the intervention, in keeping with the post-intervention results (Figure 7.5). In addition, at pre-assessment, the mean gait parameters for those with FOG were significantly different compared to those without FOG but at post-assessment the differences between these values had notably reduced. Furthermore, the improvement in dual-task reaction time and in reaction time dual-task effect seen in the FOG group was not seen in the non-FOG cohort. As a result, the pre-intervention difference in these parameters between groups was not evident following the intervention. This implies that the improvements gained during the intervention were more marked for the group with FOG, bringing the performance of the FOG group closer to that of the non-FOG group. This differential effect may be explained by a greater room for improvement in the FOG group (given the worse baseline parameters) or a specific effect of this intervention on those with FOG.

The parallel improvement in gait and cognitive performance suggests that the reduction in the dual-task effect (cognitive task) is not simply a result of participants focusing more attention on the cognitive part of the dual-task (rather than on stepping in place) but implies that such combined perceptual-motor-cognitive training has a direct effect both on cognitive dual-task performance during locomotion, and on gait parameters. This is not surprising given the well-described association between dual-task performance and gait in Parkinson's disease (Yogev-Seligmann *et al.*, 2008). This result is in agreement with other studies in healthy older populations that have also shown an improvement in balance and gait with dual-task training (Li *et al.*, 2010).

Caution should always be used in interpreting gait parameters measured from a stepping in place task as this may involve different neural and cognitive processes than required for normal locomotion in real environments. Although impairments in balance and posture are closely associated with FOG, FOG is inherently a problem with step initiation. This study focused on stepping-in-place, rather than walking in a real environment, which has been validated previously in FOG cohorts. Motor arrests during lower limb motor tasks (stepping in place or alternate stepping on pedals while seated) have been correlated

with FOG questionnaires and clinically observed FOG episodes (Nantel *et al.*, 2011; Shine, Matar, Bolitho, *et al.*, 2013). Given the close association between the breakdown of these motor performance parameters in real environments and the development of FOG, one might expect that improvements in these parameters (even in a virtual reality setting) may have a subsequent effect on gait and FOG in real environments. The noteworthy improvement in number of freezing episodes post-intervention supports this and a larger sample size may show a statistically significant improvement. To further confirm this, clinical gait assessment was undertaken in a real environment in the form of a modified Timed Up-and-Go test (Snijders *et al.*, 2012) which revealed a significant reduction in time taken to complete the test in all patients ($p=0.015$). This improvement of gait performance in a real environment provides external validation of the similar improvements in stepping gait parameters reported in the virtual environment. Further evidence of improvements in FOG comes from a subjectively reported reduction in frequency of freezing episodes from the patients who experience it and a statistically significant improvement in Question 6 of the New Freezing of Gait questionnaire ("How long is your longest freezing episode when initiating the first step?") (Nieuwboer *et al.*, 2008). There is some debate as to the usefulness of such a questionnaire in the evaluation of FOG (Shine, Moore, *et al.*, 2011). However, it remains in widespread use in the absence of an alternative quantitative method for assessment of patient-reported severity of freezing of gait, a phenomenon which is frequently elusive in clinical and experimental environments.

The specific improvement in dual-tasking ability for those with FOG is of particular interest. Firstly, this provides further evidence that dual-tasking may play a central role in the pathophysiology of FOG. This is in agreement with clinical and imaging studies that have shown links between dual-tasking and FOG (Kostic *et al.*, 2012; Peterson, Fling, *et al.*, 2014). Previous studies have found associations between altered dual-task prioritization (prioritization of cognitive task over gait) and executive function in older adults (Hobert *et al.*, 2011). There is conflicting evidence as to whether Parkinson's patients prioritize concurrent tasks over gait compared with healthy controls (Yogev-Seligmann, Rotem-Galili, *et al.*, 2012; Bloem *et al.*, 2006) . Given the differences in executive function between Parkinson's patients with and without FOG, it is likely that there are differences in dual-task prioritization between these two groups. Secondly, these results indicate that dual-task capacity can be increased in PwP. The parallel improvements seen in gait and cognitive performance seen here suggest that the findings are not simply the result of patients focusing more attention on one component of the dual-task (rather than on stepping-in-place) but implies that such combined perceptual-motor-cognitive training has a direct effect, both on cognition and on gait. These results support a multiple resource capacity model of dual-tasking whereby efficient use of resources can allow simultaneous processing of cognitive and motor

tasks (as opposed to a bottleneck model which would preclude the improvements seen in this study) (Pashler, 1994). We therefore propose that this intervention improves FOG by increasing dual-tasking ability. Longer-term follow-up was not pursued as the intervention was designed for regular home-use. It is expected that the effects seen in the current study will be temporary, requiring regular cognitive-motor training in order to provide a sustained effect. If dual-tasking is the core deficit which gives rise to FOG, then such an intervention may have a further protective role (if commenced early in the disease course) in preventing progression to FOG in PD.

Formal cognitive assessments pre- and post-intervention found noteworthy improvements in cognitive flexibility (p=0.10), as measured by the Montreal Cognitive Assessment trail-making task (TMT B). Previous studies have found trail-making task performance (TMT B-A) to be correlated with severity of freezing as assessed by the FOG-Q (Milman *et al.*, 2014) and FOG episodes during a motor task (Shine, Naismith, *et al.*, 2013). Furthermore, Shine et al. concluded that this inability to shift between competing attentional demands might play a central role in the underlying pathophysiology of FOG (Shine, Naismith, *et al.*, 2013). This finding could, therefore, be considered analogous to impaired dual-task performance. TMT B performance correlates with falls risk in older adults with cognitive impairment (Taylor *et al.*, 2014) and is also associated with altered dual-task prioritization (prioritization of cognitive task over gait) in older subjects (Hobert *et al.*, 2011). A functional MRI study during dual-tasking has isolated the right inferior frontal gyrus as an area central in the resolution of dual-task interference (Herath *et al.*, 2001). It is interesting to note that this region has been shown to be selectively atrophied in patients with FOG compared to those without FOG providing further evidence that dual-tasking may play a central role in the pathophysiology of FOG (Kostic *et al.*, 2012). Repetitive transcranial magnetic stimulation over the right inferior frontal gyrus leads to improvements in trailmaking in patients with Alzheimer's disease (Eliasova *et al.*, 2014) further supporting a link between trail-making, dual-tasking and the right inferior frontal gyrus. Although not statistically significant (p=0.10), it is likely that the trend in trailmaking performance seen in the current study is likely relevant given the close link between trailmaking and gait performance as well as the simultaneous improvements in cognitive task performance above.

Another plausible biological mechanism for these results comes from the neuroplastic effects of goal-based exercise training on motor and cognitive function in PwP (Petzinger *et al.*, 2013). Strenuous exercise (particularly exercise which pushes beyond the participants self-perceived capabilities) reduces the risk of Parkinson's disease (Xu *et al.*, 2010). Neuroplasticity is the process of development and

modification of structural and functional neural connectivity to allow changes in neural behaviour and function to be learned. Goal-based exercise therapy has long been used to improve motor function (particularly balance and posture) in PwP. More recently the concept of cognitive engagement in such training has become important in such training. This cognitive engagement is achieved by feedback, attentional demand (e.g. dual tasking) and reward-based motivation (all of which are central to the current intervention). Although many studies show benefit of motor training on gait parameters, some do not (Skidmore *et al.*, 2008) and degree of cognitive engagement may be the crucial difference. Similarly, dual-task training studies which do not incorporate exercise show that any improvements seen are merely through compensatory cortical circuits (Wu and Hallett, 2008), rather than neuroplasticity reversing the aberrant changes in circuitry in PwP. This is particularly important for those with FOG where such aberrant compensatory changes are advanced. As discussed previously, dopaminergic denervation leads to both loss of automatic motor control and impairments in executive function via frontostriatal circuits. Aerobic exercise can lead to improvement in executive function in PwP (Tanaka *et al.*, 2009) via changes in prefrontal regions (Angevaren *et al.*, 2008) in mild-moderate disease. The contributions of disease severity and cognitive impairment however, remain unclear. Animal studies suggest that such synergistic training may revers dendritic spine loss in striatal neurons containing dopamine (D2) receptors (Petzinger *et al.*, 2013). This combined effect of cognitive and motor training on cognitive and motor function has only recently been studied in PwP in general. The intervention presented in this chapter is the first to show benefits of such a synergistic approach in FOG. Whether the effect seen merely confirms that those with severe or advanced disease benefit more from such training or whether the combined training is more effective for FOG specifically is not known. Most exercise training targets balance and postural instability in PwP as this contributes to falls and morbidity. It is surprising that few such training interventions have specifically targeted FOG as this represents an equally significant threat in this population.

