Magnetic resonance imaging of saccadic eye movements in Parkinson’s disease

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Abstract

Parkinson’s disease (PD) is a progressive neurodegenerative disorder with cardinal signs of bradykinesia, tremor, rigidity and postural instability. Current PD treatment only alleviate symptoms of PD, however, there is an intense research focus on identifying disease-modifying therapies. No biochemical or laboratory marker exists for PD progression. An objective and repeatable biomarker would allow assessment of the efficacy of novel disease-modifying therapies, as well as allowing precisely tailored treatment.

Saccadic eye movements are an accurately measured and easily repeatable performance measure. Saccades show characteristic impairments in PD and show promise as the basis of a novel biomarker. Our current understanding of the causes of saccade performance deficits in PD is speculative. This thesis will examine saccades in PD using MRI, aiming to develop a better understanding of PD processes affecting eye movements and assessing MRI analyses for future studies into this area.

Eye movement and MRI data from 96 PD and 33 controls from the New Zealand Brain Research Institute was analysed in the first study using voxel-based whole-brain comparisons of structural, perfusion and diffusion MRI, with visually-guided and volitional saccade tasks. The results largely indicated no significant group differences; however subtle structural and perfusion group-differences were detected in the temporal lobe and precuneus. A characteristic PD-related perfusion pattern has been previously identified for 68 PD and 24 controls of this study, the extent of which is expressed by a network score in a given individual. This network score was compared with saccadic eye movement performance of these participants. Every eye movement measure significantly correlated with the network score, supporting the use of this score as a biomarker of general PD status. Furthermore, increased hypoperfusion in the areas described by the network score may contribute, in part, to eye movement deficits in PD.

In the next study, 16 PD and 16 controls underwent functional MRI scanning while performing reflexive and predictive saccades. Group-level activity differences were not detected, however, the medial frontal eye field (FEF) subregions demonstrated greater activity in the reflexive task compared to the predictive. This finding is novel and FEF subregions have not been explicitly described to relate to reflexive or predictive saccade tasks. This is relevant in the context of our overall understanding of eye movement control.
The final study investigated memory-guided saccades in PD using functional MRI. Blood-oxygen level dependent (BOLD) activity in the bilateral posterior parietal cortex was altered in PD patients compared to controls during this task. Additional principal component (PCA) and K-means clustering analyses were able to identify regions exhibiting similar BOLD time courses during task performance. This supports the ability of PCA to identify key components patterns related to function, from BOLD time course data.

This study of eye movements in PD using MRI has identified several areas for investigation in future studies. This study was cross-sectional but has allowed us to establish initial benchmark findings for future comparison, and has helped validate analysis methods for future longitudinal studies into eye movements and PD using MRI.
Acknowledgements

Words cannot express my overwhelming gratitude to my supervisors, Dr. Michael MacAskill, Prof. Tim Anderson, Dr. Toni Pitcher and Dr. Tracy Melzer for their most unparalleled guidance and never-ending patience. I am especially grateful for always having an open door for discussion, often needed at unsavoury hours of the day/night. Their kind accommodation and expertise offered to me during those times were what made this thesis possible.

I offer my most sincere gratitude to all the participants who had given up their time, often during the weekend, to lie in a cramped and noisy scanner, in order to further our knowledge of the human brain. I am also grateful for the financial support of the University of Otago Doctoral Scholarship during my extended course of study for the combined MBChB/PhD programme.

During my time at the New Zealand Brain Research Institute, I have worked with the most intelligent and supportive colleagues, whose attitude and humour made for the most positive and enjoyable PhD experience, even through all the difficult challenges. I must thank Ms. Leslie Livingston for her tireless recruiting effort and foresight to assess patients in my study group before I left to restart medical school midway through my research. I would like to thank Mrs. Kathryn Mulcock for her assistance in administrative issues and short-notice meeting planning. I’d like to thank my medical PhD colleagues Dr. Eng Toh and Dr. Yassar Alamri, with whom I feel privileged to have shared this combined degree experience with, and who have provided me with endless conversation and humour. I’d like to make special mention of the late-night research crew of Ms. Beth Elias, Mrs. Kyla-Louise Horne and Mr. Mustafa Almuqbel for the encouragement and drive to finish my thesis during the final stages of my write-up.

Outside of the laboratory, I must give my sincerest thanks to Dr. Ruth Helms and Mr. Stephen Sharp, along with the rest of the administrative team at the University of Otago, Christchurch School of Medicine. Your support and advocacy during my years of study really made a difference.

I would finally like to thank my family. To Mum and Dad, your unconditional love and support got me to where I am today. I hope I have made you proud.
Preface

This thesis is submitted as a requirement for the Doctor of Philosophy as part of an intercalated Bachelor of Medicine and Bachelor of Surgery and Doctor of Philosophy (MBChB/PhD) degree in the Department of Medicine at the University of Otago, Christchurch. The research conducted for this thesis was undertaken at the New Zealand Brain Research Institute (formerly known as the Van der Veer Institute for Parkinson’s Disease and Brain Research) under the supervision of Dr. Michael MacAskill, Prof. Tim Anderson, Dr. Toni Pitcher and Dr. Tracy Melzer. This study was made possible from two grants from the CMRF for the studies under the titles "Saccadic function in Parkinson's disease: an fMRI study" and "Can advanced MRI and saccade parameters faithfully measure progression in Parkinson’s disease?". Ethics approval documents are in the appendix pages 359-360.

A number of studies within this thesis were only made possible with the work and help of others. The work of collaborators is described for each chapter separately below.

Chapter 3

The imaging data and eye movement measurements used in this chapter were collected as part of a long-term longitudinal PD study at the New Zealand Brain Research Institute. Eye movement tasks for this study were originally designed by Dr. Michael MacAskill. Eye movement performance measures were obtained by Dr. Toni Pitcher and Dr. Charlotte Graham. MRI preprocessing was done by Dr. Toni Pitcher for a portion of the participants and another portion by myself. Neuropsychiatric scoring and motor assessment were performed by Dr. Toni Pitcher, Dr. Charlotte Graham and Ms. Leslie Livingston. MRI data used for this chapter was collected by Dr. Tracy Melzer, with assistance from Christchurch Radiology Group technicians Mr. Gareth Leeper, Mr. Simon Felton and Mr. Steven Kingston-Smith. I performed all the statistical analysis, using the post-processed MRI data and eye movement performance measures. I created all comparisons, tables and images for this chapter.

Chapter 4

The ASL network score was generated as part of a previous study investigating the potential of developing a perfusion based measure of PD progression by Dr. Tracy Melzer (2011). The original score used data from 61 PD patients. This score was updated with additional PD
patients, totalling 73 and was made using the same technique as described in the original Melzer paper. This updated network score is not yet published as of 2017. The participants used to construct the network score were from the same group used in the ongoing longitudinal PD study at the New Zealand Brain Research Institute as Chapter 3. Eye movement performance measures were obtained by Dr. Toni Pitcher and Dr. Charlotte Graham as in the previous chapter. Similarly, neuropsychiatric scoring and motor assessment were performed by Dr. Toni Pitcher, Dr. Charlotte Graham and Ms. Leslie Livingston, as in the previous chapter. I performed all the statistical testing using the ASL network score and eye movement measures. The graphs were initially generated by myself and were graphically refined by Dr. Michael MacAskill.

Chapter 5

The participants in this study were recruited by myself, with the help of Ms. Leslie Livingston. These participants were newly recruited to be involved in both my study and the ongoing longitudinal PD study at the New Zealand Brain Research Institute. Eye movement tasks designed to be displayed during fMRI were initially developed by Dr. Michael MacAskill, based on the tasks used in the longitudinal studies. I further developed and refined the eye movement paradigms to be used with the fMRI scanning sequence by modifying the original code to construct the task sequence to be displayed in the MRI machine. I oversaw acquisition of the MRI data for these participants with supervision from Dr. Tracy Melzer, with assistance from Christchurch Radiology Group technicians Mr. Gareth Leeper, Mr. Simon Felton and Mr. Steven Kingston-Smith. I preprocessed all scan data for this chapter and collected and measured eye movement performance for all tasks used in this chapter. I attended a workshop for the software package SPM8, hosted in Sydney Australia, which provided me the knowledge in the use of this program for neuroimaging analysis. Dr Karolina Marak provided fMRI analysis advice. I performed all the imaging analysis for the fMRI experiments and all whole-brain comparisons between MRI data and eye movement measures. All figures and plots of the study results were produced by myself.

Chapter 6

The principal component analysis (PCA) and cluster analysis was performed by Dr Tracy Melzer, with interpretation by myself. Otherwise, the contributions for this chapter are as described in Chapter 5.
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<tr>
<td>AG</td>
<td>Angular Gyrus</td>
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<tr>
<td>ASL</td>
<td>Arterial Spin Labelling</td>
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<td>BA</td>
<td>Brodmann Area</td>
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<td>BOLD</td>
<td>Blood Oxygen Level Dependent</td>
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<td>CN</td>
<td>Caudate Nucleus</td>
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<tr>
<td>CNS</td>
<td>Central Nervous System</td>
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<td>CSF</td>
<td>Cerebrospinal Fluid</td>
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<tr>
<td>DBS</td>
<td>Deep Brain Stimulation</td>
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<tr>
<td>DLPFC</td>
<td>Dorsolateral Prefrontal Cortex</td>
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<td>DTI</td>
<td>Diffusion Tensor Imaging</td>
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<tr>
<td>EEG</td>
<td>Electroencephalography</td>
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<tr>
<td>FA</td>
<td>Fractional Anisotropy</td>
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<td>FEF</td>
<td>Frontal Eye Field</td>
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<tr>
<td>FWE</td>
<td>Family-wise error</td>
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<tr>
<td>FIR</td>
<td>Finite Impulse Response</td>
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<tr>
<td>fMRI</td>
<td>Functional MRI</td>
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<tr>
<td>GLM</td>
<td>General Linear Model</td>
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<tr>
<td>GM</td>
<td>Grey Matter</td>
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<tr>
<td>GPE</td>
<td>External Globus Pallidus</td>
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<tr>
<td>GPI</td>
<td>Internal Globus Pallidus</td>
</tr>
<tr>
<td>HRF</td>
<td>Haemodynamic Response Function</td>
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<tr>
<td>ICV</td>
<td>Intracranial Volume</td>
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<tr>
<td>IPL</td>
<td>Inferior Parietal Lobule</td>
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<tr>
<td>IPS</td>
<td>Intraparietal Sulcus</td>
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<tr>
<td>LIP</td>
<td>Lateral Intraparietal Area</td>
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<tr>
<td>MD</td>
<td>Mean Diffusivity</td>
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<tr>
<td>MDS</td>
<td>Movement Disorder Society</td>
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<tr>
<td>MEG</td>
<td>Magnetoencephalography</td>
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<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>NICE</td>
<td>The National Institute for Health and Care Excellence</td>
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<tr>
<td>PC</td>
<td>Principal Component</td>
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<tr>
<td>PCA</td>
<td>Principal Component Analysis</td>
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<tr>
<td>PD</td>
<td>Parkinson’s Disease</td>
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<tr>
<td>PEF</td>
<td>Parietal Eye Field</td>
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<td>PET</td>
<td>Positron Emission Tomography</td>
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<tr>
<td>PVC</td>
<td>Parieto-insula Vestibular Cortex</td>
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<tr>
<td>PPC</td>
<td>Posterior Parietal Cortex</td>
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<tr>
<td>ROI</td>
<td>Region of Interest</td>
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<tr>
<td>SC</td>
<td>Superior Colliculus</td>
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<tr>
<td>SEF</td>
<td>Supplementary Eye Field</td>
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<tr>
<td>SMG</td>
<td>Supramarginal Gyrus</td>
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<tr>
<td>SN</td>
<td>Substantia Nigra</td>
</tr>
<tr>
<td>SNC</td>
<td>Substantia Nigra Pars Compacta</td>
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<tr>
<td>SNR</td>
<td>Substantia Nigra Pars Reticulata</td>
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<tr>
<td>SPL</td>
<td>Superior Parietal Lobule</td>
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<tr>
<td>SPM</td>
<td>Statistical Parametric Mapping</td>
</tr>
<tr>
<td>STN</td>
<td>Subthalamic Nucleus</td>
</tr>
<tr>
<td>STP</td>
<td>Superior Temporal Polysensory Region</td>
</tr>
<tr>
<td>TE</td>
<td>Echo Time</td>
</tr>
<tr>
<td>TMS</td>
<td>Transcranial Magnetic Stimulation</td>
</tr>
<tr>
<td>TR</td>
<td>Repetition Time</td>
</tr>
<tr>
<td>UPDRS</td>
<td>Unified Parkinson’s Disease Rating Scale</td>
</tr>
<tr>
<td>VBA</td>
<td>Voxel-based Analysis</td>
</tr>
<tr>
<td>VBM</td>
<td>Voxel-based Morphometry</td>
</tr>
<tr>
<td>WM</td>
<td>White Matter</td>
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1 Introduction

1.1 Parkinson’s disease

Parkinson’s disease (PD) is a progressive neurodegenerative disorder, prominently affecting the motor pathway. First described in 1817 by James Parkinson in the essay “The shaking palsy”, the understanding of the disease was further developed from contributions most notably from Jean-Martin Charcot, who first used the term “Parkinson’s disease” to describe it (Goetz, 2011). PD is the most common neurodegenerative disease after Alzheimer’s dementia, with a prevalence of 210 people per 100,000 in NZ in 2013, with this number expected to double within 25 years (Myall et al., 2017).

Classically, PD has the cardinal features of bradykinesia (slowness of movement), rigidity, tremor, and postural instability. Neuropathologically, PD is characterised by the selective degeneration of the dopamine-producing neurons within the substantia nigra pars compacta region, along with the presence of inclusion bodies known as Lewy bodies and Lewy neurites (Figure 1-3), within the substantia nigra and other susceptible neurological sites (Braak et al., 2003).

Parkinson’s-like motor symptoms can also be attributed to a number of other causes that mimic the physical symptoms of PD, under the umbrella term “parkinsonism”. Parkinsonism syndromes include progressive supranuclear palsy, multiple system atrophy and corticobasal syndrome. No diagnostic test for PD exists and diagnosis remains based on clinical criteria. It can prove difficult, particularly in the early disease stages, to accurately diagnose the disorder. Diagnostic errors are made (Hughes et al., 1992), but these are significantly less common for diagnoses by a movement disorders specialist (Hughes et al., 2002). Regular follow-up and repeated clinical assessment are vital for accurate diagnosis. Once a diagnosis has been made, disease progression is also assessed clinically by rating scales such as the Movement Disorder Society Unified Parkinson’s Disease Rating Scale (MDS-UPDRS) (Goetz et al., 2008).

Currently, all available treatment for PD is symptomatic and does not alter disease progression. A significant body of research is dedicated to the discovery of new disease-modifying therapies (AlDakheel et al., 2014; McGhee et al., 2016). Early, correct diagnosis and accurate monitoring of progression will be necessary for the development and use of future therapies. There is a need to develop objective biomarkers to assess disease progression. A good biomarker should ideally aid initial diagnosis as well as identify misdiagnosis, should progression not follow the disease profile. Imaging and saccadic eye
movement performance are potential avenues for biomarker development, with both showing significant changes in PD (Saeed et al., 2017; Srivastava et al., 2014). Eye movements are limited to three axes of rotation and are well-suited for laboratory testing. Eye movements are a precisely measured, easily implemented and easily repeatable measure of movement parameters, compared to, for example, measurements of a complex multi-joint limb. This thesis will investigate the structural and functional basis of saccadic eye movements in PD using MRI techniques. The understanding of cerebral changes underlying abnormal eye movement measures in PD could form the basis for future biomarker development.

1.1.1 Causes of PD

The causes of the majority of PD cases are unknown. A small proportion of the PD population have a familial genetic component. Familial genetic contribution to PD has been identified most commonly in mutations with the leucine-rich repeat kinase 2 (LRRK2) gene (Kumari & Tan, 2009). Other genes linked to PD include mutations of SNCA, Parkin, PINK1, DJ-1 and ATP13A2 (Klein & Westenberger, 2012). Only 10% of PD cases have a familial component however (Thomas & Flint Beal, 2007), with the majority being sporadic and of unknown cause, possibly with contributions from both genetic and environmental factors (Klein & Westenberger, 2012; Lill, 2016). Certain agents, most notably MPTP (1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine), are known to cause parkinsonism in some cases but cannot be responsible for PD itself. A recent meta-analysis of environmental associations with PD found physical activity and smoking to be associated with a lower relative risk for PD. The authors warn the reader that these are far from confirmed as causal factors and more evidence is needed to better understand the associations (Bellou et al., 2016).
1.1.1.1 **Dysfunction of the basal ganglia in PD**

The basal ganglia are a collection of nuclei (or clusters of neurons) which are located in the base of the forebrain (Figure 1-1) and have a key role in modulating movement. The striatum, composed of the caudate nucleus and putamen, form the main input region of the basal ganglia and receives inputs from nearly all cortical regions. The main output region is from the substantia nigra pars reticulata (SNr) and internal globus pallidus (GPi). The basal ganglia send modulatory outputs to the thalamocortical networks and brainstem motor networks which can inhibit or disinhibit motor activity (Albin et al., 1983; DeLong, 1990). Other nuclei, including the substantia nigra pars compacta (SNC), external globus pallidus (GPe) and subthalamic nucleus (STN), form interconnections within the basal ganglia structures and modulate basal ganglia output (a more detailed description of the basal ganglia circuit, with more emphasis on eye movement control, is provided in this chapter on page 34). In PD, selective loss of dopaminergic neurons in SNC occurs, which leads to the classically described depigmentation of the SN (Figure 1-2). The overall effect of dopaminergic neuron loss is that an increased inhibitory effect is present on motor output circuits, leading to some of the motor symptoms seen in PD.