This study has limitations as it is a pilot study with a small sample size. Differences between groups in disease duration (UPDRS) and cognitive function (MoCA, FAB) may also have greatly affected results and created a possible ceiling effect in the non-FOG group also. Improvements for the FOG group may be attributed to greater disease severity and poorer cognitive function, instead of a specificity to FOG. Nevertheless, patients with severe disease, cognitive impairment and FOG are a group who represent a significant therapeutic challenge. These results suggest that this group may derive the greatest benefit from such training. Further work is required to extract the factors which lead to this benefit. Another limitation of this study is that, although different experimental paradigms were used in the assessment

and intervention, a learning effect of stepping-in-place cannot be excluded. These factors limit this study's clinical meaning. However, this study does show the potential efficacy of recent technological advances (assessment of motor-cognitive function employing VR and portable balance board) for clinical application.

The experimental set-up of interventions is typically immobile and expensive. The results of this study support the use of a relatively inexpensive, mobile balance board and a VR environment for use in a hospital setting for a community dwelling population. It also suggests that the intervention may be easily translated to a home-based or community-centre based setting for use by this population. This would be particularly beneficial given the reduced mobility of this population. Anecdotally, all participants gave very positive feedback and it was encouraging that most expressed a desire to continue with the intervention at home. The VR platform can be customized, providing a system for answering specific research questions in these patients, as well as a customized intervention targeting specific triggers of FOG, which are often vary between patients. There is little evidence for the use of balance boards in PD for cognitive or motor training (Barry *et al.*, 2014; Hindle *et al.*, 2013; Holmes *et al.*, 2013; Santos Mendes *et al.*, 2012). However, these studies focussed on balance and posture rather than stepping-in-place and no study to date has focused on FOG. Similarly few studies have used balance boards as a method of analysing temporal gait parameters (Nantel *et al.*, 2012). The ability to record clinically important gait parameters from the intervention hardware would allow online performance to presented to the participant as feedback as well as allowing the clinician to monitor performance remotely.

Results from this pilot study support the introduction of VR environments in the clinical setting, both as a means of assessing gait in Parkinson's disease but also as a potential therapeutic intervention. Future studies should confirm these findings in a larger cohort. The role of dual-task training in Parkinson's disease and, in particular, in FOG has implications, not only in alleviating this disabling symptom, but also in furthering our understanding of this phenomenon.

7.5 Conclusions

This is the first study showing the effect of combined perceptual-motor-cognitive dual-task training on gait and dual-task performance in Parkinson's disease patients with and without FOG. Measures of dual-tasking were found to significantly improve post intervention in patients with FOG as well as a noteworthy decrease in number of FOG episodes.

The results suggest that a combined perceptual-motor-cognitive intervention can, not only improve dual task performance, but also improves severity of FOG, gait parameters and cognitive flexibility. These findings have implications for our understanding of the pathophysiology of FOG and the role of executive function and attention. This represents a promising therapeutic option for FOG but warrants further assessment in a larger patient group e.g. in the setting of a randomised controlled trial.

8. Discussion

Freezing of gait (FOG) is poorly understood and, as a result, few effective therapies which specifically target freezing exist. Many models of FOG exist, however, none are unifying. Consistently, the literature reports that freezers demonstrate perceptual, motor and cognitive abnormalities. Few studies to date, however, have quantified these difference in a way that accounts for the significant overlap between cognitive, motor and perceptual function. This has led to a poor understanding of the how individual abnormalities in these three domains contribute to FOG.

The primary aim of this thesis was to quantitatively examine differences in perceptual, cognitive and motor measures in freezers and non-freezers in order to broaden our understanding of the mechanisms underpinning this phenomenon. The secondary aim of this thesis was to develop an intervention for FOG which, rather than facilitating movement using alternative pathways (such as with sensory cueing), challenges and trains freezers to expand their ability to simultaneously process perceptual, cognitive and motor tasks. This approach has never been undertaken in an FOG intervention previously. The studies presented herein have shown:

1) PwP display unisensory and multisensory processing abnormalities in auditory and visual modalities compared with healthy controls. These abnormalities correlate with freezing of gait status and disease duration.
2) PwP can refine goal-directed movement as well as healthy controls and freezers have better goal-directed motor control than non-freezers
3) No difference in electrophysiological markers of cognitive function but earlier onset and increased amplitude of motor readiness potentials in freezers compared with non-freezers during a simple motor task while seated.
4) The findings in 3) above can be replicated while stepping in place. Dual-tasking (stepping while performing a cognitive task) introduces further premotor abnormalities (loss of N2 potential, further increase in LRP amplitude) in freezers only. Non-freezers do not develop these changes while dual-tasking.
5) Improvements in motor and cognitive dual-tasking, timed walking tests and cognitive flexibility following a combined perceptual, cognitive and motor intervention, especially for freezers.

These findings suggest significant adaptive changes in sensorimotor control in freezers which is in agreement with the neuroimaging literature (Fasano *et al.*, 2015). Specifically, multisensory processing changes, improved goal-directed control and earlier, augmented motor preparation is seen in patients with FOG. These adaptive changes may lead to inefficient sensorimotor processing which depletes cognitive resources in the presence of impaired automatic habitual motor control in PwP, especially while dual-tasking. These adaptive changes can easily become maladaptive leading to difficulties with dual-tasking, attentional set-shifting, response inhibition and response conflict. Importantly, however, the capacity to perform dual tasks can be expanded with training, resulting in improvements not only in FOG but also overall gait performance and potentially cognitive function. The studies will be discussed separately.

8.1 Quantitative Sensory Function and Freezing of Gait

In Chapter 3 (Audiovisual Processing is Abnormal in Parksinson's Disease and Correlates with Disease Duration and Freezing of Gait), PwP and age-matched healthy controls performed a simple reaction time task in response to unisensory (auditory-alone, visual-alone) and multisensory (audiovisual) stimuli in order to quantitatively assess relative sensory processing differences in PwP and, in particular, those with FOG. Sensory and perceptual disturbances are known to progress with disease duration in PwP and probably contribute to motor deficits such as bradykinesia and gait disturbances, including FOG. However, these disturbances have not been studied independent of cogntive and motor function previously. Because of the inherent delays in motor responses seen in PwP, *relative* unisensory and multisensory processing delays were assessed as these take into account the motor delays which are invariably present in PwP.

The PD group were significantly slower than controls for all conditions but auditory reaction times were significantly faster than visual for the PD group only. These relative unisensory differences are correlated with disease duration and divide the PD group by FOG status, but these factors are co-dependent. This suggests a possible adaptive response in PwP where auditory processing becomes faster relative to visual processing. This difference becomes more marked with disease duration and the development of FOG. These adaptive changes may explain some of the perceptual phenomena which are prominent in freezers such as provocation of FOG when walking through doorways or narrow spaces (Cowie *et al.*, 2012) or the powerful effect of auditory and visual cueing on FOG (Spaulding *et al.*, 2013).

Secondly, although multisensory facilitation occurs in PD, it is significantly less enhanced than in healthy controls. The multisensory processing abnormalities are independent of disease duration and FOG status and may be a potential biomarker for the disease. This suggests that whereas the unisensory abnormalities seen may reflect adaptive changes which contribute to FOG, the multisensory abnormalities do not. One must consider the possibility that the attentuated multisensory response simply represents loss of dopaminergic innervation to the basal ganglia, a multisensory hub. These results are a preliminary exploration into quantifying perceptual function in PwP and freezers. The strength of the paradigm, it's simplicity and portability, allow studies to be undertaken with other sensory modalities, more complex stimuli as well as variation of timing between stimuli to examine the effect of temporal window of integration.