![Figure 1-1 – Location of basal ganglia structures within the brain. Source - Wikipedia Commons.](image-url)
Figure 1-2 – Autopsy brain section at the level of the midbrain. Loss of dopaminergic neurons within the substantia nigra (SN) results in depigmentation, visibly seen on the left image compared to a control image on the right. Figure permission obtained. Adapted from Mandel et al., (2010).

Figure 1-3 – Round Lewy body (circle) with strand-like Lewy neurites (rectangle). Source - Wikipedia Commons.
1.1.1.2 **The Braak Hypothesis**

In recent years, PD has become thought of as a progressive multifocal neurological disease, with widespread α-synuclein pathology not limited to the substantia nigra. A prevalent theory regarding Lewy body/α-synuclein pathology is known as the Braak hypothesis (Braak et al., 2003). Braak and colleagues, in their widely cited autopsy study, described a characteristic, non-random pattern of abnormal α-synuclein spread and accumulation through the CNS in PD. Braak et al. described nigral pathology as always accompanied by extra-nigral pathology in the region surrounding and containing the dorsal motor nucleus of the vagus nerve. They used an immunohistochemical stain for abnormal α-synuclein in brains of deceased patients with known PD, along with patients with no PD diagnosis but with Lewy body presence in the associated lesions sites near the vagal dorsal motor nucleus. A staging system was described relating to pathological α-synuclein spread, made up of 6 stages. Each stage evolves from the pathology of previous stages, with changes occurring in a progressive manner. Stage 1 pathology was seen with lesions in the dorsal motor nucleus of the vagus nerve within the medulla. Stage 2 shows further lesions in the medulla, extending to the pontine tegmentum region. Stage 3 involves regions of the midbrain, notably including the SNC region. Stage 4 shows lesions in the basal forebrain and Stages 5-6 show progressive lesions into the neocortex from the prefrontal and sensory association areas to the premotor and primary sensory/motor fields. The Braak hypothesis states that the motor manifestations of PD, in fact, represent the mid to late stages of PD, which occurs after a premotor prodromal phase, before the α-synucleinopathy reaches the basal ganglia in Stage 3 of the disease (Braak et al., 2004).

1.1.1.3 **Source of abnormal α-synuclein and the “dual hit” hypothesis**

The earliest sites of Lewy pathology are seen to develop in the olfactory bulb (Berendse & Ponsen, 2006; Duda, 2010; Pearce et al., 1995) and peripherally in the enteric nerve plexus (Braak et al., 2006; Wakabayashi et al., 1989). The occurrence of two initial, separate sites of Lewy pathology has led to what’s known as the “dual hit hypothesis” for PD pathogenesis (Hawkes et al., 2007). Hawkes et al. argue it is unlikely for two regions to develop simultaneous α-synuclein pathology independently and a more probable explanation is that a common pathogen initiates the abnormal α-synuclein formation in both regions. This external neurotropic agent, possibly viral, could at first affect the upper respiratory system and the olfactory mucosa, much like a common cold. The agent, along with saliva and mucous, is swallowed into the stomach. From there, the pathogen passes through the thin single cell layered epithelium into Meissner’s and Auerbach’s plexus of the enteric nervous system. Neurons within the enteric nervous system are interconnected with the vagus nerve, which has
projections to and from the dorsal motor nucleus of the vagus of the medulla (Braak et al., 2003). Abnormal α-synuclein is thought to be transported through the vagus to the dorsal motor nucleus, where the pathological CNS aggregation process begins (Braak et al., 2003). The process by which abnormal α-synuclein develops into widespread aggregations is not yet certain but a prion-like mechanism has been postulated (Brundin et al., 2010; Dunning et al., 2012; Frost & Diamond, 2010).

1.1.1.4 Evidence and criticisms of the Braak hypothesis

Evidence for the neural propagation of abnormal α-synuclein comes from human autopsy and animal studies. Deceased PD patients who previously received grafts of embryonic mesencephalic dopaminergic neurons showed aggregated α-synuclein presence in the grafted tissue (Kordower et al., 2008; Li et al., 2008). This suggests that abnormal α-synuclein is capable of progressively propagating into unaffected non-host tissue. In animal studies when α-synuclein was injected into the intestine, pathological spread of α-synuclein was seen in the dorsal motor nucleus via the vagus nerve (Holmqvist et al., 2014). A rotenone-induced animal PD model (Cannon et al., 2009) was also associated with Lewy bodies in the enteric nervous system, dorsal motor nucleus and substantia nigra (Pan-Montojo et al., 2010). Vagotomy (severing the vagus nerve) halted this spread (Pan-Montojo et al., 2012). While evidence is present for the neural propagation of misformed α-synuclein, a number of questions remain regarding the nature of the α-synuclein spread whether it can be truly called prion-like (Visanji et al., 2013), or whether the extent to which abnormal α-synuclein spread contributes to the aetiology of PD. For example, the prion process implies there should be a contagious element to PD, which is yet to be found (Beekes et al., 2014). The association of α-synuclein and cell death is thought to be related to certain toxic oligomers of α-synuclein (Conway et al., 2000; Stefanis, 2012), but this neurotoxic effect is not yet fully understood (Benskey, Perez, & Manfredsson, 2016; Roberts & Brown, 2015). The Braak grading scale itself is based on abnormal α-synuclein spread and not directly on dopaminergic cell loss (Dickson, 2012) and it is possible for individuals to have widespread Lewy body pathology, even in the cortex, and yet not show signs of PD (Parkkinen et al., 2005). Longitudinal studies show that not all patients follow the Braak staging system (Halliday et al., 2008) and it may be that the Braak staging system may apply only to a certain subset of PD patients (Rietdijk et al., 2017). Numerous publications call for further research in this field to further elucidate and clarify the aetiology of PD (Longhena et al., 2017; Recasens & Dehay, 2014; Visanji et al., 2013).
1.1.1.5 Oxidative stress and cell death

Alpha-synuclein is not alone in its contribution to neuronal death, with neuroinflammation (Hirsch et al., 2012; Tansey & Goldberg, 2010; Wang et al., 2015), abnormal toxic dopamine metabolite formation (Hastings, 2009; Munoz et al., 2012; Segura-Aguilar et al., 2014), and mitochondrial dysfunction (Henchcliffe & Beal, 2008; Moon & Paek, 2015; Winklhofer & Haass, 2010) all having a role to play in PD-related neurodegeneration. Oxidative stress is seen as the key underlying mechanism, stemming from a combination of the above factors, and ultimately leading to dopaminergic cell loss in PD (Blesa et al., 2015; Dias et al., 2014; Hwang, 2013). Oxidative stress results from an excess of reactive oxygen species, too much for natural cellular antioxidant activity to clear (Dias et al., 2014). Reactive oxygen species damage cellular components such as DNA, RNA and mitochondria, and affect cell signalling processes, leading to cell damage and potentially death (Brieger et al., 2012). The detection of changes relating to neuroinflammation and oxidative stress is possible using certain forms of MRI and PET, and underlie the basis for the development of novel imaging strategies and potential biomarkers for PD (see later Imaging section page 53). Neuroinflammation and oxidative processes may also be the targets for future neuroprotective or disease-modifying therapies for PD (Jin et al., 2014; Wang et al., 2015).

1.1.2 Clinical features of PD

The cardinal features of Parkinson’s disease include the classical triad of bradykinesia, tremor and rigidity (Gelb et al., 1999). Postural instability is considered a fourth cardinal feature (Kim et al., 2013; Massano & Bhatia, 2012) but is more prominent in later stages of the disease (DeMaagd & Philip, 2015; Jankovic, 2008).

1.1.2.1 Bradykinesia

Bradykinesia, or the slowness of movement, is the defining clinical characteristic of PD. This causes difficulty initiating movements, prolonged reaction times and difficulties with sequencing movements or self-paced movements (Berardelli et al., 2001). It often presents as a slowness of everyday movements such a walking, putting on clothes and using utensils (Jankovic, 2008). Bradykinesia also causes a “poverty” of spontaneous movement, drooling from impaired swallowing, decreased facial expression, softer voice and smaller handwriting (Massano & Bhatia, 2012). Clinically, bradykinesia can be assessed by having the patient perform finger tapping and alternating hand/feet/limb movements as quickly and with as large an amplitude as possible (Goetz et al., 2007). Bradykinesia is thought to correlate with the degree of dopamine deficiency (off-medication or inadequately-medicated state) - more so than tremor, rigidity or postural instability (Vingerhoets et al., 1997).
1.1.2.2  **Tremor**
A resting tremor is the most visible sign and is typically unilateral at onset and affects the distal extremities of the limb first. PD tremor has a frequency of 4-6 Hz and in the fingers often manifests as a “pill rolling” motion (Findley et al., 1981; Massano & Bhatia, 2012). It can on occasion be difficult to differentiate tremor from other causes, such as essential tremor. Essential tremor tends to be bilateral, occurs at a higher frequency, and is present on posture as opposed to rest. PD tremor often disappears with movement and is most clearly seen with the affected limb resting on a supporting surface (Bain, 2007; Bhidayasiri, 2005; Günther Deuschl et al., 1998).

1.1.2.3  **Rigidity**
Rigidity is the stiffness or increased resistance of limbs/joints to movement, due to increased muscle tone. This can be felt as “lead pipe” rigidity (constant resistance throughout the range of motion) or can be felt as a notchy or ratcheting style of resistance, called “cogwheel rigidity”, which is thought to be a superposition of tremor on top of increased tone (Berardelli et al., 1983; Guttman et al., 2003; Lance et al., 1963; Massano & Bhatia, 2012).

1.1.2.4  **Postural instability**
PD patients will often show impairment of postural reflexes involved in maintaining balance. This can lead to a feeling of instability and a tendency for falls with risk of significant injury (Allen et al., 2013; Koller et al., 1989; Muslimovic et al., 2008). Postural instability can be tested by standing behind a patient (after explaining the test) and giving a firm rearward pull (Nonnekes et al., 2015). Patients with preserved reflexes should be able to maintain balance with no more than one step back. In PD however, multiple backward steps are often needed to maintain balance, or the patient may actually fall, without any compensatory responses.

1.1.3  **Non-motor PD features**

1.1.3.1  **Cognitive decline**
Features of PD are not limited to the motor system. Most PD patients will experience cognitive decline, progressing to dementia, with a cumulative prevalence as high as 78% (Aarsland et al., 2003; Emre, 2004; Hely et al., 2008). Cognitive decline places a significant additional burden on the patient and caregivers and yet no diagnostic biomarker for cognitive decline exists (Goetz et al., 2008; Meireles & Massano, 2012). The Movement Disorders Society (MDS) have proposed diagnostic criteria for mild cognitive impairment (Litvan et al., 2012). These criteria rely on prior history, requiring a gradual decline in cognition noted by
the patient/clinician, which doesn’t significantly impact daily life; impairment on a global cognitive test such as the Montreal cognitive assessment (MoCA) (Nasreddine et al., 2005) and impairment in two or more cognitive domains in a battery of neuropsychological tests assessing multiple cognitive domains (attention and working memory, executive function, language, memory and visuospatial function) (Litvan et al., 2012). The MDS have also published a set of guidelines for PD dementia diagnosis, which involves the onset of dementia after the diagnosis of PD, global cognitive score deficits, cognitive impairment significant enough to impair daily activities and impairment of at least two of the above-mentioned cognitive domains (Dubois et al., 2007).

1.1.3.2 Mood disturbances
Mood disturbances such as depression are common symptoms in PD, affecting up to half of the PD population (Aarsland et al., 1999; Ravina et al., 2007) Mood disorders are rated as one of the most troublesome symptoms in advanced PD (Politis et al., 2010). Depression is considered undertreated in PD, yet has a significant impact on quality of life (Ravina et al., 2007). There is a need to ensure the emotional disturbances are identified and treated alongside the motor symptoms (Zesiewicz et al., 2010). Anxiety was reported as the second most common mood disturbance after depression (Aarsland et al., 1999) and often co-exists with depression in PD (Wee et al., 2016). Apathy is also common in PD (Pedersen, Larsen, Alves, & Aarsland, 2009) and can be a component of depression but may also occur separately too (Richard, 2006). A number of imaging studies have identified frontal lobe and temporal lobe changes associated with increased apathy scores (Reijnders et al., 2010; Robert et al., 2012; Skidmore et al., 2013).

1.1.3.3 Sleep disturbances
Sleep disturbances, including insomnia and excessive daytime sleepiness, affect nearly half of PD patients (Brodsky et al., 2003; Gjerstad et al., 2007). Fatigue is another common symptom, reported in up to half of one cohort (Alves et al., 2004). Alves et al. found fatigue commonly relates to depression and excessive daytime sleepiness but can exist as an independent symptom in the absence of these factors as well. Rapid eye movement (REM) sleep behaviour disorders are characterised by vivid, often frightening dreams, and loss of muscle atonia with resulting motor activity during sleep. This has been described as the acting-out of dreams and is seen in PD patients, and often precedes the development of PD (Poryazova & Zachariev, 2005). REM sleep behaviour disorders may form part of a screening
criteria used to identify patients at-risk of developing PD in the future (Mahlknecht et al., 2015).

1.1.3.4 **Hallucinations**

Hallucinations, particularly visual, are commonly experienced in PD, with cognitive decline and advancing age thought to be predisposing factors (Fenelon et al., 2000; Sanchez-Ramos et al., 1996). PD medications may also induce hallucinations on their own, including both visual and auditory hallucinations, and need to be carefully monitored (Cummings, 1991; Goetz, Tanner, & Klawans, 1982).

1.1.3.5 **Autonomic dysfunction**

Autonomic dysfunction, such as orthostatic hypotension, constipation, urinary difficulties and sexual dysfunction are all known to occur in PD (Asahina et al., 2013; Brown et al., 1990; Lemack et al., 2000; Verbaan et al., 2007). Of note, these symptoms are present in multiple system atrophy (MSA), but at a more severe level (Wenning & Colosimo, 2010) and can lead to misdiagnosis in early disease stages. Constipation is frequently seen in PD, affecting over 50% of cases. From the Braak hypothesis (Braak et al., 2006), it is possible α-synuclein pathology affecting the enteric nervous system causes loss of gut motility, leading to constipation. Colonic biopsies have detected abnormal α-synuclein in PD patients (Shannon et al., 2012). Constipation can predate PD by a decade or more (Abbott et al., 2001; Savica et al., 2009). A meta-analysis by Bellou et al. (2016) found constipation to be associated with a relative risk of 2.6 for developing PD. Assessment of constipation may form a marker of early or prodromal PD (Postuma & Berg, 2016).

1.1.3.6 **Olfactory loss**

Olfactory loss is predicted by the Braak hypothesis, with early α-synuclein pathology affecting the olfactory bulb (Braak et al., 2003). This symptom may not be noticed by the patient but when tested is extremely common in PD, present in up to 90% of patients (Doty et al., 1988). Abnormalities of the olfactory system can be detected using MRI techniques (Ibarretxe-Bilbao et al., 2010; Rolheiser et al., 2011; Scherfler et al., 2006). Smell testing, however, is easily performed without the need for imaging (Doty et al., 1984). Olfactory changes may form a part of an assessment for early PD (Mahlknecht et al., 2015; Postuma & Berg, 2016).

1.1.4 **Clinical diagnosis**

There is no physiological or blood test available to diagnose PD. The diagnosis of PD remains clinical and is based upon the physician identifying the characteristic symptoms which make
up PD, while excluding other causes of parkinsonism. One widely-known diagnostic criteria was developed by the UK Parkinson’s Disease Society’s Brain Bank (Hughes, Daniel, Kilford, & Lees, 1992). The Movement Disorders Society has developed a diagnostic criteria more recently for clinical research (Postuma et al., 2015). The presence of bradykinesia, along with at least one other cardinal feature of PD, is needed at a minimum for considering PD as a diagnosis. A definitive response to dopaminergic medication is an important supportive factor and other factors, such as unilateral symptoms, rest tremor and progressive onset, all support the diagnosis of PD. The UK Parkinson’s Disease Society’s Brain Bank diagnostic criteria (Hughes et al., 1992) is included in the appendix (Table 9-3 page 358)

1.1.4.1  **Eye movements in clinical diagnosis of PD**

The current involvement of oculomotor features for diagnosis is only to exclude other causes of parkinsonism. Signs such as supranuclear gaze palsy and oculogyric crises (dystonic bilateral gaze elevation) are part of the UK Parkinson’s Disease Society’s Brain Bank diagnostic criteria (as an exclusion), and serving to exclude causes such as progressive supranuclear palsy (PSP) or encephalopathic causes.