Future research questions include:

- Are similar patterns seen with other modalities (e.g. haptic stimuli)? Can the complex relationship between proprioceptive and visual processing abnormalities be quantified in PwP using this method?
- Given theat temporal discrimination threshold abnormalities have previously reported in PwP, do PwP have a wider temporal window of multisensory integration? This may explain some including visual hallucinations and illusions commonly reported in advanced PD.
- What is the effect of dopaminergic medication on the above findings? Does dopamine augment or attenuate multisensory facilitation?
- Does deep brain stimulation have an effect on auditory, visual or multisensory processing in PwP?
- Do these perceptual abnormalities correlate with other markers of cognitive or motor performance outlined in this thesis or elsewhere? If so, they may represent a simple, non-invasive biomarker for the disease. Studies on a larger population of PwP would be required to confirm this.
- Can EEG or fMRI be used to identify the neural correlates of this task in PwP v healthy controls? This could probe whether true adaptive changes in cortical perceptual processing is occurring in PD.

With minimal modification, this paradigm could be employed to answer all of the above research questions in the future.

8.2. Cognitive Control of Motor Function in Freezing of Gait

In Chapter 4 (Motor Skill Acquisition and Goal Directed Behaviour in Parkinson's Disease and Freezing of Gait), attention is turned to the contribution of goal-directed motor control to FOG. Freezers exhibit greater deficits in stride length (Chee *et al.*, 2009), stride time, gait symmetry (Frazzitta *et al.*, 2012), rhythmicity (Hausdorff, Schaafsma, *et al.*, 2003; Plotnik and Hausdorff, 2008) and upper limb coordination (Nieuwboer, Vercruysse, *et al.*, 2009) compared with non-freezers suggesting a greater degree of motor impairment is associated with FOG. However, initiation and control of movement is what is of greatest interest in FOG. The loss of automatic motor control due to early denervation in more caudal areas of the basal ganglia leads to greater reliance on goal-directed movement in PwP. Although it has not been formally proposed as mechanism for FOG to date, loss of dopaminergic signalling in more anterior areas of the basal ganglia which govern goal-directed movement could explain the development of FOG in advanced disease. This paradigm has never been employed in PwP previously and allows for quantitative measurement of motor function which takes into account the slowness of movement seen in PwP. Since performance on the Movement Learning trials are normalised by performance on the Continuous Cue trials, the paradigm obtains a measure of motor control which is independent of bradykinesia.

In spite of the hypothesis above, both freezers and non-freezers were able to refine goal-directed behaviour to a level that was comparable to healthy controls. Furthermore, those with FOG performed even better than those without FOG suggesting that, contrary to the above hypothesis, freezers rely to an even greater degree on goal-directed control (probably as a result of longer disease duration). This is further evidence that these patients with advanced Parkinson's disease develop adaptive methods for motor control to compensate. In the absence of automatic habitual control of movement, greater reliance on goal-directed movement takes its place. However, goal-directed movement competes with other cognitive processes. This explains why the dual-task effect is particularly prominent in freezers. Over-reliance on the prefrontal cortex for movement can lead to interference from other cognitive tasks which used similar networks.

Nevertheless, the ability to improve motor performance, as demonstrated in this study is crucial to the success of any training paradigm targeting FOG. Improvements in motor and cognitive function achieved through cognitive engagement and exercise in PwP requires the ability to acquire a motor skill (action acquisition). This study has shown that freezers can acquire motor skills as well as, if not better than,

non-freezers. This has significant implications for the successful rehabilitation of persons with FOG.

Future research questions arising from this chapter include:

- Is there a longterm retention of motor skills? Do PwP retain some ability to convert these tasks to habitual control?
- How do PwP perform on tasks which are entirely habitual rather than goal directed? Are PwP able to switch between goal-directed and habitual control?
- What is the effect of dopaminergic medication on goal-directed learning and motor skill acquisition?
- What is the effect of deep brain stimulation on goal-directed learning and motor skill acquisition?
- In certain genetic forms of PD (such as Parkin), dopaminergic denervation is limited only to nigrostriatal pathways with no involvement of cortex. How does this subset of PwP perform on such a task?
- What are the neural substrates of goal-directed behaviour in PwP vs healthy controls? Does functional MRI reveal increased activation in frontal areas during this task in PwP compared with healthy controls? Do freezers have even greater activation in these areas compared to non-freezers?

8.3. Electrophysiological Markers of Cognitive and Motor Function in Freezing of Gait

There are strong associations between cognitive impairment (in particular, executive dysfunction) and freezing of gait. The main evidence for this comes from bedside neuropsychological testing and behavioural studies of patients with and without FOG which are relatively insensitive ways to assess this. The primary aim of Chapter 5 (Getting Ready To Freeze: Motor Preparation Rather Than Decision-Making Differentiates Parkinson's Disease Patients With And Without Freezing of Gait) was to examine quantitative electrophysiological measures of cognitive function in freezers and non-freezers while seated. Given the previously reported differences in neuropsychological measures of cognitive function, one would expect such differences to be detectable from scalp EEG responses during a cognitive task, i.e. event-related potential (EPR) analysis.

The standard method of ERP analysis was performed initially and revealed differences in P3b amplitude (a robust marker of cognitive function) between freezers and non-freezers. However, the distribution of the response suggested significant frontal activation which was interfering with the centroparietal P3b signal in freezers, leading to an underestimation of its amplitude. Subsequently, a higher resolution approach (current source density, CSD) revealed two distinct signals, a cognitive decision making signal (centroparietal positivity, CPP) and a simultaneously active movement related cortical potential (lateralised readiness potential). The CSD analysis, contradicts the finding from the ERP analysis, showing that the cognitive decision making potential is equivalent between freezers and non-freezers, an unexpected result in view of the literature. The lateralised readiness potential, however, is significantly larger and onsets earlier in freezers than in non-freezers, implying that freezers require a greater amount of cortical activation to initiate movement for a simple task (such as a button press) compared with non-freezers. It has previously been suggested that this greater amplitude may be the result of alternative motor initiation pathways in parkinsonism. It is important to note that these motor preparation differences were seen in freezers in spite of equivalent motor scores and reaction times between groups.

It has previously been suggested that the executive function deficits seen in PwP may be the result of an overload of frontal cortical systems rather than a frontal executive deficit. The correlation seen between lateralised readiness potential amplitude and frontal assessment battery scores seen in Chapter 5 lends support to this theory. The findings support a model in which excessive cortical activation is required for movement to occur, which overloads frontal networks involved in other cognitive processes. When other cognitive processes are required, such as a second cognitive task or manoeuvring through a complex or narrow space, interference occurs. There may also be a threshold effect where the lateralised readiness potential needs to reach a certain amplitude before movement is triggered. If this threshold is higher in freezers it could further explain the all-or-nothing response which occurs with FOG.

These findings create an important insight into the mechanisms underlying FOG, as the known association between executive dysfunction and FOG correlates with excessive motor preparation abnormalities rather than impairment in executive decision making. The earlier, larger lateralized readiness potentials in those with FOG may reflect excessive recruitment of lateral premotor areas to compensate for dysfunction of the supplementary motor area and resultant loss of automatic motor control. It is possible that, even for the simple motor task employed, overload on frontal processing

networks could occur, leading to an apparent impairment in executive function, rather than a primary executive dysfunction. Moreover, with more complex tasks or secondary cognitive tasks, premotor interference could lead to motor impairment and freezing of gait, as well as the behavioural deficits frequently reported in FOG (attentional set-shifting, dual-tasking, response inhibition and conflict) which have previously been attributed to a primary deficit in executive function.

This study provides electroencephalographic evidence of premotor abnormalities exist in freezers compared with non-freezers, even when it is not evident from performance on the motor task. This finding may represent an important biomarker for predicting those PwP who will develop FOG and cognitive impairment, ever before the clinical motor manifestations occur. Some PwP never develop FOG and it is not understood why this is the case.

In addition, the ERP findings raises questions regarding previous ERP studies in PwP (see Appendix II). The vast majority of these have shown delayed or attenuated P3b potentials. If interfering signals such as the lateralised readiness potential were not taken into account in these studies, the potential for misinterpretation of P3b differences exist. The CSD approach undertaken in this study permits separation of these two distinct signals allowing the morphology of each to be accurately assessed and should be considered in all future studies in this population.

Future research questions arising from this chapter include:

- Can greater insight into the interaction between motor preparation and decision making be gained by using alternative tasks, e.g. a Go-No-Go task? This would allow response inhibition as well as decision making to be studied in the context of motor preparation.
- Does spectral analysis of specific frequency bands (e.g. beta frequencies which are associated with motor performance in PwP) shed further insight on motor preparation in FOG?
- What is the effect of dopaminergic medication and deep brain stimulation on decision-making and motor preparation?
- How does the amplitude of CPP and LRP in freezers and non-freezers compare with age-matched healthy controls?
- Using local field potential recordings from the stimulating electrodes of deep brain stimulators along with the methods described here, can the propagation of these cortical signals throught the basal ganglia – cortical network be analysed? This would allow a greater understanding of

movement execution and decision making in a basal ganglia disorder.

8.4 Cognitive Function and Motor Preparation While Stepping

Ambulatory electroencephalography (EEG) is difficult due to the artefact introduced by walking. It has only been employed in human studies in the last 5 years. Surprisingly few ambulatory EEG studies have been undertaken in PwP to date. The primary aim of Chapter 6 ('Neurophysiological Correlates of Decision Making and Motor Preparation While Stepping in Place') was to attempt to reproduce the findings of Chapter 5 while stepping in place. In addition, these data were used to examine the effect of locomotion on decision making (CPP morphology) and motor preparation (LRP morphology) in freezers and non-freezers. No study has examined event-related potentials in PwP or FOG.

The CPP and LRP were readily detectable while stepping in place and showed the same pattern of abnormality as seen in Chapter 5. That is, no significant difference in CPP morphology is seen between freezers and non-freezers. However, the onset of the LRP is earlier in freezers and the amplitude is greater prior to activating a response. Performing the task while stepping, freezers had a greater slowing of reaction time than non-freezers, suggesting a greater dual-task interference. This was associated with loss of the N2b potential and further early recruitment of frontal networks leading to an even earlier onset of LRP.

These findings suggest that, as freezers dual-task, the frontal networks become overloaded, and freezers compensate by initiating movement even earlier. This overload could lead to significant dual-task interference, difficulty with shifting sets and response inhibition errors, all of which have been reported frequently in the FOG literature. The loss of the N2b potential in freezers while walking is significant as this potential has been associated with response monitoring and control as well as response inhibition. Areas associated with response inhibition and resolution of dual task interference include the right inferior frontal gyrus, which is selectively atrophied in freezers compared with non-freezers. Furthermore, the right inferior frontal gyrus inhibits responses via the hyperdirect pathway. This pathway is deficient in PwP compared with healthy controls and functional imaging studies have shown that freezers create adaptive connections with other motor pathways in order to compensate. These findings are in keeping with the abnormal motor preparation seen in the current study.

EEG has two advantages over standard neuroimaging methods: it allows excellent temporal resolution to explore the dynamics of an evolving freezing episode which other neuroimaging studies do not allow;

and it permits data collection while patients are standing, walking or stepping, rather than lying supine in an MRI scanner for example.

Future work should include:

- Using independent component analysis to match the patients stepping frequency and remove artefact caused by locomotion may help to improve the signal-to-noise ratio of the data collected via this method.
- Repetition of this study using cycling rather than stepping in place. Cycling is frequently reported to ameliorate freezing. Is this achieved via to an effect on motor preparation or decision making?
- Further study of instantaneous cortical activity at the onset of actual freezing episodes and and how cortical potentials influence the gait cycle.
- Consideration of other cognitive tasks, effect of medication and deep brain stimulation as described for the sitting study above.

8.5 Combined Sensory, Cognitive and Motor Training for Freezing of Gait

As stated previously, few interventions exist specifically designed to target FOG. Cueing-based physiotherapy approaches which encourage the use of alternative neural pathways to facilitate gait, may further promote adaptive cortical processing during locomotion. The findings in the previous chapters, that difficulty with processing multimodal information information may underlie FOG, motivated the design of an intervention for FOG which aimed to train freezers to process sensory, motor and cognitive information simultaneously. Thus, the information processing deficiencies were probed with a training program which consistently presenting freezers with situations in which FOG is likely to occur. Rather than facilitate alternative locomotor networks, it forces participants to overtrain the traditional locomotor pathways in the face of competing and interfering information from other modalities. This training program, presented in Chapter 7 (Combined perceptual, motor and cognitive intervention for FOG) has shown improvements in gait parameters while stepping in place, walking speed in real environments, dual-tasking ability, cognitive flexibility and self-reported freezing of gait.

It is clear that the combination of multiple modalities in training has beneficial effects, particularly in the freezing cohort. It is unclear whether this was due to greater room for improvement in the freezing

group (which had an overall greater disease duration and worse motor performance at baseline) and a ceiling effect in the non-freezers or whether it represents a specific effect on FOG.

Recently the literature has suggested that cognitive-based training, rather than physical training, may be beneficial in treating FOG (Walton *et al.*, 2014) as early cognitive interventions can improve both cognition and gait in PwP (Milman *et al.*, 2014). Similarly, there is an emerging literature showing that goal-directed exercise improves motor and cognitive function in PwP via effects at the molecular, synaptic and network level (Petzinger *et al.*, 2013). However, the potentially additive effect of these two approaches has not been explored until now. None of these studies have focused on cohorts with advanced PD or FOG. The current study, however, showed benefit in patients with advanced cognitive and motor dysfunction and it is possible that the intervention may have even greater effects if implemented early in the disease course. Incorporating such training from the time of diagnosis may even prevent or delay onset of FOG in PwP and have positive effects of overall gait and cognition.

There is a significant amount of future work to be undertaken in this area. Remaining research questions include:

- What are the relative contribitions of the perceptual, cognitive and motor elements in the improvements seen here? Isolating which elements of the intervention have the greatest effect will allow refinement of such interventions in the future.
- Were the greater improvements seen in the freezing group due to a specific freezing effect or the result of a ceiling effect on the less severely affected non-freezing group? The study would need to be repeated with a larger sample size including non-freezeres with advanced and severe PD to explore this.
- Is this intervention more effective than standard treatment approaches? A blinded randomised controlled trial including a large number of PwP with and without FOG should be undertaken to examine the efficacy of this intervention compared to standard physiotherapy or cueing-based techniques.
- Can this intervention be translated safely and effectively in a home environment? If so would remote monitoring of performance improve patient outcomes and quality of life?
- What is the effect of this intervention on patients with new / recent onset FOG? Could it potentially reverse the appearance of freezing in patients before significant adaptive changes take place?

- If such a training program was initiated prospectively in patients in early PD, could it prevent or retard development of FOG and/or cognitive impairment and would it have an overall effect of progression of motor impairment and severity of disease?

8.6 Impact of Current Work

Our present knowledge of FOG pathophysiology is limited. Evidence suggests that perceptual/sensory, motor and cognitive elements are at play. However, quantitative measures of perceptual, motor and cognitive function in PwP are lacking. Consistent quantitative measures of these factors are required to allow reliable FOG studies with different experimental designs and research questions to be compared in a meaningful way. Without them, each FOG study becomes an isolated island of information, an incremental piece in the jigsaw puzzle. In addition, these quanitative measures could represent metrics of response to any targeted therapy for PD or FOG. Importantly, the measures need to be independent of each other in order to allow a greater understanding of their individual contribution to FOG.

This thesis presents a number of novel studies:

- Chapter 3 presents the first study quantitatively assessing pure unisensory and multisensory processing differences between modalities in PwP, showing relative sensory changes in PwP which correlate with FOG. This provides quantitative evidence (independent of speed of movement or cognitive function) to support the neurobehavioural literature suggesting sensory disturbances in FOG.
- In Chapter 4, goal-directed motor skill acquisition is studied for the first time in FOG, confirming superior performance in a goal-directed task compared with non-freezers.
- In spite of the wealth of literature supporting cognitive and motor preparation deficits in FOG, no study has examined event-related potentials or motor readiness potentials in FOG. Chapter 5 reveals that there are no significant differences in markers of cognitive function between freezers and non-freezers but that freezers require significantly greater cortical activation to initiate movement. The CSD approach used here shows the advantage of its superior spatial resolution in separating disparate cortical potentials. Only one study to date has employed this approach in PwP (van Wouwe *et al.*, 2014).

- No study has performed ambulatory event-related potentials in PwP. The study in Chapter 6 is the first ambulatory ERP study in PwP or FOG and one of only a few ambulatory EEG studies in PwP or FOG. It confirmed that these evoked potentials can be readily detected while stepping in place in PwP and reinforced the findings of Chapter 5. In addition, the effects of dual-tasking on cognitive and motor potentials in freezers has shown additional premotor abnormalities occur (loss of N2b potential) only in the freezing group while stepping. This links these premotor abnormalities with response inhibition and conflict resolution which is frequently described in FOG.
- Chapter 7 presents many novel findings. It includes the first study combining perceptual, motor and cognitive training for PwP or FOG which shows significant benefits in dual-tasking, gait speed, self reported freezing and possibly cognitive flexibility. The platform presented here combines an assessment paradigm as well as an intervention using different virtual reality scenarios for each. This allows translation of the portable device for home use, where both assessment and training can take place with remote monitoring.