Current scales of PD progression do not have eye movement components. The Hoehn and Yahr scale (H&Y) is a simple, five-stage scoring scale, classifying only broad motor symptoms of PD occurring unilaterally or bilaterally. Compared to the H&Y, the MDS-UPDRS covers a more in-depth range of PD symptoms. Part III of the MDS-UPDRS covers motor symptoms of PD such as the severity of tremor, rigidity and gait. However, the MDS-UPDRS also currently does not have eye movement criteria as part of the scoring. By contrast, the Unified Huntington’s disease rating scale (UHDRS) (Huntington Study Group, 1996), which is modelled on the UPRDS, has 6 oculomotor items as manifest disease markers. The oculomotor features of Huntington’s disease (HD), such as reduced saccade velocity, are however, more clinically obvious in HD than in PD.

1.1.5  **Parkinsonism syndromes**

Symptoms such as bradykinesia, tremor and rigidity are not exclusively linked to Parkinson’s disease. A number of other neurodegenerative processes may also present with these features. These are collectively known as parkinsonism or parkinsonian syndromes and can be difficult to differentiate from PD in early stages of the disease. Many of the exclusion criteria in the UK PD society diagnostic guidelines are an attempt to exclude these disorders from the diagnosis of PD.
1.1.5.1 **Dementia with Lewy bodies**

There is current discussion over what separates dementia with Lewy bodies (DLB) and Parkinson’s disease with dementia (PD-D) (Berg et al., 2014; McKeith, 2009; Richard, Papka, Rubio, & Kurlan, 2002). The diagnostic criteria of DLB were until recently based on the 1-year rule, whereby if dementia develops 1 year or more after initial motor parkinsonism symptoms, a diagnosis of Parkinson’s disease with dementia is given. If dementia develops in the same year as or before parkinsonism motor symptoms, a diagnosis of dementia with Lewy bodies is given (Gomperts, 2016). The one year time period is an arbitrary length of time and the MDS have suggested omitting the 1-year rule, giving a diagnosis of PD to anyone who meets PD diagnostic criteria regardless of the timing of dementia onset (Berg et al., 2014).

The underlying pathology of DLB and PD-D may differ, with an added vascular and amyloid pathological component in DLB as opposed to predominant α-synuclein pathology in PD-D (Berg et al., 2014). The Lewy Body Dementia Association Scientific Advisory Council argues for retaining the diagnosis of dementia with Lewy bodies as separate from PD-D, until underlying mechanisms are better understood (Boeve et al., 2016). The Dementia with Lewy Bodies Consortium has recently published recommendations for the diagnosis of DLB, with core clinical features including REM sleep disorder, visual hallucinations and fluctuating cognition, with at least one cardinal parkinsonism feature and existing dementia (McKeith et al., 2017).

1.1.5.2 **Progressive supranuclear palsy (PSP)**

Also known as Steele-Richardson-Olszewski syndrome, classic PSP is characterised by an inability to voluntarily look up or down, axial rigidity, postural instability with falls and cognitive decline (Ling, 2016; Lubarsky & Juncos, 2008). PSP is a tauopathy. Tau is a protein found in neurons and it stabilises microtubules - structures needed for maintaining cell shape and axonal transport. Abnormal aggregations of tau can accumulate and give rise to a tauopathy (Buee, Bussiere, Buee-Scherrer, Delacourte, & Hof, 2000). A subset of PSP patients have bradykinesia, rigidity and tremor (PSP-P), and they are commonly misdiagnosed as PD (Williams et al., 2005).

1.1.5.3 **Multiple system atrophy (MSA)**

MSA or Shy-Drager syndrome is characterised by autonomic dysfunction along with cerebellar ataxia and parkinsonism (Wenning et al., 2004). Microscopically, MSA is a glial disorder (Wenning et al., 2008) characterised by glial (oligodendroglial) cytoplasmic inclusions (Papp et al., 1989) that are stained by α-synuclein sensitive antibodies (Lin et al.,
MSA has a rapid onset, has more severe autonomic symptoms than PD, and has parkinsonism symptoms that are poorly responsive to levodopa (Wenning & Krismer, 2013).

1.1.5.4 Corticobasal degeneration
Corticobasal degeneration, the commonest cause of corticobasal syndrome, is a rare tauopathy that presents with progressive asymmetric symptoms, usually affecting one limb at first. Corticobasal degeneration causes dystonia, rigidity, inability to mime hand gestures (ideomotor apraxia), a sensation of disconnect from the limb (alien limb phenomenon), cognitive decline and features of parkinsonism (Armstrong et al., 2013; Mathew et al., 2012). Corticobasal degeneration is associated with tau protein accumulation in the frontal, temporal and parietal lobe, along with the basal ganglia (Forman et al., 2002). The unilateral parkinsonism seen in corticobasal degeneration is poorly responsive to levodopa (Wenning et al., 1998).

1.1.5.5 Vascular parkinsonism
Vascular parkinsonism refers to the idea that lacunar infarcts in the basal ganglia can cause parkinsonism (Sibon & Tison, 2004). This idea is controversial since not all basal ganglia infarcts cause parkinsonism and no correlation of infarct location and parkinsonism has been made (Peralta et al., 2004; Reider-Groswasser et al., 1995). Recently, attempts have been made to clarify features of vascular parkinsonism, with a call to develop clear diagnostic criteria (Kalra et al., 2010; Vale et al., 2015).

1.1.5.6 Essential tremor
While not a parkinsonian disorder, essential tremor is prevalent among the general population and more importantly, can on rare occasion be difficult to distinguish from PD (Thenganatt, 2012). Essential tremor classically affects both hands symmetrically and is postural or action-related (Deuschl et al., 1998). More severe essential tremor can, however, manifest at rest (Cohen et al., 2003). Essential tremor may not always be bilateral (Louis et al., 1998) and even bradykinesia has been observed in essential tremor patients (Christian et al., 2006). Essential tremor can co-exist with PD (Benito-Leon et al., 2009) and misdiagnosis does occur (Jain et al., 2006).

1.1.5.7 Diagnosing “prodromal PD”
A concept of a “prodrome” phase of PD has emerged in recent years (Berg et al., 2014). The prodrome phase is described as a period when initial neurodegeneration has occurred, but before the development of the motor features needed for PD diagnosis. During the prodrome phase, other motor and non-motor aspects from initial neurodegeneration may be present,
which could herald the development of PD (Berg et al., 2015). This concept is in line with what we know from the Braak studies, which demonstrated α-synuclein pathology developing in the olfactory bulb and in the enteric nervous system before CNS involvement. This is supported by studies showing that olfactory loss and constipation have a possible association with future PD (Abbott et al., 2001; Berg et al., 2013; Ross et al., 2008; Tunc et al., 2015). Non-motor symptoms such as REM sleep behaviour disorder are also frequently reported prior to PD diagnosis (plus the other synucleinopathies, MSA and DLB) (Mahlknecht, et al., 2015; Poewe, 2008; Postuma et al., 2012; Postuma et al., 2015). No single symptom can be considered a harbinger of PD but a combination may be able to give a good prediction of the risk of PD development (Berg et al., 2013; Mahlknecht, et al., 2015; Tunc et al., 2015). This idea has led the MDS to develop criteria for prodromal PD (Berg et al., 2015).

The prodromal criteria developed by the MDS are intended purely as a research tool. Berg et al. (2015) state that the prodromal symptoms cannot be reliably used to predict the timing and development of PD in the individual, only overall risk. In the absence of disease-modifying drugs, ethical issues are encountered if discussing the future probability of developing PD. In addition, the number of studies used in the construction of the criteria was limited. The MDS does not recommend that this criterion be used in a clinical context at this stage, and state the need for these criteria to be continually updated as more information is available.

Imaging has been considered as potential means of diagnosing a prodromal PD (Liu et al. 2017). PET techniques have been trialled using specially radioactively tagged compounds whose uptake in the GI system may reflect levels of parasympathetic dysfunction via impaired vagal nerve activity in PD (Gjerløff et al., 2015). Olfactory changes are also part of the prodromal PD concept. The use of MRI in detecting reduced olfactory bulb volume in PD patients has been the subject of a meta-analysis (Li et al., 2016), which did find significant olfactory volume differences in established PD patients and controls. This meta-analysis however did not track progression of non-PD patients into PD. As the bulb changes are known to occur before PD diagnosis, future studies could consider olfactory bulb imaging in patients meeting current prodromal criteria and validating the olfactory bulb MRI measures against progression of non-PD into PD patients as a longitudinal cohort study.

### 1.1.6 PD Treatment

A number of pharmacological and surgical treatments are available for PD. Current medical therapy is focused on treating the symptoms of PD, but new neuroprotective strategies are being developed, with the aim of modifying disease progression. The decision to initiate PD
treatment depends on the extent of functional impairment experienced by each individual patient. Important factors to consider are the effect of the disease on the dominant hand and the extent to which the disease affects work or activities of daily living, particularly if significant bradykinesia is present or walking is affected (Olanow et al. 2001). A UK multidisciplinary panel, The National Institute for Health and Care Excellence (NICE) (2006), published a set of guidelines for PD management. Key suggestions were that patients with suspected PD be referred to a specialist service for diagnosis and that this diagnosis is reviewed every 6 to 12 months. Also noted was the importance of non-pharmacological management, such as physiotherapy and specialist nursing care, plus the need to treat the non-motor symptoms of PD throughout the disease stages. The opportunity for people with PD and their family to discuss palliative care issues should also be given.

1.1.6.1 Medication use in PD

A range of pharmacological classes are used in the treatment of PD. The goal is to provide the best relief of patient-specific symptoms, minimising off-time (the re-emergence of PD symptoms after levodopa treatment has worn off) and medication side-effects. The approach to PD medication is highly individual, needing to take into account patient preferences, with ongoing monitoring of patient symptoms needed (National Collaborating Centre for Chronic Conditions, 2006).

Levodopa has been the gold standard treatment for PD for over 40 years (Davie, 2008). Levodopa provides the greatest symptomatic benefit for those with PD with fewer side-effects (such as sleepiness, impaired impulse control and hallucinations) compared to other classes, such as dopamine agonists (Davie, 2008). The need to treat Parkinson’s symptoms (too little voluntary movement) is balanced with the side-effect of dyskinesias (excessive involuntary movements). Young-onset PD patients are at an increased risk of dyskinesias and as a result, tend to be given dopamine agonists initially (Wickremaratchi et al., 2009). As PD progresses, medications are adjusted accordingly with regard to adverse effects such as dyskinesias. The elderly (age over 60) have a greater disposition to develop cognitive and psychiatric side-effects from dopamine agonists and as a result, levodopa is preferred in the elderly patient population (Connolly & Lang, 2014). Monoamine oxidase inhibitors (MAOIs) are used in early and mild PD and have been shown to provide moderate benefit for mild PD symptoms (Parkinson study group, 1989). Medications such as anticholinergics and amantadine are also
occasionally used in younger PD. The use of each individual medication class is covered in further detail below.

1.1.6.2 **Levodopa**

Levodopa is the most effective treatment of motor PD symptoms, especially if used in treating bradykinesia that has reached the point of affecting daily life for the patient (Connolly & Lang, 2014; Ferreira et al., 2013).

**Pharmacology**

Levodopa is a dopamine precursor and is able to pass through the blood-brain barrier to reach the CNS. Here, the levodopa is converted to dopamine by the enzyme DOPA decarboxylase. This helps replenishes the dopamine deficit caused by the death of dopamine-producing cells in PD. Levodopa, before passing through the blood-brain barrier, is converted by peripheral decarboxylase into dopamine. This peripheral dopamine causes such side-effects as nausea/vomiting and orthostatic hypotension. To prevent this, levodopa is always used in combination with a peripheral decarboxylase inhibitor – examples include carbidopa and benserazide. This reduces this peripheral conversion process and hence side-effects from levodopa (Connolly & Lang, 2014).

**Dosing regimens**

Combination carbidopa and levodopa are available in different ratios and may come under the commercial name of Sinemet or Kinson. Benserazide and levodopa is also a common combination and is known as Madopar. Treatment begins with a small dose and is titrated up, according to effect. Tolerance and side-effects are monitored during this process. The majority of PD patients will have a clinical response at a moderate levodopa dose. If no response is achieved, the diagnosis of PD is placed in doubt and an alternative parkinsonism syndrome should be considered.

A dosing strategy is needed to minimise off-time. Levodopa can be divided into smaller but more frequent doses to reduce off-time. Levodopa is started at two to three times a day, but this frequency can be increased if needed. Levodopa also comes in immediate-release and controlled-release formulations. The use of controlled formulations is often for convenience purposes once the therapeutic dose has been determined. These controlled-release preparations are often used at night to reduce nocturnal akinesias (loss of voluntary movement at night). There does not appear to be a difference in the symptom control between the use of
either long- or short-acting levodopa after several years of use (Koller, Hutton, Tolosa, & Capilldeo, 1999).

**Motor complications**

A phenomenon that affects levodopa users after a few years is known as motor fluctuations. These are fluctuating responses to the established levodopa dose, ranging from a faster transition to the “off” period, despite being on the same medication, to the other end of the spectrum, where patients experience abnormal involuntary movements (dyskinesias) while “on” levodopa. It is likely that the development of dyskinesias is due to progressive destruction of dopamine terminals in the nigrostriatum, limiting dopamine uptake and release (Olanow et al., 2001). The DATATOP study reported that up to 30% of patients developed motor complications after 2 years of levodopa use (Penney et al., 1996). A number of studies suggest these motor fluctuations are related to age of onset of PD and to higher levodopa doses. Block, Liss, Reines, Irr, & Nibbelink (1997) found a smaller proportion of patients developed motor complications after 5 years of treatment with relatively lower levodopa doses. Motor fluctuations were seen more in PD patients with a younger age of onset (Kumar et al., 2005; Quinn et al., 1987). Similarly, the STRIDE-PD study reported that the risk for dyskinesias and the wearing-off phenomena was most strongly related to age of PD onset and levodopa dose (Olanow et al., 2013). Some studies have suggested initial treatment with pramipexole (a dopamine agonist) is associated with a later onset of motor fluctuations compared with initial levodopa treatment (Holloway et al., 2004; Parkinson Study Group, 2000). Other studies report no effect of initial medication on subsequent motor fluctuations and dyskinesias (Gray et al., 2014; Katzschlager et al., 2008; Parkinson Study Group CALM Cohort Investigators, 2009).

Despite an unclear picture on the cause of motor fluctuations, current recommendations are to use the lowest levodopa dose for therapeutic response. This potentially decreases the risk of motor fluctuations and increases quality of life through a better-titrated dose regime. If a repeatable biomarker can be developed without needing a subjective component, it may allow better titration of levodopa, minimising dose.

1.1.6.3 **Dopamine agonists**

Dopamine agonists are synthetic compounds which directly stimulate dopamine receptors. There are five known dopamine receptor types: “dopamine receptor D1 – D5” or simply D1-5. D1-like (D1 and D5) receptors are linked to adenylate cyclase. D2 like (D2, 3, 4) are not and have the opposite effect of D1 like receptors. D1 and D2 receptors are concentrated in the
striatum (caudate nucleus and putamen) and are considered important in movement control. 
D3 is also thought to be involved in movement control but is mainly located in the limbic 
system and is believed to play a role in behaviours and emotions (Missale et al., 1998).
Dopamine agonists can be used as monotherapy for treating PD symptoms or in combination 
with other antiparkinsonian medication. Examples of dopamine agonists include lisuride 
(Dopergin), pergolide (Permax; but now rarely used), ropinirole (Requip or Ropin), 
bromocriptine (Alpha-Bromocriptine), apomorphine (Apomine) injections and rotigotine 
(Neupro) transdermal patches.

Dopamine agonist use
Initially, dopamine agonists were used as adjuvant therapy with levodopa to reduce off 
periods, but studies have shown that dopamine agonists are an effective monotherapy in their own right (Stowe et al., 2008), with a lower risk of developing dyskinesias (Rascol et al., 2000). Levodopa, however, has been found to provide better symptom control (Holloway et al., 2004) and most patients will need levodopa eventually even if starting on dopamine agonists. By initially starting on dopamine monotherapy, it was believed the use of levodopa can be “saved” until later in the disease (Kurlan, 1988). The idea that levodopa response has a finite limit is, however, unproven as previously mentioned, with a number of studies showing initial therapy does not change motor fluctuation symptoms (Gray et al., 2014; Katzenschlager et al., 2008; Parkinson Study Group CALM Cohort Investigators, 2009). The dopamine agonists ropinirole and pramipexole are effective in early PD as monotherapy. Pramipexole also has antidepressant properties. Rotigotine is provided as a transdermal patch, which avoids food interactions and first pass metabolism. Apomorphine is given as a subcutaneous injection to treat (or “rescue”) off-episodes or as a continuous infusion to minimise fluctuations.

Dopamine agonist side-effects
Medication side-effects can be minimised by using the lowest possible dose. Side-effects of dopamine agonists include nausea and vomiting, orthostatic hypotension, peripheral oedema, confusion and hallucinations (Stowe et al., 2008). Valvular heart disease can be caused by pergolide (Baseman et al., 2004; Pritchett et al., 2002; Van Camp et al., 2004), which is no longer recommended in the treatment of PD. Dopamine dysregulation syndrome can occur with dopamine agonist use though more commonly encountered with excessive levodopa intake. Patients with this syndrome develop an addictive compulsive use of the medication, despite developing drug-related dyskinesias (Giovannoni et al., 2000). Impulse control
disorders, such as pathologic gambling, may also develop with dopamine agonist use in particular (Voon et al., 2006). On abrupt withdrawal, dopamine withdrawal syndrome can develop (Rabinak & Nirenberg, 2010). This resembles cocaine withdrawal, giving anxiety, panic attacks, depression, sweating, nausea, pain, fatigue and cravings. The dosing and tapering of dopamine agonists need to be carefully monitored.

1.1.6.4 Monoamine oxidase type B (MAO B) inhibitor

Monoamine oxidase type B (MAO B) inhibitors are used in the treatment of mild PD symptoms (Lewitt et al., 2009; Riederer & Laux, 2011). Monoamine oxidase is an enzyme that exists in two isoforms - Type A and B (Johnson, 1968). Both types are found throughout the periphery and CNS, but MAO B is the predominant form found in the basal ganglia (Youdim & Weinstock, 2004). Early use of non-selective MAO inhibitors caused serious side-effects in the form of food interactions, leading to severe headache and hypertensive crisis – known as the “Cheese effect” (Finberg & Rabey, 2016). Since then, the use of selective MAO inhibitors has reduced this occurrence. Some examples of MAO-B inhibitors include selegiline, rasagiline and safinamide. Selegiline (L-deprenyl) intake is associated with a significant increase in dopamine concentrations in the basal ganglia of PD patients (Riederer & Youdim, 1986). A meta-review by Ives (2004) reported improved UPDRS scores in PD patients on a MAO-B inhibitor vs placebo. The use of selegiline is reported to delay the initiation of levodopa. Selegiline treatment also lowers the dose of levodopa needed to provide symptomatic relief. A modest reduction in motor fluctuation, but not dyskinesia, was reported in patients taking both levodopa and selegiline relative to levodopa alone. This was thought to be an effect of either a lower levodopa dose with concurrent selegiline use or a neuroprotective effect of selegiline itself. No difference in mortality was noted by Ives. Other reviews have also supported the findings of Ives, but noted the neuroprotective properties of selegiline were not apparent (Turnbull et al., 2012). Most studies have used selegiline in their comparisons but a more recent alternative rasagiline has also shown benefit in the treatment of PD symptoms and also possible neuroprotection in animal models (Chen, Swope, & Dashtipour, 2007; Parkinson Study Group, 2002, 2004). Side-effects of MAO inhibitors include nausea and headache (Horn & Stern, 2004). Hallucinations may also be related to selegiline use in advanced PD patients on multiple other treatments (Kamakura et al., 2004).

1.1.6.5 Anticholinergics

Anticholinergics were the first medication used to treat PD, before the widespread use of levodopa (Olanow et al., 2001). This class of medication inhibits muscarinic acetylcholine receptors. The exact mechanism behind anticholinergic action in PD remains unknown.
(Brocks, 1999) but it is thought that neurotransmitters dopamine and acetylcholine exist in an equilibrium state in basal ganglia. Dopamine depletion in PD creates an imbalance in the cholinergic system which an anticholinergic is thought to correct (Olanow et al., 2001). Examples of anticholinergics in use include trihexyphenidyl, benztpine, orphenadrine and procyclidine. Anticholinergics are mostly used in a relatively younger population (under 70 years of age), experiencing tremor as the main symptom (Connolly & Lang, 2014). Anticholinergics are used with caution in the elderly population, as they are more susceptible to adverse effects including memory impairment, hallucinations and confusion. Other side-effects include urine retention, constipation, dry mouth and blurred vision. A Cochrane review found the side-effects of anticholinergics to be a more common reason for discontinuation than lack of efficacy (Katzenschlager et al., 2003).

1.1.6.6 **Amantadine**

Amantadine is an antiviral that has some antiparkinsonian properties (Schwab et al., 1972). The mechanism of action is not entirely understood but is thought to be from antagonistic activity at N-methyl-D-aspartate (NMDA)-type glutamate receptors (Kornhuber et al., 1994), which increases extracellular dopamine levels (Mizoguchi et al., 1994).

Trials have found amantadine improves bradykinesia and rigidity (Parkes et al., 1974; Schwab et al., 1972b). Guidelines have recommended the use of amantadine for PD treatment as monotherapy or adjuvant (Ferreira et al., 2013; Fox et al., 2015). Side-effects of amantadine include ankle oedema, confusion and hallucinations (Horn & Stern, 2004).

1.1.6.7 **COMT inhibitors**

Catechol-O-methyl transferase (COMT) is an enzyme which metabolises levodopa and dopamine to 3-O-methylldopa (Axelrod & Tomchick, 1958; Guldberg & Marsden, 1975; Lotta et al., 1995). This decreases the concentration of available levodopa and dopamine. COMT inhibitors impede this process. Entacapone and tolcapone are both COMT inhibitors. Entacapone acts peripherally (Brooks, 2003) while tolcapone can cross the blood-brain barrier and act centrally in the CNS (Truong, 2009). COMT inhibitors are used to treat patients experiencing motor fluctuations while on levodopa. There appears to be no additional benefit in adding a COMT inhibitor to levodopa use if motor fluctuations are not present (Olanow et al., 2004). Side-effects of entacapone and tolcapone are due to increased dopamine stimulation and include dyskinesia, hallucinations, confusion and nausea (Horn & Stern, 2004).
1.1.6.8 **Surgical treatment**

*Deep brain stimulation (DBS)*

Currently, deep brain stimulation is the preferred form of surgical management for PD (Wagle et al., 2014; Weaver et al., 2009). DBS of the STN is used in the advanced stages of PD for patients experiencing problematic motor fluctuation with levodopa (Günther Deuschl et al., 2006; Limousin et al., 1998). From the physiology of PD, it is known the loss of dopaminergic neurons in the SN causes aberrant STN activity. This activity increases activation of the internal globus pallidus, which leads to increased inhibition of the outputs to the motor thalamic nuclei, and in turn, reduces excitation of motor cortical areas (Benazzouz et al., 2000). The mechanism of DBS is not fully understood. Initial studies found an inhibitory effect of DBS on the subthalamic nucleus (Benazzouz et al., 2000) but more recent observations have shown this idea to be too simplistic. The high-frequency impulses from DBS are now thought to modulate the basal ganglia-thalamocortical network through both excitatory and inhibitory effects, with an overall effect of decreasing pathological neural signals (Miocinovic et al., 2013). Surgical ablation techniques performed on the thalamus, subthalamic nucleus and globus pallidus have been used in the past (Krack et al., 2000; Patel et al., 2003; Tasker, 1990) but have largely been replaced by DBS (Bronstein et al., 2011). DBS electrodes can be situated in either the subthalamic nucleus or globus pallidus, both having good efficacy, and result in equal improvement in motor scores or quality of life measures (The Deep-Brain Stimulation for Parkinson’s Disease Study Group, 2001; Weaver et al., 2012). Some recent reports have also suggested that DBS of the pedunculopontine nucleus could improve PD with postural instability and gait disorder (Tykocki et al., 2011; Wang et al., 2017). STN DBS has been reported to allow a greater reduction in dopaminergic medication use compared to GP DBS (Liu et al., 2014). The risks of DBS include mainly surgical complications of bleeding, infection and dysfunction/displacement of the electrode leads (Bronstein et al., 2011).

*Intestinal gel infusion*

Levodopa can also be combined with gel and continuously infused into the jejunum via a percutaneous endoscopic gastrojejunostomy tube (PEG-J) (Fernandez & Odin, 2011; Nyholm et al., 2003). This is an alternative to DBS and, has been shown to reduce levodopa “off time” (Olanow et al., 2014). The risks of continuous gel infusion relate to the surgical procedure to insert the PEG-J, abdominal pain from the device and device malfunction (Lang et al., 2016). Jejunal infusion is recommended to only be used in advanced cases with motor fluctuation and needs attention and monitoring to ensure the condition of the pump and infusion and to
monitor for potential infections. Regular monitoring by a gastrointestinal expert is recommended (Epstein et al., 2016).

*Tissue transplantation*

Surgical transplantation of dopamine-producing neural tissue has been previously performed for PD, the idea being that the graft tissue can secrete dopamine, making up for host deficits (Olanow et al., 1996). This is no longer a recommended treatment due to conflicting study results on efficacy and observed side-effects of graft-related “runaway” dyskinesias (Freed et al., 2001; Kordower et al., 2017; Olanow et al., 2003).

1.1.6.9 *Neuroprotective treatments*

Neuroprotective or disease-modifying therapies would revolutionise PD treatment. Neuroprotection is the idea that a therapy is able to slow neurodegenerative disease progression. Currently, all available therapy for PD is considered symptomatic, giving relief of symptoms, but ultimately not affecting the progressive disease course. A number of trials have been undertaken in the past to test out potential neuroprotective agents.

MAO-I's are thought to help prevent reactive oxygen species formation from the oxidation of dopamine and may prevent cell death by apoptosis. This, in theory, may confer a neuroprotective effect (Jenner, 2004). The TEMPO trial (Parkinson Study Group, 2004) found patients started on rasagiline earlier had a smaller increase in UPDRS scores than those with later treatment. However, this could have been from the symptomatic properties of rasagiline itself (Suchowersky et al., 2006). The ADAGIO trial (Olanow et al., 2009) found a potential disease-modifying effect of 1 mg rasagiline but not a 2mg daily when measured on week 72 of the trial. The interpretation of this result is difficult, with the higher dose having less effect.

The DATATOP study (Parkinson Study Group, 1993) investigated the use of selegiline for treating early PD. Selegiline was found to delay the initiation of levodopa treatment by 9 months, but again, this could have been due to symptomatic improvements provided by selegiline itself, rather than a neuroprotective benefit. Olanow et al. (1995) did report a possible mild neuroprotective benefit with selegiline-treated patients having a slower decline in UPDRS after a two month withdrawal of the medication. This study lasted 14 months total. A study three years after the initial DATATOP results was published found no long-term effect of selegiline treatment on the original study group (Penney et al., 1996). Dopamine agonists have anti-oxidant properties and have been shown to protect dopaminergic neurons in cultures and animal models (Olanow et al., 1998). The REAL-PET study (Whone et al., 2003) and CALM-PD study (Parkinson Study Group, 2002b) compared dopamine agonists.
with levodopa treatment. Both studies found less reduction in positron emission tomography (PET) markers of dopaminergic neuron depletion in the dopamine agonist groups vs levodopa. These results are controversial due to a number of important limitations. For example, no placebo control group was used. The studies also did not have a washout period and the use of PET measures to assess neuron depletion is uncertain (Ravina et al., 2005).

Coenzyme 10 is involved in mitochondrial function, which is known to be disrupted in PD. However, studies have found no support for neuroprotection with coenzyme 10 (Beal et al., 2014; Storch et al., 2007).

Vitamin E is an antioxidant and was included in the DATATOP study. No neuroprotective benefit was found (Parkinson Study Group, 1993). Uric acid, the precipitant for gout, also has antioxidant properties (Ames et al., 1981). Several epidemiological studies have noted a lower prevalence of PD in gout sufferers (Alonso et al., 2007; de Lau et al., 2005). Lower uric acid levels have even been described as a potential PD biomarker (Wen et al., 2017). No study has yet been completed showing a neuroprotective effect of uric acid but any benefit would need to be weighed against the increased risk of developing gout.

Caffeine is an adenosine receptor agonist and caffeine intake has been linked with a lower relative risk of PD (Ascherio et al., 2001; Palacios et al., 2012). Adenosine receptor agonists are seen as a target for future investigation for therapeaic treatment and neuroprotection (Schwarzschild et al., 2006) but no studies have yet shown a neuroprotective effect with an adenosine receptor agonist.

As neuroinflammation is considered to contribute to cell loss, anti-inflammatory medications such as ibuprofen have been investigated for possible neuroprotective benefits (Bassani et al., 2015). Ibuprofen and non-aspirin NSAIDs were found to possibly contribute a neuroprotective effect for PD (Gagne & Power, 2010; Samii et al., 2009).

As previously mentioned, PD pathogenesis is associated with accumulating α-synuclein pathology. Factors increasing α-synuclein toxicity such as phosphorylation processes, the formation of toxic oligomers and the prion-like properties of α-synuclein are all targets for potential neuroprotective treatments (Kalia et al., 2013).

No current therapy is considered to be neuroprotective but vigorous research is ongoing in this field (AlDakheel et al., 2014; McGhee et al., 2016; Rascol, 2009). A reliable biomarker for PD progression is needed to aid identification and assessment of putative neuroprotective
therapies. One potential avenue for biomarker development in PD is assessment of saccadic eye movements.

1.2 Eye movements

1.2.1 Introduction

The human eye is a light detecting organ that detects external visual stimuli, which are sent to and interpreted by the brain. In order to attain sharp central vision, the external light image must be focused on the fovea. The fovea is a pit in the retina at the back of the eye and offers the highest visual resolution (Rossi & Roorda, 2010). In order to keep images focused on the fovea, an adjustable lens is needed to account for varying image distances and also, a system for aligning the eye with the visual target and stabilising the eye in response to head/body motion. A number of eye movement classes exist to maintain this alignment of the image on the retina/fovea such as vestibular eye movements, smooth pursuit, vergence, saccades, nystagmus and fixation (Leigh & Zee, 2000).

In Parkinson’s disease, an eye movement type known as saccades show characteristic changes. The most notable change is hypometria or the undershooting of the saccade, however, changes in saccade reaction time have also been demonstrated (Anderson & MacAskill, 2013; Chan et al., 2005). The primary aim of this thesis is to investigate the hypothesis that this altered saccade performance can reflect brain health in PD as detected using MRI. The aims of this thesis will be the subject of discussion in extensive detail in later sections. Prior to this, I present the fundamental background information regarding saccade testing and saccade control, which are essential to appreciate before a detailed discussion of the study aims and methods can take place.

Saccades are a certain type of eye movement. For completeness, I have provided a brief description below of other non-saccade eye movement types. Although control of different eye movement types may interact, the non-saccade eye movements are controlled by different neural processes compared to saccades and are not related to the studies in this thesis. These non-saccade eye movements are listed in brief but not further discussed. In contrast, the neural control of saccades will be discussed extensively following this.

1.2.1.1 Non-saccade eye movement types

Smooth pursuit

Smooth pursuit is responsible for tracking continuously moving objects and keeping an image of the object constantly projected upon the fovea. Visual information is transformed into eye
movement commands to initiate the smooth pursuit movement. Separate mechanisms are thought to adjust the eye movement to match changes in target motion and to maintain steady-state pursuit (Ono, 2015). Smooth pursuit allows constant, clear vision of moving targets in the visual field. Smooth pursuit also interacts with the vestibulo–ocular reflex to allow the eye to continuously maintain gaze upon an object during self-motion (Huebner et al., 1992; Lanman et al., 1978).

**Vestibulo–ocular reflex**

The vestibulo–ocular reflex or VOR is a reflex for gaze stabilization during head and/or body movement detected by the vestibular system. This occurs by the generation of eye movements equal and opposite the displacement of the head. This system works by using vestibular signals from the labyrinth system, which is integrated with visual information in the brain to generate a compensatory eye movement (Dieterich & Brandt, 1995; Seidman et al., 1995).

**Vergence**

Vergence eye movements are simultaneous movements of each eye in the opposite direction i.e. towards (convergence) or away from the midline (divergence). As the two eyes are separated by a small distance, vergence movements are necessary to keep the same image focused on the fovea of each eye, with closer objects requiring a higher degree of midline convergence, and further objects requiring less (Mays, 1984). Of note, vergence movements were found to be slowed in PD, with patients also showing hypometric divergence movements (Hanuska et al., 2015). As mentioned previously, vergence movements have a separate mechanism of control to saccades and are not further investigated in this thesis. The remaining sections on eye movements will focus solely on saccades.

1.2.1.2 **Saccadic eye movements**

Saccadic eye movements are fast ballistic movements. These rapid eye movements last only tens of milliseconds and quickly redirect the eye to new areas in the visual field. Saccadic movement occurs by a rapid acceleration of the eye which tapers with a rapid deceleration to standstill. The maximum speed of a saccade occurs from about one third to halfway through eye movement travel. Peak velocity is reached relatively earlier in larger saccades (Boghen et al., 1974; Robinson, 1964).

After the initial saccade, the eyes may drift to compensate for slight undershoots and any eye disconjugacy (caused by the two eyes not moving exactly together). This drifting is known as a “glissade”. Another process called “dynamic overshoot” may occur. This is a small saccade
made in the opposite direction to the main saccade movement. Dynamic overshoots are thought to be caused by a reversal in saccadic neural input which stops the main saccade as well as moving the eye in the opposite direction (Bahill et al., 1975). However, another study has suggested that dynamic overshoot may also be a result of physical tissue properties rather than innervation changes (Robinson et al., 1990).