The results from the studies presented in this thesis imply that FOG is not problem caused by primary motor dysfunction but by overload of perceptual, motor and cognitive information, leading to paroxysmal loss of motor output. The loss of efficient, automatic, habitual control in parkinsonism forces PwP to rely on resource-intensive cognitively controlled goal-directed behaviour in order to perform the simplest of motor tasks. This requires excessive cortical activation to perform complex motor tasks such as locomotion, which becomes sensitive to interference from dual tasks. These dual tasks can take the form of a second motor task or a cognitive task or even the processing of sensory information during the primary task. In freezers, the activation required to prepare a movement is significantly greater than in non-freezers. This means that dual-tasking is likely to have a much greater effect on freezers given the limited cortical resources available. Furthermore, the way in which PwP process multimodal sensory information is also altered. However, it remains unclear whether this is a primarily pathological phenomenon or merely an adaptive process to compensate for the parkinsonian state. It is clear, however, that the processing of sensorimotor information is highly abnormal in PwP and that these changes are greater for patients with FOG than those without. It is likely that this excessive information at the cortical level in freezers reaches a bottleneck at the level of the basal ganglia, which is deficient, leading to excessive inhibition from the basal ganglia output nuclei, leading to motor arrests.

It would be naïve to suggest that FOG results from either cortical or subcortical dysfunction alone. It is clear that more severe striatal dopaminergic denervation and additional cortical dysfunction occur in freezers. The striatal dopamine depletion, however, appears to place an extra strain on compensatory cortical processing. The cortex of freezers must therefore adapt to this extra burden and may do so in a maladaptive way. These neurodegenerative and subsequent adaptive/maladaptive changes are insufficient to explain the episodic nature of freezing. Further burden from a perceptual, motor, cognitive or emotional stressors may be required to provoke a freezing episode.

Superior goal-directed cognitive control of movement as well as equivalent electrophysiological markers of cognition support that, although apparent differences in cognitive function exist between freezers and non-freezers, it may reflect an overall problem with cortical processing of information rather than a primary deficit in cognition. Abnormalities in premotor and sensorimotor processing in individuals who lack the efficient streamlined automatic habitual control of movement clearly leads to problems where percetual, motor and cognitive processes compete for limited resources, resulting in overload. It may however be possible to train this capacity to reduce this overload effect. In order to maximise training capacity, interventions should include simultaneous sensory, motor and cognitive processing.

8.7 Limitations

The sample sizes in the studies presented in this thesis are small. As a result, subgroup analyses have not been possible. They were designed as preliminary studies in order to investigate the hypotheses outlined in Chapter 2. These studies represent a significant departure from the current state of the art in FOG research. For this reason, determining sample sizes with sufficient power was not always possible and sample sizes were chosen that were both practically possible and similar to the studies in the current FOG literature. If the studies were repeated with larger samples, examination of particular subtypes of FOG would be possible (e.g. on-FOG vs off-FOG, FOG triggered by initiation vs turning vs narrow spaces). It has been suggested that FOG may be a final common pathway for a number of different impairments and exploring the relative perceptual, motor and cognitive effects in each subgroup may shed light on the clinical spectrum of FOG. This, in turn, may help focus therapeutic intervention targets for each clinical subtype.

All studies were performed on medication. Although this makes the studies more ecological, this complicates interpretation of results as dopaminergic therapy influences perceptual, motor and cognitive function in PwP. Many of the participants in these studies has severe, advanced parkinsonism and stopping their medications would have precluded partipation in these studies for many of them. Although there were no statistically significant differences in medication doses and timings in group comparisons, a medication effect can not be excluded. The studies in this thesis should be repeat in the off-medication state where possible to examine this effect. However, the ecological validity of studying Parkinson's patients in the dopamine withdrawal state has its own limitations and may complicate interpretation.

The experimental paradigms and analysis methods employed here provide a set of tools to quantitatively examine perceptual, motor and cognitive function independently. Although it was applied to a specific clinical group (PwP with FOG), these methods could be utilised to answer specific research questions in Parkinson's disease in general, such as:

- The effect of dopaminergic medication of sensory processing, goal-directed motor control, motor preparation and executive function In PD.
- The effect of deep brain stimulation (at various sites) on perceptual, motor and cognitive function in PD.
- The differences in sensorimotor and cognitive function in subtypes of PD (tremor predominant vs postural instability and gait disorder vs rapidly progressive) and genetic forms of PD.

Finally, these tools could be applied to other movement and neurodegenerative disorders creating a framework for examining perceptual, motor and cognitive function in a wide range of pathological states.

8.8 Conclusion

Early perceptual and motor signalling is impaired and inefficient in people with FOG. These processes, which are crucial to complex tasks such as locomotion, can be compensated for at the expense of attentional and cognitive resources, which are limited. Therefore, when multiple motor and cognitive tasks take place in a complex sensory environment, this dual-task capacity becomes easily depleted

probably leading to FOG. Fortunately, this capacity can be expanded with training, resulting in improvements not only in FOG but also overall gait performance and potentially cognition.

A. Appendix I: Supplementary Studies of Motor Skill Acquisition

A.1 All PwP vs Healthy Controls (Pooled Right and Left Hands)

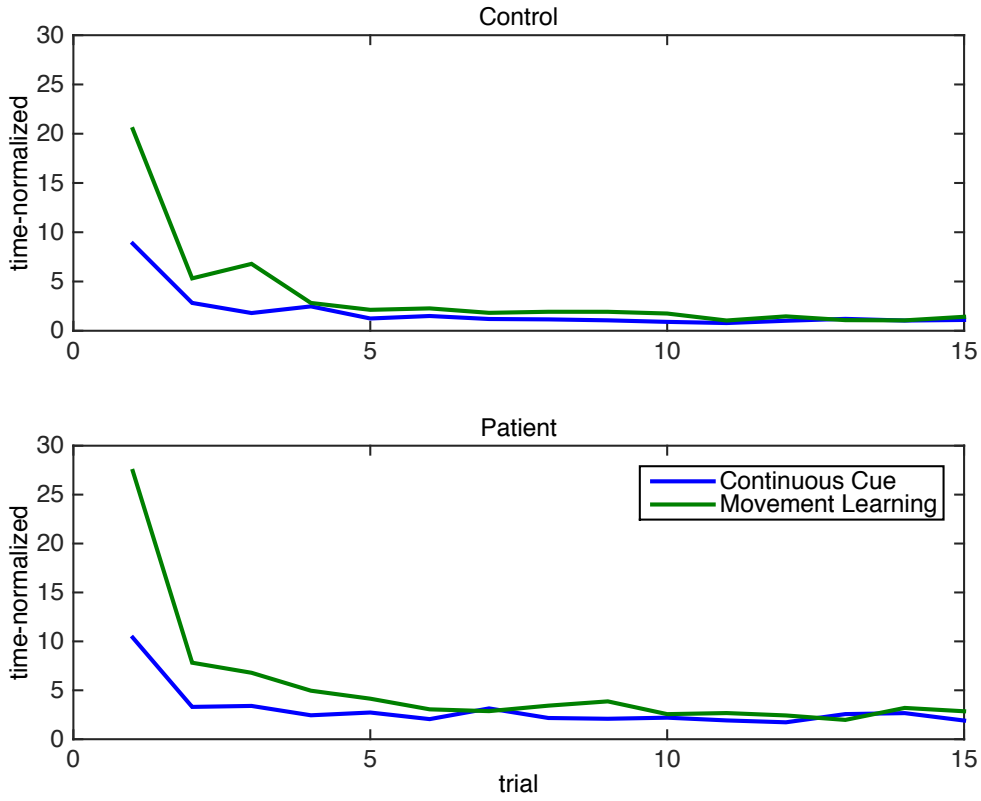


Figure A.1. Motor learning for all PwP vs Healthy Controls (pooled right and left hands): *Mean learning curves (time taken to find target in secs vs trial number) of Movement Learning task (green) and Continuous Cue task (blue) for all trials (right and left hand trials pooled) of healthy controls (upper plot) and PwP (lower plot).*