The duration of movement has a linear relationship with the angular distance the eye has to travel and the peak velocity of the saccade is also dependent on the saccade amplitude but in a logarithmic fashion rather than linear (Bahill et al., 1975). Only the amplitude of the saccade can be controlled consciously. Once initiated, saccades cannot be modified: new corrective saccades are required if the original saccade does not fall on target (Leigh & Zee, 2000).

There appears to be greatly reduced vision during saccadic movements. As the eye jumps to a new location, no rapid sweeping motion of the image is seen, despite the movement of light across the retina. One theory is that the blurred image during the saccade would be masked out by the stable images before and after the saccade. It has been shown that suppression of visual pathways occurs during saccadic eye movements: the magnocellular pathway (high temporal resolution with low spatial resolution) is found to be selectively suppressed, with the parvocellular pathway (low temporal resolution but high spatial resolution) unimpaired (Burr, 1994).
1.2.2 Saccadic eye movement experimental classes.

Saccadic behaviour can be experimentally classified into reflexive and volitional tasks. The tasks used in this study are the reflexive task, predictive task, memory-guided task and antisaccade task. Figure 1-4 and Figure 1-5 illustrate the task sequences which were presented to participants.

A Reflexive

B Predictive

C Antisaccade

Figure 1-4 – Diagram of the saccadic tasks, with coloured squares representing task stimuli and numbers on each frame indicating the task sequence. The grey arrow indicates the direction of the saccadic eye movement made for each task. (A) Reflexive task - participants followed a stimulus as it jumped horizontally at pseudo-random intervals and distances. (B) Predictive task - participants followed a stimulus as it jumped horizontally between two fixed positions at a regular interval. (C) Antisaccade - participants fixating on central green target made a saccade to the mirror opposite location of the stimulus.
1.2.2.1 **Reflexive saccades**

These are saccadic eye movements made to new unanticipated targets that appear or move suddenly in the environment. Reflexive saccades, also known as visually-guided saccades or prosaccades, are made by instructing the subject to look at a stimulus as it appears, and presenting the subject with a series of stimuli that appear randomly in time or location, or both. Variations of the reflexive task can be used. The “gap” reflexive task has a short time period from when the original stimulus is extinguished until the new stimulus appears (Saslow, 1967). This offset of stimulus presentation has the effect of disengaging fixation before a saccade is made. The “overlap” reflexive task, by contrast, has a short time period after the appearance of the new stimulus when the previous stimulus remains (Ross & Ross, 1980; Saslow, 1967). This has the effect of requiring the subject to disengage fixation while the fixation stimulus is still present in order to make a saccade to the new stimulus (Leigh & Zee, 2000).

1.2.2.2 **Volitional saccades**

These are deliberate saccades which can be classed by experimental requirements:

Predictive saccades are a type of volitional saccade made to an anticipated appearance of a visual target in the environment (Bronstein & Kennard, 1987). Predictive saccade trials can...
consist of an alternating stimulus, which jumps between two locations at predictable regular time intervals.

Antisaccades are saccades required to be made in the opposite direction to a suddenly-appearing target to its imagined, mirror-opposite position (Fischer & Weber, 1992; Hallett, 1978). Antisaccade trials begin with a central fixation stimulus (illustrated as a green square in Figure 1-4). After a set time, a peripheral stimulus (a red square is used to illustrate) is presented to the left or right of centre, with participants instructed to make a saccade to the mirror opposite location of this stimulus as quickly as possible. This task involves the suppression of a reflexive saccade to the peripheral stimulus and the generation of a volitional saccade in the opposite direction. Error trials occur when the subject makes a saccade towards the target, as opposed to away from it.

Memory-guided saccades are saccades made to a remembered location where a stimulus was previously (Figure 1-5) (Funahashi et al., 1993). Memory-guided tasks involve an initial fixation period, followed by a “cue” or “flash”, where a peripheral stimulus is briefly presented, with the subject required to maintain fixation during this period. The subject is required to retain the location of the cue stimulus for a “delay” period after the cue disappears. Then the fixation stimulus disappears, signalling the subject to make a saccade towards the position of the remembered cue. Error trials occur when the subject is unable to maintain fixation and makes a saccade directly to the cue. The memory-guided saccade task is sometimes known as the oculomotor delayed-response task (Tsujimoto & Postle, 2012).

1.2.3 Saccadic control system

1.2.3.1 Muscular control

Extraocular motor muscles need to overcome viscous forces acting the eye and pull it to fixate a new target in the quickest amount of time (de Corte, 1982). Once the eye is in the new position, it must be held in a stable position for clear vision. This movement of the eye occurs with a powerful contraction of the extraocular muscles (Collins et al., 1975). This contraction is then followed by a stable tonic contraction to hold the eye in position (Robinson, 1964).

The corresponding neural input has the characteristics of a “pulse-step” innervation, described by Robinson in his seminal work (Robinson, 1964). This is a simple model of saccade neurophysiology, with the “pulse of innervation” giving a brief period of intense stimulation of the extraocular muscles, which rapidly contract, moving the eye to a new position (Robinson, 1970). Following this, the muscles hold the eye in the new position against elastic
forces that tends to restore the eye to a central resting position. An isometric (muscle held in
tension without changing in length) contraction of the extraocular muscles occurs after the
pulse and is the result of a steady stream of action potentials to the extraocular muscle. This is
known as the “step of innervation” (Leigh & Zee, 2000; Robinson et al., 1990).

1.2.3.2  **Saccade neural control**

In order to carry out a saccade, the brain converts the spatial coding of visual information to a
motor command. The amount of motor activity in the muscle is determined by action
potential frequency and duration (Robinson, 1970).

Six extraocular muscles move the eye in three rotational axes. These muscles are controlled
by the extraocular motor neurons which carry the neural impulses that shift the eye into the
required spatial position (von Noorden, 1996). The extraocular motor neurons arise from
cranial nerves 3, 4 and 6 which correspond to the oculomotor, trochlear and abducens nuclei
respectively (Sharpe & Wong, 2005). These nuclei receive inputs from various burst neurons
which encode the saccadic movement (Henn et al., 1989). Outside of saccades, burst neurons
are completely inactive. This is due to the gating effect of omnipause neurons (Furuya &
Markham, 1982). Only during saccades do omnipause neurons deactivate, allowing burst
neurons to fire (Evinger et al., 1982; Fuchs et al., 1985).

1.2.3.3  **Burst neurons**

There are two main types of excitatory burst neuron, called the medium-lead burst neuron
(premotor burst cells) and long lead burst neuron. Inhibitory burst neurons also exist (Leigh &
Zee, 2000; Sharpe & Wong, 2005).

**Premotor burst neurons**

Premotor burst cells show excitatory activity approximately 12 ms before saccade initiation
(Scudder et al., 2002; Van Gisbergen et al., 1981). This is responsible for the pulse of
innervation. Premotor burst neurons can be excitatory or inhibitory (Strassman et al., 1986).
Excitatory burst neurons are responsible for generating motor activity while inhibitory burst
neurons fire to stop movement antagonist muscles during saccades (Leigh & Zee, 2000;
Strassman et al., 1986).

The control of horizontal saccades arises from premotor burst neurons within the paramedian
pontine reticular formation (Henn et al., 1989). These reach the extraocular motor neurons via
axons running in the medial longitudinal fasciculus (Horn et al., 1996). Vertical and torsional
saccades are controlled by midbrain premotor burst cells arising from the rostral interstitial
nucleus of medial longitudinal fasciculus (riMLF) (Henn et al., 1989). riMLF pathology
causes vertical gaze deficiencies in PSP (Bhidayasiri et al., 2001) and Niemann-Pick type C
disease (Salsano et al., 2012).

**Long lead burst neurons**

Long lead burst neurons (LLBN) start to show activity 40 ms or more prior to the saccade and
continue to fire during the saccade (Leigh & Zee, 2000). The LLBN are linked with the
central mesencephalic reticular formation and receive input from the superior colliculus and
have projections with the pontine excitatory burst neurons, medullary inhibitory burst neurons
and omnipause neurons (Scudder et al., 1996). Other LLBN are found in the nucleus
reticularis tegmenti pontis and project to the cerebellum (Scudder et al., 1996). Some neurons
project from the cerebellum to the paramedian pontine reticular formation (Scudder et al.
1996). Interactions of the LLBN are speculated to be involved in a feedback loop or a
resettable integrator for saccades (Waitzman et al., 1996). The LLBN connection with
omnipause cells also suggests a role in the start/stop control of saccades (Hepp & Henn, 1983;
Scudder et al., 1996).

1.2.3.4  **Omnipause neuron**

These neurons inhibit burst cells arising from the PPRF and riMLF (Nakao et al., 1989;
Nakao et al., 1980). These cells are constantly firing (i.e. tonic) (Strassman et al., 1987) and
only cease before and during saccades (Fuchs et al., 1985). In animal studies where the
omnipause cells are chemically excited, saccade velocity is decreased,(Kaneko, 1996). It is
believed omnipause neurons inhibit burst neurons until a saccade is made. This helps to
control burst cells to ensure they fire only when necessary (Leigh & Zee, 2000).

1.2.3.5  **Cortical and subcortical control of saccades**

Several cortical and subcortical areas are involved in saccadic control. These areas have
mainly been investigated using functional MRI techniques and transcranial magnetic
stimulation (TMS)(Milea et al., 2005). An illustration of cortical regions involved in saccade
control is provided (Figure 1-6). A circuit diagram of saccadic eye movement control is
provided in Figure 1-7.
Figure 1-6 – Diagram of cortical regions of eye movement control location. Figure permission obtained (Leigh & Kennard, 2004).

Figure 1-7 – Circuit diagram of basal ganglia and cortical structures connecting to the brainstem saccade generator. Abbreviations: FEF - frontal eye field, SEF - supplementary eye field, DLPFC – dorsolateral prefrontal cortex, PEF – parietal eye field, IMP – internal medullary lamina, SNpr – substantia nigra pars reticulata, STN – subthalamic nucleus, NRTP – nucleus reticularis tegmenti pontis. Adapted with permission (Leigh & Kennard, 2004).
1.2.3.6 **Superior colliculus**

The superior colliculus (SC) is a major site for sensorimotor integration needed to direct the head and eyes towards objects of interest (May, 2005; Platt, Lau, & Glimcher, 2003; Sparks & Hartwich-Young, 1989; Sparks & Mays, 1990). The SC receives input from all cortical eye fields and the basal ganglia. The SC is in the final pathway where connections converge from basal ganglia and cerebral cortical regions (Hikosaka et al., 2000; Terao et al., 2013) and as a result, has been described as the bottleneck of neural structures responsible for saccades. The SC projects to critical brainstem areas that are involved in the circuits (comprising excitatory and inhibitory burst neurons) which generate premotor commands for saccades (Moschovakis, 1996). These include the paramedian reticular formation of the pons, the nucleus prepositus hypoglossi and central mesencephalic reticular formation for horizontal saccades. Vertical saccades involve connections with the rostral interstitial nucleus of the medial longitudinal fasciculus, the interstitial nucleus of Cajal and the nucleus of the posterior commissure. The SC is also connected to omnipause neurons in the nucleus raphe interpositus (May, 2005).

The SC anatomically consists of seven layers. The superficial layers (stratum zonale, stratum griseum superficiale, and stratum opticum) receive retinal information for visual processing. The intermediate and deep layers (stratum griseum intermedium, stratum album intermedium, stratum griseum profundum, and stratum album profundum) respond to a number of sensory modalities (visual, auditory, proprioception and vestibular input) and integrates these inputs into a topographic sensory map (Wallace, Wilkinson, & Stein, 1996). The intermediate layers also send motor output signals to brainstem saccade generating regions prior to saccades (Gandhi & Katnani, 2011; May, 2005; Moschovakis, 1996).

The intermediate layers of the SC containing neurons coding for saccade output are organized to form a retinoscopic motor map, with the location of the neural activation in the SC corresponding to a specific saccade amplitude and direction. Rostral neurons within the SC discharge for small-amplitude saccades, whereas caudal neurons discharge for large saccade amplitudes. Medial SC regions are most active during upward saccades and the lateral regions for downward (Sparks et al., 1976). The saccade activity is not from single neuron activity but occurs as a Gaussian “mound”, involving a spread of SC neurons – up to 28% of saccade-related neurons discharging for every saccade (Munoz & Wurtz, 1995), with the activity peak centred on the SC location corresponding to the desired saccade vector. Aside from location, the SC neural discharge also codes for saccade velocity by discharge firing rate (Sparks & Mays, 1990). Saccade-related output activity of the SC is thought to be mapped to retinal
coordinates and not the environment. Activation of a specific SC location corresponds to a fixed retinal location (Klier et al., 2001).

The rostral pole of the SC was thought to contain a “fixation” zone (Munoz & Wurtz, 1992, 1993a, 1993b). However, this has been debated recently. Neurons in this area were seen to fire during small saccades. Activity in this area doesn’t directly correspond with omnipause neuron activity and the rostral SC neurons have no different characteristics from the saccade-related neurons aside from their location (Gandhi & Katnani, 2011; Krauzlis et al., 2017). A current proposal states that fixation is achieved with equilibrium between both SC hemispheres. Each SC simultaneously contributes a motor saccade signal for the target which is balanced out, achieving no overall eye movement, i.e. fixation. This is known as the “equilibrium hypothesis” (Goffart et al., 2012; Hafed et al., 2008; Krauzlis et al., 2017).

SC ablation in the primate results in increased reflexive saccade latencies to the contralateral side, with an elimination of fast “express”-type saccades (Schiller et al., 1987). Frontal eye field lesions (FEF) in the same study did not result in these findings, leading Schiller et al. to conclude that the SC, rather than the FEF, is responsible for saccade reaction times and express saccades. It is known that PD patients make more express-type saccades (Chan et al., 2005), which could possibly indicate PD-related changes in the SC (or a circuit with inputs to the SC). The SC is a small structure deep within the brain and is located close to pulsating vessels (Poncelet et al., 1992). These factors make this region difficult to image using functional MRI (fMRI), but studies using cardiac gating have been able to identify SC activation (DuBois & Cohen, 2000; Guimaraes et al., 1998).

1.2.3.7 Basal ganglia

As mentioned in the general introduction, the basal ganglia are a group of nuclei located in the base of the forebrain. Here, I discuss their role in eye movement control. The basal ganglia consists of the caudate nucleus (CN), putamen and the nucleus accumbens (collectively known as the striatum) (Hikosaka et al., 2000), substantia nigra (pars compacta and pars reticulata, SNC and SNR respectively), globus pallidus (internal and external, GPe and GPi respectively), subthalamic nucleus (STN), ventral tegmental area, and the olfactory tubercle (Heimer, 1978; Xiong & Wesson, 2016). The basal ganglia have been thought to have a role mainly in motor function as well as cognitive functions such as working memory and sequence learning (Helie et al., 2013). The basal ganglia are also involved in saccade control through a circuit which mainly involves the CN, SNR, STN and GPe (Figure 1-8) (Hikosaka et al., 2000). The basal ganglia control saccades by sending connections to the SC. Outputs from
the basal ganglia reach the SC via substantia nigra pars reticulata (SNr), which can be seen as the primary output region of the basal ganglia for saccade control. The SNr sends inhibitory connections to the SC that regulates the excitatory inputs entering the SC (Leigh & Kennard, 2004). In primates, it has been observed that electrical stimulation of the SNr, which increases inhibition of the SC, reduces the amplitude of both visually-guided and memory-guided saccades (Basso & Liu, 2007). The influence of the SNr appears to be more prominent for volitional saccades, with SNr stimulation having a greater effect on memory-guided saccades than visually-guided saccades (Basso & Liu, 2007). A similar result was shown by Mahamed, Garrison, Shires, & Basso (2014). In a memory-guided task, when the SNr was stimulated during the memory period, saccades occurred less frequently in the contralateral direction (to the stimulation site), with a longer latency (slower reaction time). The SNr is modulated by the caudate nucleus. The caudate nucleus has an intermittent (i.e. phasic) suppressive effect on SNr activity which, in turn, transiently removes the SNr inhibitory effect on the SC (Hikosaka et al., 1989). These signals from the SNr and caudate nucleus are greatly influenced by behavioural contexts, such as attention and expectation (Hikosaka et al., 2000).

The caudate nucleus can be seen as the input region for the basal ganglia. The caudate nucleus receives numerous connections from nearly every region of the cortex including the FEF, SEF and DLPFC (Graybiel & Ragsdale, 1979; Stanton et al., 1988), which are thought to modulate caudate activity.
Figure 1-8 – A simplified diagram of the basal ganglia saccade circuit. The caudate nucleus (CN) is seen to have D1-expressing connections to the SNr (direct pathway) and D2-expressing connections to the GPe (indirect pathway). The SNc produces dopamine and is affected in PD leading to dopamine depletion of the basal ganglia system. Figure permission obtained (Watanabe & Munoz, 2011).

Direct and indirect pathways

Saccade-related neurons of the CN output to SNr neurons through the direct dopamine receptor D1 (or simply D1) pathway. This inhibits the SNr, removing the SNr inhibitory effect on the SC and facilitating saccades. The CN can also exert influence on SNr indirectly, via the GPe in the indirect dopamine receptor D2 (D2) pathway. The indirect pathway has an overall inhibitory effect on saccades – opposite to the direct pathway. Caudate projections inhibit the GPe, which normally inhibits the SNr. Therefore, an inhibited GPe, leads to increased SNr activity, which is inhibitory on the SC (Hikosaka et al., 2000; Watanabe & Munoz, 2011).

Dopamine depletion causes opposite effects on D1 and D2 receptor-mediated neurons, with decreased D1 (direct pathway) activity and enhanced D2 (indirect pathway) activity. The increased indirect pathway activation means in theory, for parkinsonism syndromes, the inhibitory activity of the SNr will be enhanced, suppressing the SC and inhibiting eye movements. In primate studies with unilateral dopamine depletion limited to one side of the
CN, fewer spontaneous contralateral saccades are made. Decreased amplitude was seen for contralateral saccades for both visually-guided and memory-guided saccades but a decrease in latency was only seen for memory-guided saccades (Kori et al., 1995). This observation does mirror in some aspects that which is seen in PD eye movement studies. PD patients make frequent fast express saccades in visually-guided tasks and generate hypometric (undershooting) memory-guided saccades (Chan et al., 2005). However, the predominance of the indirect pathway in dopamine depletion and subsequent inhibitory effect on the SC does not explain the increased propensity for express saccades. PD patients also tend to make more reflexive erroneous anticipatory saccades in the antisaccade task and are less able to inhibit reflexive saccades in a delayed response task (Chan et al., 2005). It is possible other mechanisms are also contributing to eye movement changes in PD (van Stockum et al., 2008), such as attention deficits or dysfunctional signals from cortical regions causing an increase in excitability in saccade-related SC neurons.

CN activation has been found to be stronger for antisaccades compared to prosaccades (Brown et al., 2006; Dyckman et al., 2007; Ettinger et al., 2008; Sweeney et al., 1996). Watanabe & Munoz (2009) postulated a conflict system within the CN which occurs during antisaccades. The caudate nucleus is proposed to have separate regions for automatic reflexive saccades, voluntary contralateral saccades and voluntary ipsilateral saccades. In the antisaccade task, competition is thought to occur between mechanisms behind the erroneous reflexive saccade and the correct voluntary saccade within the basal ganglia. The caudate is thought to help generate the correct voluntary saccade by facilitating a saccade via the direct pathway in the correct (opposite) direction, while using the indirect pathway to inhibit a saccade in the incorrect (reflexive) direction.

The STN modulates SNr activity through a direct glutamatergic connection. The STN also has reciprocal connections with the GPe, which projects to the SNr itself. The STN is a key target for deep brain stimulation (DBS) in Parkinson’s disease (see treatment section). DBS of the STN is thought to modulate aberrant pathological signals which arise in the dopamine depleted basal ganglia. DBS is believed to enhance inhibition of the SNr via the STN connection and the STN, GPe, SNr circuit. DBS may inactivate the increased burst neuron activity of the STN, resulting in a decrease of SNr activity. DBS improves certain saccade performance measures in PD. Fawcett et al. (2010) found latencies for visually-guided saccades were decreased and saccade amplitude for memory-guided and antisaccades were improved. Similarly, Yugeta et al. (2010) found similar results with DBS improving reaction times and saccade amplitudes for reflexive and antisaccades. Memory-guided saccades only
showed improved amplitude. Error reflexive saccades were reduced in the memory-guided task with DBS. As the mechanisms behind DBS are not fully understood, from these studies, we can only speculate on the mechanisms behind eye movement changes in PD with DBS.

One theory of basal ganglia function involves certain frequencies of neural oscillations, known as the “beta band”. These frequencies are detected in basal ganglia electrode recordings and are enhanced in PD patients “off” medication. The beta band is thought to be “akinetiс” in nature (Brown & Williams, 2005; Weinberger et al., 2009). DBS could disrupt these pathological akinetic frequencies, facilitating the generation of saccadic eye movements (Yugeta et al., 2010). DBS leads to increased glucose metabolism (observed using PET) in cortical regions distant to the immediate electrode stimulation sites, including regions in the frontal lobe, temporal lobe and parietal lobe (Hilker et al., 2004). This observation may indicate increased activity in these distal cortical regions induced by DBS. Fawcett et al. (2010) speculated that cortical eye control regions such as the SEF may be influenced by DBS, leading to improvements in saccadic eye movement measures. Temel et al., (2008, 2009) found decreased latencies in the reflexive task for DBS patients. When plotting the latencies Temel et al. found the results to correlate well with a statistical model predicting latency distributions called the LATER model (Carpenter & Williams, 1995; Noorani & Carpenter, 2016). The nature of the correlation indicated neural signals involved in reflexive saccade generation rose faster to saccade threshold in DBS patients.

The remaining basal ganglia nuclei and the influence each has on eye movements are complex and incompletely understood. A review article by (Watanabe & Munoz, 2011) further explored the theories behind each individual nuclei but discussion on this aspect is beyond the scope of this discussion and the studies in this thesis.

1.2.3.8 Frontal eye field

The FEF is described as a cortical region within the frontal lobe involved in the control and triggering of saccadic eye movements (Pierrot-Deseilligny et al., 2002). FEF activity is consistently seen in imaging studies of eye movements: It is active in fixation (Petit et al., 1995) and nearly all types of saccadic eye movement tasks: reflexive and memory-guided saccades (Anderson et al., 1994), delayed-response saccades (Sweeney et al., 2002), predictive saccades (O’Driscoll et al., 2000), antisaccades (Brown et al., 2006; Kimmig et al., 2001; O’Driscoll et al., 1995; Darby et al., 1996; Matsuda et al., 2004; McDowell et al., 2005), saccade preparation (Connolly et al., 2005; DeSouza et al., 2003), suppression of reflexive saccades (Cornelissen et al., 2002) and self-paced saccades (McDowell et al., 2002). Much of our understanding of the FEF is derived from primate studies, with knowledge of a
frontal lobe region related to eye movements going back to the late 1800s. These early studies found electric stimulation of the monkey frontal lobe was able to trigger saccades (Ferrier, 1874; Ferrier, 1876). Later studies localised this primate FEF to the arcuate sulcus in Brodmann’s area 8 (Tehovnik et al., 2000). Electrode recording and electrostimulation studies in epilepsy patients have found the human FEF equivalent in the posterior middle frontal gyrus and adjacent superior frontal sulcus (Blanke et al., 1999, 2000). Imaging studies in the human have found FEF activation in the precentral sulcus (Paus, 1996; Petit et al., 1995) precentral gyrus (Anderson et al., 1994; Fox et al., 1985; Sweeney et al., 1996) and middle frontal gyrus (Kawashima et al., 1998).

The FEF receives connections from the substantia nigra pars reticulata, superior colliculus and cerebellar dentate nucleus (Lynch, Hoover, & Strick, 1994) as well as the supplementary eye field (SEF) (Schall, Morel, & Kaas, 1993), dorsolateral prefrontal cortex (DLPFC) (Pierrot-Deseilligny et al., 2005), posterior parietal cortex (PPC) (Ferraina et al., 2002) and primary visual areas (Schall et al., 1995). The FEF projects connections to the contralateral FEF (Künzle & Akert, 1977), SEF (Stanton et al., 1993), the caudate nucleus, superior colliculus, pontine nuclei (Stanton et al., 1988) and the parietal cortex (Stanton et al., 1995). The FEF plays a key role in saccade initiation, with connections to SC. The saccade command is thought to arise from the FEF to SC to trigger a saccade (Helminski & Segraves, 2003; Komatsu & Suzuki, 1985; Schlag-Rey et al., 1992; Sommer & Wurtz, 2000). The SC also sends projections back to the FEF, with SC signals thought to modulate pre-saccade activity (Berman et al., 2009).

The main role of the FEF is in triggering voluntary saccades, with less involvement in reflexive or visually-guided saccades (Müri & Nyffeler, 2008; Pierrot-Deseilligny et al., 2004). The FEF in each hemisphere triggers saccades to the contraversive side (Connolly et al., 2005; Everling & Munoz, 2000). The FEF is seen to be involved in predictive, memory-guided and antisaccade tasks – all voluntary saccade tasks (Everling & Munoz, 2000; Gaymard et al., 1999; Hanes & Schall, 1996; Müri & Nyffeler, 2008; Pierrot-Deseilligny et al., 2004). Single neuron studies in the primate show neurons in the FEF active only after purposeful saccadic eye movements (Bruce & Goldberg, 1985). Lesion studies by Gaymard et al. (1999) and Rivaud et al. (1994) have found the FEF to be associated with increased latencies for antisaccade and memory-guided saccades, and a decreased gain (decreased accuracy) in the predictive task.
In contrast to the strong evidence of FEF involvement in voluntary tasks, studies have produced mixed results on the impact of impaired FEF function in reflexive saccade tasks. A lesion study by Rivaud et al. (1994) found increased latencies in the reflexive overlap task and an increased gain in the gap reflexive task with FEF lesions. A lesion study carried out by Pierrot-Deseilligny et al. (1987) found the reaction time of gap reflexive saccades was shortened in patients with lesions affecting the FEF, but latencies were unchanged in a later study (Pierrot-Deseilligny et al., 1991). Further lesions studies by Gaymard et al. (1999) and Rivaud et al. (1994) described saccade latency to not be affected in the gap reflexive task in FEF lesions, but increased in the reflexive overlap task. From these observations, the authors have proposed that the role of the FEF relates more to fixation disengagement than reflexive saccade generation.

Transcranial magnetic stimulation (TMS) studies have attempted to clarify FEF function in reflexive saccades. TMS can be thought of as generating temporary “lesions” by a focussed magnetic pulse delivered to a specific cortical region, temporarily disrupting function. TMS of the human FEF was found not to affect visually-guided saccades but caused significantly prolonged antisaccade latency (Müri et al., 1991). Priori et al. (1993) reported that TMS to the vertex (approximate FEF) can disrupt visually-guided reflexive saccades. TMS over the FEF increased the latency of reflexive prosaccades (Nagel et al., 2008) but van Donkelaar, Lin, & Hewlett (2009) observed that FEF TMS induced a greater proportion of saccades made with decreased latencies, along with hypometria, necessitating multiple corrective saccades. This pattern occurred on the side ipsilateral to the TMS application, suggesting a disruption in the balance between the hemispheres (van Donkelaar et al., 2009). As with lesion studies, conclusions from TMS studies into reflexive saccade FEF function remain uncertain. The application of TMS is also confounded by an audible click during stimulation, which may act as an auditory signal to initiate a saccade through a non-visual, auditory pathway. Despite the variable results from lesion and TMS studies, imaging studies have consistently observed FEF activity during reflexive saccades (Anderson et al., 1994; Connolly et al., 2005; Grosbras et al., 2001), indicating that the FEF is involved in the generation of reflexive saccades. Studies using pharmacological inactivation of the FEF report a resulting “ocular motor scotoma” (Dias, Kiesau, & Segraves, 1995; Dias & Segraves, 1999), with severe impairment in both visual and memory-guided saccades, with fewer saccades initiated to retinotopic sites of FEF inactivation.

Much like the SC, the FEF is noted to have a spatial motor map (Bruce et al., 1985; Savaki et al., 2014; Schall, 2002) with smaller saccades arising from the ventral-lateral and larger
saccades from the dorso-medial region. Regions of the FEF are also involved in vergence movements (Alkan et al., 2011; Jampel, 1960). Stimulation of certain regions of the FEF can promote fixation (Hanes et al., 1998) and FEF microstimulation can also suppress saccades in visually-guided and memory-guided saccade tasks (Izawa et al., 2004a). Izawa, Suzuki, & Shinoda (2004b;2005) have suggested the FEF helps fixation by saccade inhibition, noting FEF lesions cause difficulty suppressing reflexive “express” saccades to inappropriate targets (Braun et al., 1992; Guitton., 1985). Pharmacological inactivation of FEF also impairs fixation control with deviation of gaze towards the inactivated FEF side (Dias et al., 1995; Dias & Segraves, 1999).

The FEF exhibits preparatory activity before saccade generation for the antisaccade task (Connolly et al., 2002; DeSouza et al., 2003). Preparatory FEF activity correlates with saccade latency in fMRI studies of pro and antisaccade tasks (Connolly et al., 2005), an observation also made in monkey electrophysiology trials (Everling & Munoz, 2000). It is proposed that preparatory activity reflects some form of pre-saccade attention control (Smith et al., 2005).

The FEF is part of an attention network involving a number of other cortical regions including the visual cortex, parietal lobe and superior colliculus (Armstrong & Moore, 2007; Bisley & Goldberg, 2010; Buschman & Miller, 2007; Coe et al., 2002; Corbetta & Shulman, 2002; Ignashchenkova et al., 2004; Noudoost et al., 2010; Premereur et al., 2014; Wardak et al., 2011). The attention component of the FEF can influence saccade generation (Schafer & Moore, 2007). FEF neurons activate to form a visual saliency map which is involved in the direction of overt (with gaze shift) and covert (without eye movement) attention shifts (Awh et al., 2017; Beauchamp et al., 2001; Monosov & Thompson, 2009; Thompson & Bichot, 2004). Certain neurons within the FEF have been shown to evaluate stimulus importance (Wurtz & Mohler, 1976) with the FEF implicated in maintaining spatial information and target selection (Armstrong et al., 2009; Cohen et al., 2010; Schall, 2002; Schiller & Tehovnik, 2005). Magnetic stimulation over FEF interferes with visual search tasks. and target discrimination (Muggleton, 2003; O’Shea et al., 2004).

The FEF also has a role in error control. This is relevant to predictive and memory-guided saccades, which are made to a location with no visual target. Occasionally corrective saccades are made without any visual feedback. An investigation of these corrective saccades concluded that the FEF encodes for eye movement errors without external visual input (Ferrera & Barborica, 2010). Others have detected post-task FEF activity (albeit from a visual
selection task and not a saccade task), and proposed that the FEF optimises future responses by processing errors made in previous responses (Teichert, Yu, & Ferrera, 2014).

Some have suggested the FEF may have functionally different subregions (Ettinger et al., 2008; Grosbras et al., 2001; Lobel et al., 2001; Luna et al., 1998), with involvement in either reflexive or predictive type tasks (Gagnon et al., 2002; Simo, 2005) but no firm conclusions have yet been drawn. This issue is discussed further in Chapter four.

1.2.3.9 Supplementary eye field

The supplementary eye field (SEF) is located in the medial frontal lobe in Brodmann area 6 (BA6), rostral to the supplementary motor area (SMA) (Grosbras et al., 1999). The SEF is connected to all cortical eye movement control areas – the FEF, DLPFC and the parietal cortex as well as the caudate nucleus of the basal ganglia and SC (Huerta & Kaas, 1990; Schlag & Schlag-Rey, 1987; Shook et al., 1991). No cohesive theory of SEF function has thus far been put forward. Rather, the SEF is seen to be active in nearly every type of saccade task. SEF activity has been reported in imaging studies for visually-guided saccades (Luna et al., 1998), self-paced saccades (Petit et al., 1993), memory-guided saccades (Heide et al., 2001) and antisaccades (Doricchi et al., 1997; O’Driscoll et al., 1995). For antisaccades, the SEF is thought to be more involved in antisaccade preparatory activity, rather than the antisaccade itself (DeSouza et al., 2003). Primate studies have detected SEF neural activity in relation to saccade performance and error/reward monitoring (Amador et al., 2000; Stuphorn et al., 2000), saccade task instruction learning (Chen & Wise, 1995), determination of eye movements relative to object position (Olson & Gettner, 1995) and internally processing saccade-related decisions (Coe et al., 2002). A region immediately anterior to the SEF has been termed the pre-SEF (Pierrot-Deseilligny et al., 2003). The pre-SEF is more involved in the learning of the saccade sequence and the SEF proper responsible for initiating the saccade sequence.

A consensus view is that the SEF is involved in internally generated complex eye movement tasks such as coordinating a memorised sequence of several saccades (Heide et al., 2001; Lu et al., 2002). This idea has been challenged by Parton et al. (2007) using a patient with a focal unilateral SEF lesion. This patient showed no impairment in sequencing learning but rather an impairment in switching between eye movement tasks and learning a new (arbitrary association) saccade task. Also of note was that this patient generated hypometric saccades in memory-guided saccades, similar to PD patients.
Outside of direct saccade control processes, the SEF is thought have a role in “metacognition”. Simply put, metacognition is thinking about one’s own thoughts. This process is thought to occur in animals as well as humans (Smith et al., 2014). In a saccade task involving a betting component, metacognitive thought processes are needed to monitor and make decisions as opposed to making arbitrary choices. Neural activity, specifically believed to be related to metacognitive processes, was detected in the primate SEF between the decision making process and executing the “bet” (making the saccade) (Middlebrooks & Sommer, 2012).