ML-CC	Controls		PwP		Difference
	Mean	SD	Mean	SD	p-value
Trials 1-5	4.07	16.19	5.77	21.12	0.223
Trials 6-10	0.78	3.12	0.82	7.90	0.921
Trials 11-15	0.18	1.75	0.47	6.10	0.432

Table A.1. Group differences by trial for all PwP vs Healthy Controls (pooled right and left hands): *Mean and standard deviations of difference (in secs) between movement learning (ML) and continuous cue (CC) task for all trials (right and left hands) of healthy controls and PwP.*

A.2 Dominant Hands vs Non-Dominant Hands (Healthy Controls)

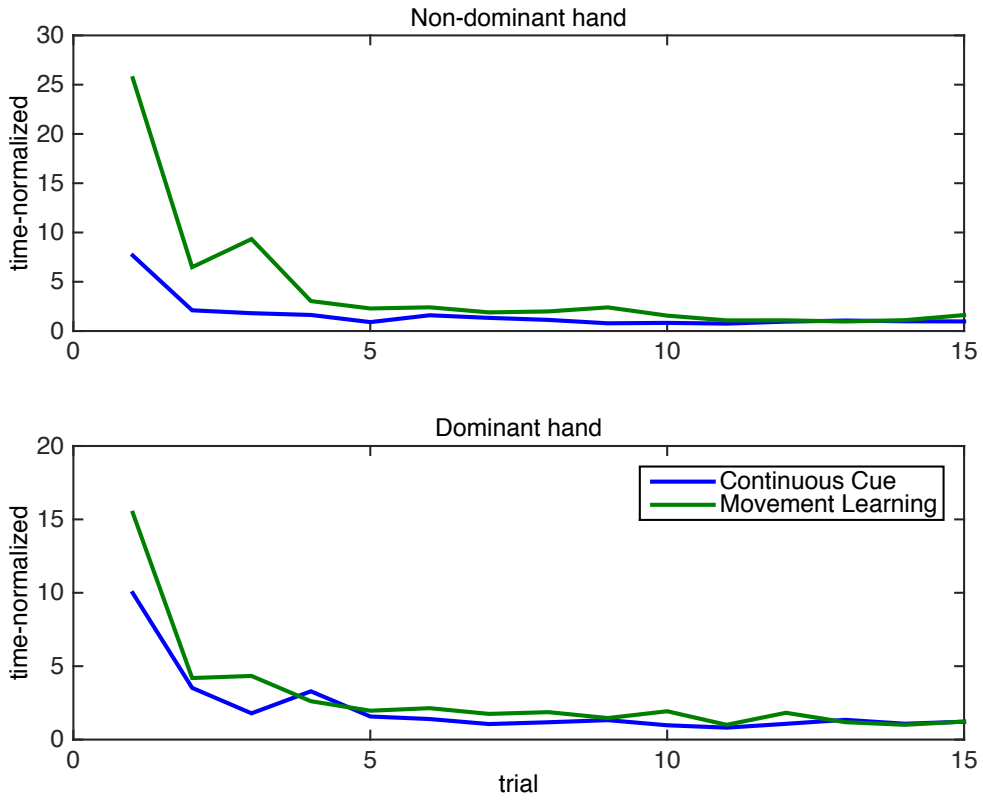


Figure A.2. Motor learning for dominant hands vs Non-dominant hands (pooled right and left hands): *Mean learning curves (time taken to find target in secs vs trial number) of Movement Learning task (green) and Continuous Cue task (blue) for all trials of non-dominant hands (upper plot) and dominant hands (lower plot) of healthy controls.*

ML-CC	***Non-Dominant***		***Dominant***		***Difference***
	Mean	*SD*	*Mean*	*SD*	*p-value*
Trials 1-5	6.54	19.76	1.68	11.33	**0.010***
Trials 6-10	0.92	3.38	0.64	2.85	0.456
Trials 11-15	0.22	1.34	0.14	2.08	0.702

Table A.2. Group differences by trial for dominant hands vs non-dominant hands: *Mean and standard deviations of difference (in secs) between movement learning (ML) and continuous cue (CC) task for non-dominant hand trials and dominant hand trials of healthy controls.*

A.3 Most-Affected Hand vs Least Affected Hand (Healthy Controls)

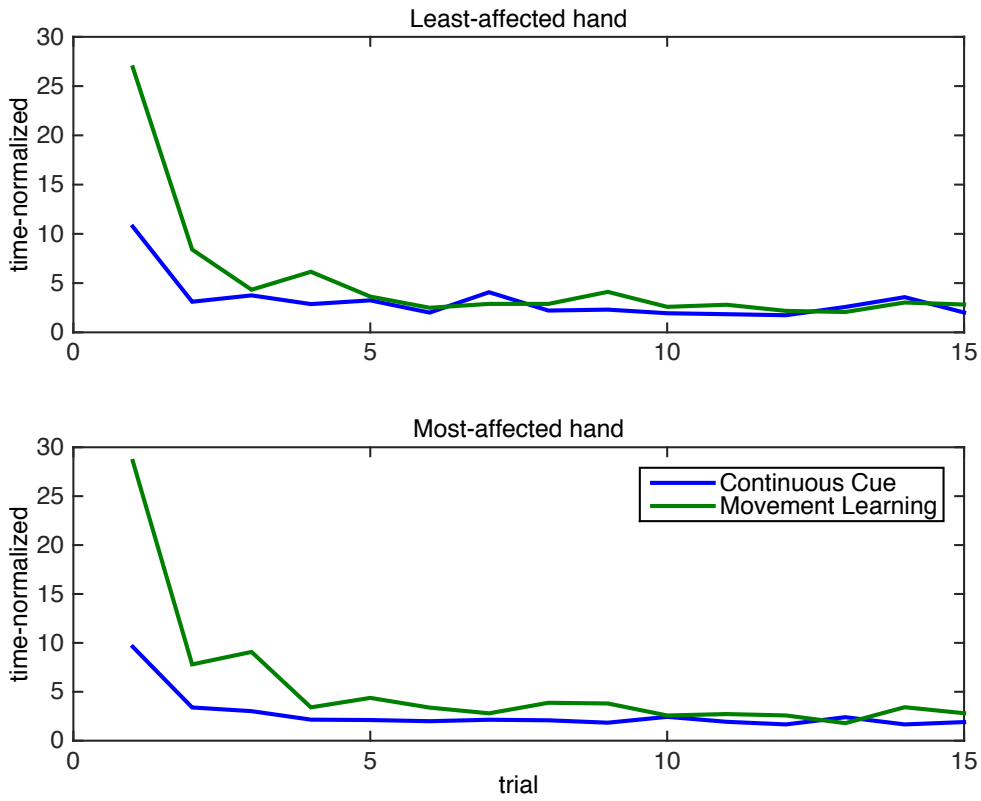


Figure A.3. Motor learning for most-affected hand vs Least-affected hands (pooled right and left hands): *Mean learning curves (time taken to find target in secs vs trial number) of Movement Learning task (green) and Continuous Cue task (blue) for all trials of least-affected hands (upper plot) and most-affected hands (lower plot) of PwP.*

ML-CC	***Least-Affected***		***Most-Affected***		***Difference***
	Mean	*SD*	*Mean*	*SD*	*p-value*
Trials 1-5	5.14	17.45	6.59	24.08	0.388
Trials 6-10	0.49	8.45	1.19	6.97	0.259
Trials 11-15	0.23	6.46	0.75	5.49	0.278

Table A.3. Group differences by trial for least-affected hand vs most-affected hand: *Mean and standard deviations of difference (in secs) between movement learning (ML) and continuous cue (CC) task for least-affected hand trials and most-affected hand trials of PwP.*