1.2.3.10 Dorsolateral prefrontal cortex

The dorsolateral prefrontal cortex (DLPFC) is located in the frontal lobe in area BA46 (Pierrot-Deseilligny et al., 1991) and has connections with the FEF, SEF, PPC, PEF and SC (Pierrot-Deseilligny et al., 2005). The DLPFC is linked to executive processing functions. These are a set of higher-order cognitive processes such as decision making or adaptation of behaviour to changing rules and working memory (MacDonald et al., 2000; Mansouri et al., 2009; Miller & Cohen, 2001; Petrides, 2005). Executive function processes are known to be deficient in PD (Monchi et al., 2007; Owen, 2004; Zgaljardic et al., 2004) and imaging studies have found altered DLPFC function in PD related to deficits in executive function tasks (Cools et al., 2002; Hirano et al., 2012; Lewis et al., 2003).

DLPFC lesions were seen to have detrimental effects on saccade prediction, saccade inhibition and memory-guided saccade accuracy (Pierrot-Deseilligny et al., 2003). This suggests that the DLPFC plays a role in saccade response selection, prediction, inhibition and short-term spatial working memory i.e. saccade tasks requiring a component of executive function (Pierrot-Deseilligny et al., 2003; Pierrot-Deseilligny et al., 2005; Schall & Thompson, 1999). An electroencephalography (EEG) study found pre-saccade activity was higher in the prefrontal cortex in antisaccade trials compared to prosaccade trials. This was thought to fit with the DLPFC’s role in executive control (Clementz et al., 2007). The DLPFC is also involved in reflexive saccade inhibition. In the antisaccade task, a reflexive saccade needs to be suppressed during the presentation of a peripheral target. A higher number of error reflexive saccades are generated in patients with prefrontal impairment (Guitton et al., 1985; Hodgson et al., 2007; Kitagawa, Fukushima, & Tashiro, 1994). Condy et al. (2004) proposed that the DLPFC suppresses reflexive saccades stimuli through direct SC inhibition.

The DLPFC is consistently seen to be involved in memory-guided saccades in neuron recordings and imaging studies (Chafee & Goldman-Rakic, 2000; Chafee et al., 2011;
O’Sullivan et al., 1995; Pierrot-Deseilligny et al., 1991; Sweeney et al., 1996). The prevalent theory states that the DLPFC is involved in spatial working memory (Goldman-Rakic, 1995). Neurons in the DLPFC exhibit sustained activation during spatial memory retention (Funahashi et al., 1989) and DLPFC lesions cause deficits in spatial memory function (Funahashi et al., 1993). Spatial working memory processes are thought to involve a number of distributed regions including the PPC (Chafee & Goldman-Rakic, 2000; O’Sullivan et al., 1995; Sweeney et al., 1996) and the parahippocampal cortex within the medial temporal lobe (Ploner et al., 2000; Ploner et al., 1999). A model proposed by Pierrot-Deseilligny et al. (2002) describes the PPC as being involved in the first 300 ms of visuospatial integration, with the DLPFC involved in short-term spatial memory subsequently afterwards, out to approximately 20 seconds. After this period, the parahippocampal cortex becomes involved in medium-term memory, from 20 seconds to several minutes, before long-term memorisation occurs in the hippocampus. Memory-guided saccades in PD are impaired, showing a greater impairment in accuracy during shorter (less than 20 seconds) delay periods compared to controls. DLPFC impairment in PD is thought to contribute to this difference (Le Heron et al., 2005).

The DLPFC is not exclusively involved in working memory processes even within the proposed 20-second window. A model involving the PPC, DLPFC and parahippocampal cortex has been proposed as the “serial information pathway” (Nyffeler et al., 2002). An alternative “parallel pathway”, involving visuospatial information passed directly from the PPC to the parahippocampal cortex and bypassing the DLPFC has been posited (Nyffeler et al., 2004). Both pathways may operate together as a mixed model (Nyffeler et al., 2004). Other studies have observed frontal/FEF (Courtney et al., 1998; Gaymard et al., 1999; Srimal & Curtis, 2008) and visual cortex (Harrison & Tong, 2009; Serences et al., 2009) involvement in visual working memory tasks. A study by Mackey et al. (2016) has recently challenged the viewpoint of human DLPFC involvement in short-term visuospatial memory. Mackey and colleagues have shown, in their sample of patients with isolated DLPFC lesions, no impairment in a memory-guided saccade task, using delays of 3-5 seconds. Only patients having lesions extending into the FEF showed impairment in saccade accuracy. Mackey et al. (2016) have suggested these findings could indicate remodelling of cortical function, with other regions compensating for the loss of the DLPFC, or a more prominent role of the human FEF, rather than the DLPFC in visuospatial memory, which would indicate a mismatch between animal and human study findings.
Regarding functional imaging, Riggall & Postle (2012) have challenged the idea that working memory processes can be detected as sustained BOLD activity during the memory period. Instead, they state any sustained activity could relate to other task demands such as attention or preparation processes. Riggall and Postle argue memory processes may, in fact, be distributed amongst the multiple cortical regions, which may not reach detection threshold using current imaging analysis techniques. Riley & Constantinidis (2015) argue that persistent activity during memory periods does correspond to working memory processes in the DLPFC, with computational models of persistent prefrontal activity accurately predicting memory-related task outcomes (Wimmer et al., 2014). Riley and Constantinidis, however, similarly to Riggall and Postle, state that current MRI methods may be limited and do not offer the resolution necessary to accurately delineate activity from closely spaced prefrontal cortex regions.

1.2.3.11 Parietal cortex
The parietal cortex has a number of densely-packed regions responsible for the transformation of sensory information into spatial representations needed to guide motor output (Andersen, 1989; Colby & Goldberg, 1999). One of these regions seen in the primate brain, named the lateral intraparietal cortex (LIP), is thought to be responsible for these spatial transformations needed for saccadic eye movements (Andersen et al., 1992; Barash et al., 1991; Colby & Goldberg, 1999). Every time an eye movement is made, the retinal image changes, yet we do not get a sensation of movement. This is because a stable internal image of the visual environment is maintained, despite changing visual input. The LIP, through constant calculations and compensation for eye position changes, is able to form this stable internal representation (Duhamel et al., 1992). The LIP is also involved in attention and responds to salient visual stimuli (Colby & Goldberg, 1999). LIP neurons are active when detecting environmental targets for possible saccades. LIP activity is detected during fixation tasks, even with no eye movements, if a novel visual stimulus appears in the visual field (Robinson et al., 1978). LIP neurons also fire when a stable object already present in the environment become salient e.g. cueing the monkey to make a saccade to the stable object without any change or new appearance of the object itself (Gottlieb et al., 1998).

The LIP has projections to the SC (Lynch et al., 1985; Paré & Wurtz, 2001) and FEF (Barbas & Mesulam, 1981; Huerta et al., 1987; Tian & Lynch, 1996). The LIP has been described as the equivalent to the parietal eye field (PEF) in humans and the terms are often used interchangeably (Andersen et al., 1992; Culham et al., 2006).
Studies report the human LIP/PEF to be located in the intraparietal sulcus (IPS) (Astafiev et al., 2003; Brotchie et al., 2003; Culham et al., 2006; Konen & Kastner, 2008; Medendorp et al., 2003; Schluppeck et al., 2005; Sereno, 2001). The IPS divides the superior and inferior parietal lobules, which altogether forms the posterior parietal cortex (PPC). The PEF may also involve parts of the superior parietal lobule (Konen & Kastner, 2008; Sereno, 2001) or both the inferior and superior parietal lobule (Luna et al., 1998).

The PEF is thought to have a role in the triggering of reflexive saccades (Pierrot-Deseilligny et al., 2002). Lesion studies show reflexive saccade prolongation with parietal cortex damage (Gaymard et al., 2003; Heide et al., 1998; Pierrot-Deseilligny et al., 1987) with similar results from parietal TMS disruption (Kapoula et al., 2001).

As mentioned previously (see DLPFC section), the PPC is thought to be involved in the early stages of visuospatial memory, up to 300 ms in a model known as the “serial information pathway” (Nyffeler et al., 2002; Pierrot-Deseilligny et al., 2002). TMS studies have found PPC disruption during this early memory phase to disrupt memory-guided saccade accuracy (Brandt et al., 1998). Despite being only supposedly involved for the first 300 ms of spatial memory, functional imaging has found sustained PPC (and IPS) activity during significantly longer memory periods, of up to 15 seconds, in memory-guided saccade tasks (Brown et al., 2004; Curtis & D’Esposito, 2006; Schluppeck, 2006; Schluppeck et al., 2005). This may indicate memory, or an as-of-yet unidentified process, persists in the parietal cortex past the initial 300 ms memory period, which isn’t explained using the previously proposed “serial information pathway” of spatial memory. Figure 1-9 depicts the general location of regions involved in saccadic eye movement control (Pierrot-Deseilligny et al., 2004).

In the antisaccade task, greater activity in the PPC is seen compared to prosaccades (Doricchi et al., 1997; O’Driscoll et al., 1995; Sweeney et al., 1996). This increase in activity occurs during the “preparatory” process, prior to generating the antisaccade (Ford et al., 2005). This is thought to be from vector transformation processes, i.e. the process of inverting the saccade direction for the antisaccade task, which is thought to occur in the PPC (Moon et al., 2007; Nyffeler et al., 2007; Zhang & Barash, 2000).
**PPC subregion anatomy**

Our knowledge of the role of the PPC in eye movement control is founded on primate studies. A summary diagram of primate neural recording study findings of the IPS is provided in Figure 1-10. This thesis will only discuss saccade functions of the IPS/PPC area. However, serves to illustrate the closely spaced and varied functions of regions within the primate IPS, highlighting the issues in identifying equivalent regions in the human brain using non-invasive, less spatially sensitive methods such as fMRI.
Interspecies differences exist in PPC anatomy between humans and primates. This was noted as early as 1909 by Brodmann, who reported the human PPC is much expanded compared to the primate (Brodmann & Garey, 2006). The human IPS contains more regions than seen in the primate, such as additional regions sensitive to 3D motion (Orban et al., 2006). Although challenging, human equivalents of PPC regions have been detected using fMRI (Orban, 2016; Shikata et al., 2008). However, there is no firm consensus on the location of the human LIP (PEF) equivalent. This region has been described to be located within the IPS and possibly SPL (Konen & Kastner, 2008). A diagram shown earlier in the introduction by Leigh & Kennard (2004) (Figure 1-6 page 32) places the PEF in the IPL. Likewise, no consensus exists for the human 7a location but it has been suggested to comprise Brodmann areas 39 and 40 in the inferior parietal, as well as the IPS and SPL regions (Konen & Kastner, 2008; R J Leigh & Zee, 2000). Likely due to the lack of a consensus of human PPC subregion location,
studies have reported parietal activations using a number of anatomical descriptors including the IPS, LIP, PEF or PPC. This variability used in naming gives rise to confusion when comparing and interpreting the various studies reported in the literature. This is evident particularly in Chapter 6 of this thesis and has been the subject of further discussion further within that chapter.

1.3 Eye movements in PD

Components of the saccadic eye movement control system are from the same circuits controlling skeletal muscle movements. Notable for PD research, the same basal ganglia circuit provides modulatory control of both skeletal and eye movements. The dopaminergic dysfunction within the basal ganglia in PD results in abnormalities for both movement types. As described in previous sections, pathological neurological changes in PD are not limited to the basal ganglia. Widespread cortical abnormalities such as grey matter atrophy and perfusion changes are present, particularly in later stages of the disease. These affect regions of the frontal lobe and parietal lobe, which are known to be involved in saccadic eye movements. The research into eye movements provide a window for researchers for investigating movement disorders such as PD (Leigh & Zee, 1999). The knowledge of the impact of PD progression on eye movement performance and cortical activity may form the basis of an easily administered and objective biomarker of PD progression in the future.

1.3.1 Eye movement changes in PD

A number of characteristic deficits in PD eye movements have been described. Hypometria is the dominant finding in saccades in PD, with volitional saccades showing more prominent amplitude deficits when studied in the laboratory (Anderson & MacAskill, 2013). The initial saccade made to a target tends to fall short and be compensated for by corrective saccades, with PD patients using a multistep sequence to reach the desired eye position (DeJong & Jones, 1971). This is sometimes called a fragmentation of gaze shift (Kimmig et al., 2002) and some even consider this fragmentation to be a biomarker of PD (Blekher et al., 2009). A number of eye movement task types have been used to assess saccade performance in PD. Findings are briefly summarised below in sections 1.3.1.1 to 1.3.1.4.

1.3.1.1 Reflexive saccades

Studies of reflexive saccades in PD patients have shown mixed results in the past. Prolonged reaction times have been reported (Chen, & Tsai, 1999; Mosimann et al., 2005; Sauleau et al., 2008; White et al., 1983) as well as reduced (Chan et al., 2005; Kingstone et al., 2002) or no change (Briand et al., 1999; Crawford et al., 1989; Shaunak et al., 1999; Ventre et al., 1992).
A meta-analysis by Chambers & Prescott (2010) suggested methodological differences for the disparity in results. Terao et al. (2011) found reflexive latencies to be prolonged in association with PD motor status but did not find a uniform association. A study by MacAskill et al. (2012), with 210 total participants, has shown impairments in reflexive saccade latency to be associated to with cognitive impairment in PD. Reflexive saccade amplitude has been shown to be reduced in a number of studies (Mosimann et al., 2005; Terao et al., 2011), with MacAskill and colleagues finding reflexive saccade amplitude influenced mainly by PD motor status, with cognitive status more related to latency.

1.3.1.2 **Predictive saccades**

Studies investigating predictive saccades in PD have reported the tendency for PD patients to produce fewer anticipatory saccades in response to the predictable stimulus sequence of the task. PD patients, instead, tended to make visually-guided saccades after the stimulus appearance (Bronstein & Kennard, 1985). Another study showed PD patients eventually learned to anticipate the predictable target but were slower to implement this strategy compared to controls (Crawford et al., 1989). However, other studies have not found latency differences, nor predictable sequence learning differences between groups (O’Sullivan et al., 1997; Ventre et al., 1992). The common finding with predictive saccades in PD is decreased saccade amplitude (hypometria) compared to controls (Bronstein & Kennard, 1985; Crawford et al., 1989; Ventre et al., 1992).

1.3.1.3 **Memory-guided saccades**

PD patients tend to make multi-stepping saccades to memorised spatial locations with the initial saccade hypometric (Crawford et al., 1989; Hodgson et al., 1999; Lueck et al., 1992; Rivaud-Péchoux et al., 2000; Vermersch et al., 1994). Final eye position has been reported as reduced (Rivaud-Péchoux et al., 2000; Vermersch et al., 1994) or normal (Crawford et al., 1989; Hodgson et al., 1999). Latency has been reported as normal (Hodgson et al., 1999; Vermersch et al., 1994) or increased (Le Heron et al., 2005; Rivaud-Péchoux et al., 2000). Terao et al. (2011) has found memory-guided saccade latency to prolong relative to disease stage (Hoehn and Yahr scale) and also reported an increased number of inadvertent reflexive saccades made to the presented “cue” when fixation should have been maintained.

1.3.1.4 **Antisaccades**

PD patients show decreased gain, increased latency and a higher number of inadvertent erroneous saccades in the antisaccade task (Briand et al., 1999; Kitagawa et al., 1994).
Antisaccade latencies were slower and more errors were made particularly in PD with dementia compared to PD without dementia (Mosimann et al., 2005).

1.3.2 Cortical basis for eye movement changes in PD

Excessive SC inhibition is thought to be a core factor contributing to saccade performance abnormalities seen in PD (Terao et al., 2013). In PD, basal ganglia dopamine deficiency causes reduced activity of the direct pathway, increased activity of the indirect pathway with the overall effect of increased activity of the inhibitory GABAergic neurons from the basal ganglia to the SC (see Figure 1-8 in basal ganglia section of eye movement control page 36). Therefore, the SC is excessively inhibited in PD, which should in theory exert a suppressive effect on all saccadic eye movements. This, however, does not explain the differences between voluntary and visually-guided saccades in PD. Both saccade types are hypometric but this effect is much more prominent in voluntary saccades.

This may be explained by the two separate pathways controlling reflexive saccades and voluntary saccades, which converge in the SC (Hikosaka et al., 2000; Massen, 2004). It is believed that the initiation of reflexive saccades in response to visual stimuli arises in the PPC, which is directly connected to SC (Pierrot-Deseilligny et al., 2004). This route largely bypasses basal ganglia. As a result, visually-guided saccade impairments are thought to be mainly driven by excessive SC inhibition, without being influenced by excessive inhibitory activity from the basal ganglia (Terao et al., 2013). Hence visually-guided saccades experience inhibitory effects only at one point through its pathway. Voluntary saccades, however, have been shown to use a pathway involving the basal ganglia. Saccades made under voluntary control, such as the memory-guided saccade and antisaccade (Briand et al., 1999; Kori et al., 1995; Pierrot-Deseilligny et al., 2004), are believed to be generated in the frontal cortex, which sends connections to the basal ganglia, which then travel to the SC via the substantia nigra (Kimmig et al., 2002; White, Saint-Cyr, Tomlinson, et al., 1983). Kimmig et al. (2002) have argued that the pathway through the basal ganglia to the SC becomes abnormally weak in PD. Terao et al. (2013) similarly posit that the basal ganglia to SC pre-oculomotor drive becomes weak and in addition, the SC is also inhibited. This combined effect results in prominent voluntary saccade hypometria, more so than for reflexive saccades.