B. Appendix II: P3b Studies in Parkinson's Disease

STUDY	SUBJECTS	METHOD	RESULTS
IJIMA, ET AL., BEHAV NEUROL, 2000	20 Non-demented PD patients; 55 age matched healthy controls	2-stimulus auditory oddball task	P300 latency increased in PD which correlated with subcategories of Wisconsin Card sorting test No difference in P300 amplitude
LOPES ET AL., ARQ. NEURO-PSIQUIATR., 2014	44 Non-demented PD patients; 33 age matched controls	2-stimulus auditory oddball task	Increased P300 latency in PD patients and correlates with disease severity as measured by H+Y stage in older subjects but does not correlate with UPDRS III. A correlation was found with UPDRS II however.
MATSUI ET AL., PARKINSONISM RELAT DISORD., 2007	40 PD patients (13 with dementia, 27 without)	2-stimulus auditory oddball task	Increased P300 latency in those with dementia compared to those without which correlated with scores of attention on Dementia Rating Scale.
KATSAROU ET AL., PERCEPTUAL AND MOTOR SKILLS, 2004	45 non-demented PD patients; 40 age matched controls	2-stimulus auditory oddball task	Increased P300 latency in PD which correlates with Raven Colored Progressive Matrices and the Wisconsin Card-sorting Test.
MAESHIMA ET AL., BRAIN INJURY 2002	30 PD patientsl 118 healthy controls	2-stimulus auditory oddball task	8/30 patients had increased P300 latency. Correlated with MMSE score and cognitive items on Functional Independence Measure. P300 amplitude correlated with performance IQ Raven's Coloured Progressive Matrices and motor items of the Functional Independence Measure
BODIS-WOLLNER ET AL., J NEURAL TRANSMISSION, 1995	30 Non-demented PD patients	2-stimulus auditory and visual oddball tasks	Correlation between P300 and N2 latencies and cognitive scores
HANSCH ET AL., ANN NEUROL 1982	20 PD patients and 20 age-matched controls	2-stimulus visual oddball task	Increased P2 and P300 latency in PD group. P300 latency correlated with Symbol Digit Modalities test.
GOODIN ET AL., ANN NEUROL, 1986	28 patients with PD (14 with dementia, 14 without)	2-stimulus auditory oddball task	Increased N1, N2, P3 latency in demented group.
PRABHAKAR ET AL., NEUROLOGY INDIA, 2000	25 newly diagnosed PD patients before, 15 days, 3 and 6 months after commencement of dopaminergic therapy; 20 healthy controls	2-stimulus auditory oddball task	No significant difference in P300 between patients and controls before dopaminergic therapy. P300 latency reduced at 15 days post-treatment but increased again at 6 months.
JIANG ET AL. PHYSIOLOGY AND BEHAVIOR, 2000	12 Non-demented PD patients; 9 age matched healthy controls	2-stimulus auditory oddball task	N1 and P300 latencies were significantly longer in PD than in control subjects. N100 latencies progressively lengthened with habituation in PD group only.
ELWAN ET AL, J NEUROL SCI, 1996	43 PD patients; 37 healthy controls	2-stimulus auditory oddball task	P300 latencies prolonged in PD. No correlation with age, duration of illness, UPDRS or cognitive markers.
O'DONNELL ET AL., BIOLOGICAL PSYCHOLOGY, 1987	16 PD patients; 11 controls	2-stimulus auditory oddball task	No difference in N1 latency, but N2 and P3 latency prolonged in PD which correlated with cognitive testing.
SOHN ET AL, J NEUROL SCI, 1998	19 de novo PD patientsm 18 PD patients on long term	2-stimulus auditory oddball task	In levodopa-treated patients, the P300 latency was longer than controls but shorter than de novo patients. Longterm levodopa therapy shortened the P300 in the de novo

	dopaminergic therapy, 15 age-matched healthy controls		patients
NASKAR ET AL, PARKINSONSISM RELAT DISORD, 2010	10 PD patients, on- and off-STN-DBS	2-stimulus auditory oddball task	No change in P300 amplitude or latency with DBS on. Increase in N100 latency after DBS switched on.
TODA ET AL.,J GERIATR PSYCHIATRY NEUROL, 1993	35 PD patients (9 with dementia and 26 without) and 15 age-matched healthy controls	2-stimulus visual oddball task	P300 latency and reaction time prolonged compared with controls. Only reaction time prolonged in non-demented patients compared with controls
WANG ET AL., DOC OPHTHALMOL, 2001	13 PD patients; 18 age matched healthy controls	2 stimulus visual oddball task	Reduced N1 latency and amplitude in PD. N1 correlated with rCBF in a global region.
GERSCHLAGER ET AL., J NEUROL 2001	8 PD patients, on- and off-STN-DBS ; 8 age matched healthy controls	2-stimulus auditory oddball task	Prolonged P300 latencies in PD off stimulation which did not improve with stimulator on.
TANAKA ET AL., DEMENT GERIATR COGN DISORD, 2000	29 PD patients; 11 age-matched healthy controls	2-stimulus auditory oddball task	Increased N1 and P300 amplitude and total power in non-demented PD. Power and P300 latency increased with cognitive impairment (as measured by MMSE)
LAGOPOULOS ET AL, MOV DISORD, 1998	15 PD patients; 50 normal controls	2-stimulus auditory oddball task	Reduced N2 amplitude with topographical N2 differences at central and temporal regions
LAGOPOULOS ET AL, NEUROL RES, 1998	15 PD patients; 50 normal controls	2-stimulus auditory oddball task	Increased P300 latency in PD group
WRIGHT ET AL., PARKINSONISM RELAT DISORD, 1996	17 Non-demented PD patints; 28 age matched controls	2-stimulus auditory oddball task	Reduced N1 amplitude to target and standard stimuli in PD, but no difference in P2, N2, P300 amplitudes. P2, N2, P300 latency increased in PD when targets counted. Only N2 latency increased when targets identified by button press.
ANTAL ET AL., J NEURAL TRANSM., 1996	20 non-demented PD patients; 20 age-matched controls	2-stimulus visual oddball task	No difference in P300 latency. P300 amplitude and N200 amplitude differed in PD patients and controls. Central processing time (P300 latency – P1 latency) increased in younger PD patients compared with older.
PRASHER ET AL., JNNP, 1991	27 de novo PD patients, pre- and post-dopaminergic therapy; 27 age-matched controls	2-stimulus auditory oddball task	No difference in N1, N2 or P300 latency between PD and controls. Increased P3 latency following dopaminergic therapy.
GREEN ET AL., MOV DISORD, 1996	10 younger and 10 older unmedicated PD patients diagnosed within last 4 years, 10 age-matched healthy controls	2-stimulus auditory oddball task	Enlarged P300 amplitude at Cz and Pz in early PD patients (unaffected by age). No difference in P300 latency compared with controls.
RUMBACH, ET AL., J NEUROL SCI, 1993	26 patients with PD and age-matched controls	2-stimulus auditory oddball task	Increased latency of P300 in PD which correlated with disease duration but not cognitive measures. N2 and P300 latencies increased with dopaminergic therapy in spite of motor improvements.
EBMEIER ET AL., BIOL PSYCHOL, 1992	16 Non-demented PD patients; 16 age-matched healthy controls	2-stimulus auditory oddball task	Increased P2 and N2 latency but no increase in P300 latency in PD. No difference in response initiation or reaction time. Increased peak latencies (esp N2) were associated with motor impairment and visuospatial task performance.
TARD ET AL., PARKINSONISM	30 patients with PD (15 with and 15	2-stimulus auditory	No difference in P300 amplitude between groups.

RELAT DISORD, 2014	without FOG); 15 age matched controls	oddball task	
KLOSTERMANN ET AL., MOV DISORD, 2010	10 PD patients on and off STN-DBS; 10 healthy controls	2-stimulus visual oddball task	Increased P300 latencies in PD. No effect of DBS on ERP.
WANG ET AL., JNNP, 1999	38 Non-demented PD patients; 24 age-matched healthy controls	2-stimulus visual oddball task	Progressive increase in P300 latency with increasing ISI. Increased P300 latency in PD compared with controls at long ISI only Reduced P300 amplitude and delayed N2 at all ISIs
WANG ETA L., J NEUROL SCI, 1999	28 Non-demented PD patients; 24 age matched healthy controls	2-stimulus visual oddball task	P300 latency correlated with agem age at onset and duration of illness.
KOVACS ET AL, PARKINSONISM RELAT DISORD, 2008	23 PD patients, on- and off-STN-DBS ; 14 healthy controls	2-stimulus auditory oddball task	No change in ERP components on v off DBS in spite of improved behavioural responses. Frontocentral P300 amplitudes correlated with optimal stimulations voltage and P300 latency correlated with disease duration
LI ET AL, PARKINSONISM RELAT DISORD, 2008	34 PD patients; 26 controls	2-stimulus visual oddball task	Increased P1 amplitude, reduced N1 latency,, increased N1 amplitude, increased P2 amplitude, increased N2 latency, reduced N2 ampkitudem reduced P300 amplitude in PD. Abnormal ERP changes correlated with performance on Wechsler Adult Intelligece Scale-Revised and UPDRS.

Table B.1. Review of two-stimulus oddball tasks in PwP to date: *Authors and title, number and type of participants, task and results are given.*

C. Appendix III: Individual Participant Responses

C.1 Individual Participant ERP Responses

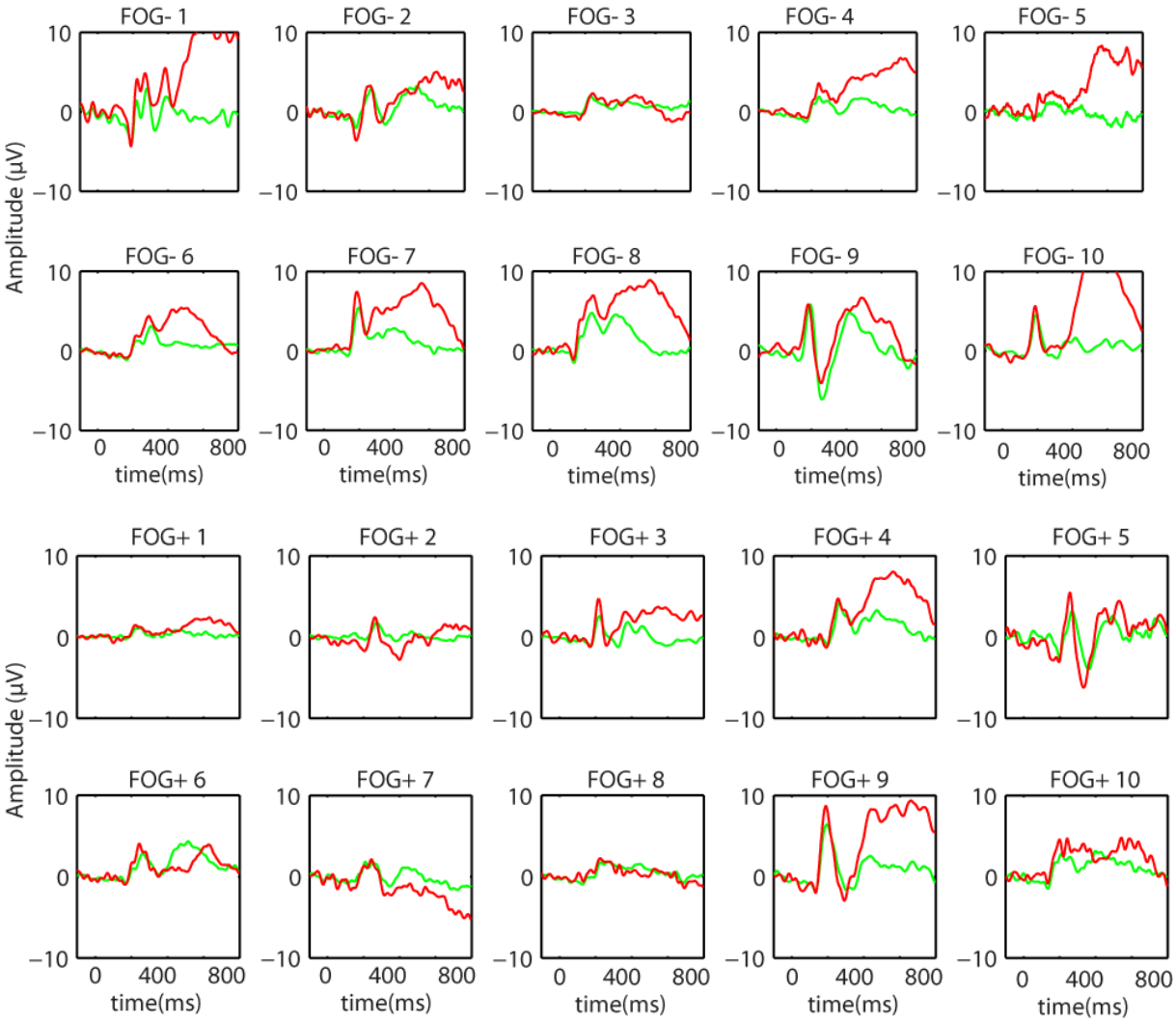


Figure C.1.Individual Participant ERP Responses. *Individual participant waveforms for ERP response to target (red) and standard (green) stimulus for the ten non-freezers (FOG-, upper plots) and the ten freezers (FOG+, lower plots).*

C.2 Individual Participant CSD Responses

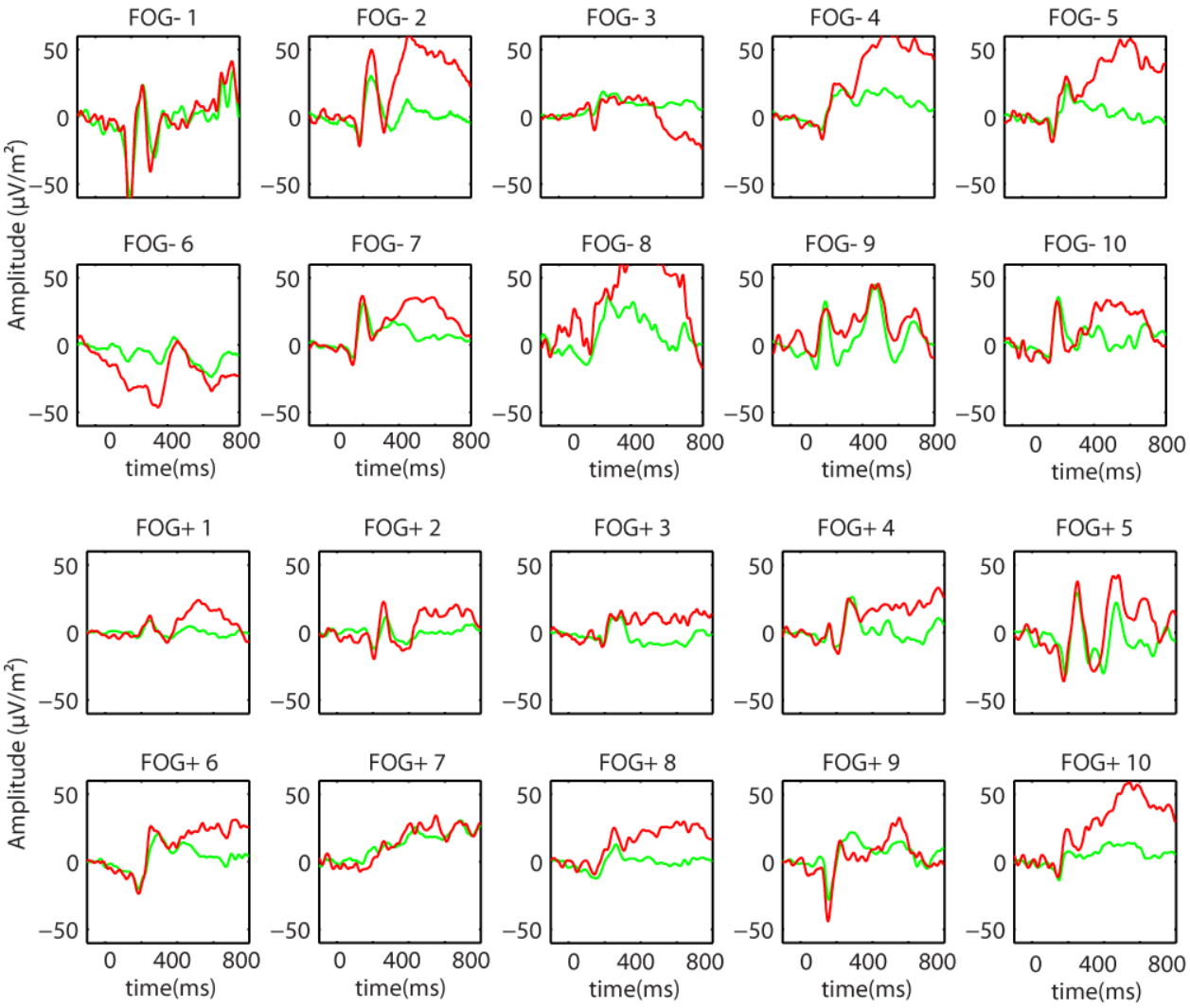


Figure C.2.Individual Participant CSD Responses. *Individual participant waveforms for CSD response to target (red) and standard (green) stimulus for the ten non-freezers (FOG-, upper plots) and the ten freezers (FOG+, lower plots).*

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