Terao et al. (2011) investigated how eye movement performance changes with PD disease stage (Hoehn and Yahr scale). It was found that the amplitude of both visually-guided and memory-guided saccades were progressively impaired with PD disease severity. Reductions in visually-guided saccade performance stabilized after Hoehn and Yahr stage 1-2, with
memory-guided saccades showing increasing impairment with advanced disease. MacAskill et al. (2012) found that reflexive latency was impaired later in the disease in the presence of more substantial motor and cognitive impairment. Mosimann, Muri, et al. (2005) found antisaccade latencies were slower in PD in dementia patients compared to non-dementia PD patients. For voluntary saccades, Terao et al. (2011) speculate that the frontal cortex (thought to be involved more with voluntary saccades) and the basal ganglia circuit (as opposed to the parietal-SC circuit of reflexive saccades) deteriorates with the disease. Yet Shaikh et al., (2011) have hypothesised increased frontal eye field activity develops compensatory to an inhibited SC. The sources that lead to eye movement deficits in PD remain unresolved.

1.3.2.1 Saccade hyper-reflexivity in PD

As well as impairments in voluntary saccade generation, some peculiar differences have been noted for certain types of reflexive saccade in PD. PD patients are noted to make express saccades (extremely rapid reflexive saccades made under laboratory conditions), more so than unaffected controls (Chan et al., 2005). Also, while PD patients show impairment in generating voluntary saccades, patients often cannot suppress reflexive saccades, even when instructed to. This leads to errors in the memory-guided task and the antisaccade task (Briand et al., 1999; Terao et al., 2011).

Express saccades are thought to be generated by an increase in excitability of SC neurons during fixation point offset in a “gap” reflexive task (Munoz et al., 2000). Terao et al. (2013) have described a “leaky” inhibition of the SC in PD. Impaired basal ganglia inhibitory control of the SC or cortical changes can adversely affect the control of SC excitability, leading to more express saccades in PD. In addition, the DLPFC have projections to SC cells and have an inhibitory effect on saccade generation (described as the tonic inhibitory model) (Pierrot-Deseilligny et al., 2005; Sereno, 1996). DLPFC impairment may release the inhibition applied to the SC, leading to more hyper-reflexive saccades (Crevits, & De Ridder, 1997; Pierrot-Deseilligny et al., 2004). Thus, one possibility is that DLPFC dysfunction in PD contributes to the increase in hyper-reflexive “express saccades” and decreased ability to suppress reflexive saccades.

Studies have attempted to reconcile the apparent disparity between impaired voluntary saccade generation and excessive reflexive saccade generation in PD. A model of inhibition control known as the tonic inhibition model has been proposed in a number of studies (Amador et al., 2006; Chan et al., 2005; Sereno & Holzman, 1995, 1996) which states the voluntary saccade system usually maintains an ongoing tonic inhibitory effect on the reflexive
saccade system. This function involves frontal-striatal processes which are disrupted in PD. This leads to impaired voluntary saccades and uninhibited, overactive reflexive saccades (Amador et al., 2006; Chan et al., 2005). However, this model predicts that impairment of voluntary saccade function should associate with excessive reflexive saccade generation, which was not clearly demonstrated (van Stockum et al., 2008), suggesting there may be alternative sources of reflexive saccade disinhibition in PD, possibly arising from the cortex or though impaired attention pathways.

1.3.2.2 Medication effects on eye movements

Studies have investigated the effect of PD medication on saccadic eye movements. The relationship between levodopa and eye movement performance is complex and not fully understood but it is thought that levodopa treatment may cause dopamine levels to exceed the natural optimum level, which may cause an increase in response time (Michell, 2007). Nakamura et al. (1991) have shown that in a group of 24 PD patients, the vast majority (21) showed eye movement performance abnormalities. When this group was given dopaminergic medication, only one patient showed improvements compared to before the treatment. Rascol et al. (1989) found that amplitude improved but no effect on latency was found. Later studies have, however, found prolonged saccade latency in patients on levodopa, but with high intersubject variability (Michell, 2007). In the review by Terao et al. (2013), it is reported that there does not appear to be a great change in saccade amplitude induced by levodopa. A recent study comparing levodopa and DBS with eye movement performance found levodopa to worsen saccade measures, leading to longer latency and smaller gain whereas DBS improved saccade performance (Dec-Ćwick et al., 2017).

1.4 Parkinson’s disease and magnetic resonance imaging

Recent years have seen a number of studies use advanced magnetic resonance imaging (MRI) techniques to further investigate the neurological basis and progression of PD. MRI is a non-invasive radiological technique used for imaging various internal bodily structures, including the human brain. MRI is distinct from computed tomography (CT) or positron emission tomography (PET) in that it does not use ionising radiation. Instead, a powerful magnetic field and radio-frequency pulses are used to produce images. MRI is particularly suited for brain imaging, being able to clearly differentiate between grey matter, white matter and cerebral spinal fluid (CSF). Compared to CT, MRI scans give much improved soft tissue contrast between muscle, fat and blood. Regions such as the brainstem are shown with more clarity using MRI due to the increased contrast and the lack of artefacts from the surrounding bone structure. MRI also does not expose the patient to potentially harmful ionising radiation. The
principle on which MRI relies is that certain nuclei under a strong magnetic field will yield a net magnetization, and this magnetization can be perturbed by a radio-frequency pulse. A signal is then recorded by the scanner as the magnetization returns to equilibrium and information about the tissue composition of the scanned area can be used to render an image.

1.4.1 Overview of the MRI process

MRI relies on tissue properties at the subatomic level. In the nucleus of an atom, protons and neutrons spin on their axes, giving a small magnetic field. If there is an even number of both protons and neutrons in the nucleus, the spins of these particles cancel out, giving a spin number of 0. However, if the nucleus contains an odd number of protons or neutrons, the magnetic field in the nucleus cannot be fully cancelled out. The presence of this small magnetic field in the nucleus allows interaction with an external magnetic field.

Medical MRI is mainly concerned with the hydrogen nucleus, which consists of a single proton. Hydrogen is found abundantly in the body, being part of the water molecule and fats. Normally, the axes on which the individual hydrogen nuclei spin are completely random. However, when exposed to a strong magnetic field, the spin axes of each of the nuclei tend to align with the direction of the external magnetic field, producing a small net magnetisation, and spins begin to precess. This magnetised sample is then subjected to a hydrogen-specific radio frequency pulse, which forces the net magnetization to tilt out of magnetic alignment.

The net magnetization vector itself precesses (Figure 1-11), in which the axis traces out a cone-like shape as the net magnetization vector reverts back to the magnetic alignment. As the net magnetisation precesses, it induces an electromotive force in the detector coil (receive coil) of the scanner, leading to the raw signal recorded by the scanner.
1.4.1.1 **Image Generation**

The method used to obtain images from the raw measured signals is commonly achieved using a method called the “Fourier transformation”. Three “gradient magnets” are built into the MRI machine. These are much smaller and weaker than the main magnet and have a variable (linear) magnetic field. When applied briefly across the main field, the smaller magnetic field causes a magnetic “gradient” across the image plane. This causes the speeding up or slowing down of the precession movement of the nuclei, depending on the position of the nuclei within the magnetic field. The result of this is a “phase difference” between nuclei at different locations. The points then can be localised based on this phase difference. The field from the gradient magnets can be adjusted, giving the ability to image all parts of the body within the magnetic field without moving the patient. These images can be rendered in 3D allowing the data to be seen as slices in any plane.

1.4.1.2 **Structural MRI - T1 and T2 Weighting**

A T1-weighted MRI scan is a basic scan with fats showing up as bright regions and water as dark. T2 is also a basic scan but with water showing up as bright instead. The differentiation of fats and water relies on the “relaxation” process. “Relaxation”, or the reverting of the excited nuclei to the original magnetised orientation, is a measurement used to give contrast to
different tissue types. The time taken for relaxation to occur is a constant for each specific tissue type.

There are two relaxation constants, named spin-lattice relaxation time (T1) and spin-spin relaxation time (T2). Spin-lattice relaxation or T1 refers to the loss of energy by the transfer of energy to the other molecules surrounding the nuclei, termed the “lattice”. Spin-spin relaxation refers to the loss of phase between the spinning nuclei caused by interactions between the spins. Every tissue has a different T1 and T2 value which can be exploited to differentiate and highlight certain tissue types. Fats tend to have shorter T1 and T2 values while fluid has longer T1 and T2 values. By adjusting MRI settings, it is possible to highlight areas of different tissue composition such as grey and white matter in the brain.

1.4.1.3 **Diffusion MRI**

Diffusion, or Brownian motion, refers to the random motion of molecules in a liquid. On the microscopic level, individual water molecules move in a random pattern due to their thermal energy and collisions with other molecules. Diffusion MRI causes the signal to be dependent on the diffusion of water and uses this information to infer tissue microstructure. Hence, diffusion MRI is a technique used for imaging fibre tracts within the body. In fibre tracts, the diffusion of water is limited by barriers such as cell membranes and myelin that prevent the water from diffusing randomly. Instead, the water tends to diffuse primarily along the fibre, instead of perpendicular to the fibre. This directionality can be detected by MRI and each voxel (3D pixel) can be given a shade reflecting the diffusion characteristic.

A method by which diffusion is measured involves the spin-echo technique. Two radiofrequency pulses are applied to the magnetised scan area. The first is an excitation pulse 90 degrees to the magnetic field, which causes the nuclei to tilt. The tilted nuclei are also precessing. This precessing movement, however, is not identical for all nuclei, due to inconsistencies in magnetic fields in different areas, with some nuclei precessing faster than others. A second, refocusing pulse at 180 degrees to the magnetic field is then applied afterwards. This flips the spins around so that the slower spins are now leading the faster spins. When the faster spins catch up to the slower spins, an echo signal is produced due to the alignment of the spins. In diffusion MRI, two diffusion weighting gradients are applied before and after the 180 degree refocusing pulse. These diffusion gradients are magnetic gradients deliberately affecting the speed of the precession of the nuclei depending on position. The two diffusion weighting gradients would cancel each other out if all nuclei remained in their initial positions. This is not the case, however, with water molecules in the
brain. The diffusion moves the molecule away from its original position and as a result, the second gradient does not completely cancel out the phase change from the first gradient. This causes dephasing among areas of high water diffusion, as not all of the spins are aligned for the echo sequence, which gives a lower signal.

This process, however, is only sensitive to information along the direction of the two weighting gradients. In order to provide a three-dimensional assessment of diffusion, multiple diffusion-weighted acquisitions must be performed in multiple, non-collinear directions. The data from these acquisitions is entered into a mathematical model called a “tensor”, which is used to describe the direction and degree of water diffusion. This process is used to visualise fibres and connections within the brain, as well as to derive quantitative metrics throughout the brain.

1.4.1.4 Arterial Spin Labelling MRI

Arterial spin labelling (ASL) is a non-invasive imaging technique used to measure perfusion in the brain. This has the advantage over traditional blood flow imaging in that ASL does not require the injection of a radioactive or exogenous tracer. Instead, ASL relies on magnetically “labelling” the water within the normal arterial blood supply via an external radio-frequency pulse, with no need for injections (Liu & Brown, 2007). Another advantage is the ability to characterise absolute perfusion values in ml min\(^{-1}\) to a tissue-specific weight (e.g. ml min\(^{-1}\) 100g\(^{-1}\)), as opposed to relative blood flow measures as from PET (unless the concentration of radiotracer is determined by direct arterial sampling – often not done). Lastly, ASL is easily and immediately repeatable, whereas repeat PET scanning requires a delay and the injection of additional radioactive material.

For ASL imaging, a radiofrequency inversion pulse is applied to a volume under the region of interest, for example, the neck, containing the carotid arteries supplying the brain. This inversion pulse results in the demagnetisation of the water in the arteries within that volume. When this demagnetised or “tagged” water enters the brain tissue, it reduces the MR scanning signal and image intensity. This image is called the “tag image”. This procedure is repeated, this time without the inversion pulse, which produces the “control image”. The “tag image” is subtracted from the “control image” giving a resulting “perfusion-weighted image”. The difference between the intensity of the control image to the tag image is proportional to blood flow, and knowing a number of specific parameters, perfusion can be quantified.
1.4.1.5 **Functional MRI**

As opposed to structural MRI, which can be considered a three-dimensional still photograph of the brain, functional MRI aims to detect and show the active regions in the brain involved in a particular task over time. Neurological activity is not measured directly by the MRI machine. Rather, it is the change in blood oxygenation level during cortical activity that is detected. This is known as the blood-oxygen-level dependent (BOLD) contrast. Oxygenated and deoxygenated haemoglobin each give a different MR signal. This phenomenon was first noted using T2 MRI, where it was seen that highly oxygenated areas would give a stronger MR signal to those areas less oxygenated (Ogawa et al., 1990). The BOLD contrast depends on the balance of oxygenated haemoglobin supply in the brain and consumption (Hyder, 2004). Explained in the most simplistic terms, active neurons need oxygen and glucose to run cellular processes, such as transmitting action potentials and releasing neurotransmitters (Shulman et al., 2004). During cortical activity, vascular processes occur which increase the blood flow to the active neurons for a task. This supplies the active cortical region with oxygenated haemoglobin and carries away the deoxygenated haemoglobin. This regional change in blood oxygenation then is able to be detected by the MRI machine by the BOLD signal (Logothetis & Wandell, 2004). The interaction between BOLD contrast and underlying neurological activity is complex and a complete model that describes this relationship has not been developed. The BOLD contrast is related to the concentration of deoxyhaemoglobin, which is affected by a combination of cerebral blood flow (CBF), cerebral metabolic rate of oxygen (CMRO$_2$) and cerebral blood volume (CBV) (Buxton, 2013; Ekstrom, 2010).

Neurological activity increases all three but increasing each value has a different effect on the BOLD signal. For example, a higher CBF is able to remove deoxygenated haemoglobin whereas a higher CMRO$_2$ increases the deoxyhaemoglobin. An increase in arterial CBF decreases deoxyhaemoglobin and the opposite occurs with venous CBF increases (Buxton, 2013).

1.4.1.6 **Metabolism and contribution to BOLD**

The metabolic processes that affect CBF and CMRO$_2$ have a direct effect upon the BOLD signal, so having a basic appreciation of these processes is necessary prior to making interpretations of BOLD signal changes. These processes were historically studied using positron emission tomography (PET). PET is a functional imaging technique, which unlike fMRI, can directly measure the uptake of oxygen and glucose through the use of radioactive tagging. Respiration is the process by which compounds such as glucose are broken up by enzymes to release energy for cellular processes. This process can involve oxygen (aerobic
respiration) or not (anaerobic respiration). Aerobic respiration is an energy generating oxidative process, converting glucose and oxygen to carbon dioxide and water. In anaerobic respiration, a process called glycolysis occurs, where glucose is converted to lactate without needing oxygen. Should glycolysis occur in the presence of oxygen, it is termed aerobic glycolysis. A PET study by Fox & Raichle (1986) found during visual stimulation that glucose metabolic rate (and CBF) increased by about 50%. However, the metabolic rate for oxygen only increased 5%. Reasons for this apparent mismatch are not fully understood, with some studies suggesting aerobic glycolysis occurs (Lunt & Vander Heiden, 2011; Vaishnavi et al., 2010). Other studies have suggested a mostly oxidative metabolism providing the energy for cortical processes (Buxton, 2013; Chih et al., 2001; Lin et al., 2010). Some studies suggest an initial non-oxygen-requiring process occurs which switches to an aerobic process during longer sustained activation (Frahm et al., 1996; Mintun et al., 2002). A complex mechanism through which metabolic processes within the neuron interact with surrounding astrocyte cells, called the “astrocyte neuron lactate model” has been described (Magistretti & Pellerin, 1999; Pellerin et al., 2007; Shulman et al., 2001). For a review of this process, see Magistretti & Allaman, (2015).

The BOLD signal depends on the concentration of deoxyhaemoglobin. For the BOLD signal to increase, the concentration of deoxyhaemoglobin must decrease. In order for this to occur while oxygen is being consumed by cellular processes, an excess of oxygenated haemoglobin must be supplied to the region, above what oxygen is consumed by respiration - this is exactly what occurs. PET studies found oxygenated blood supplied by the CBF far exceeded what is consumed (Fox et al., 1988; Fox & Raichle, 1986). This gives an overshot of oxygenated haemoglobin, flushing out deoxygenated haemoglobin and giving the BOLD MR signal. Buxton & Frank (1997) have suggested a disproportionate increase in CBF is needed to maintain oxygen supply, due to a decrease in oxygen extraction fraction during higher blood flow rates. Another study by Mintun et al. (2001) has found that the increased CBF is not necessary to maintain tissue oxygen concentration and have speculated that other factors may be behind the CBF increase. There is little oxygen reserve to act as a buffer in the brain (Leithner & Royl, 2014) and more recently, it has been proposed that CBF oversupply may be necessary to provide adequate oxygen to cortical regions more distant from the vascular supply (Devor et al., 2011) and to act as a safeguard to prevent damage from potential pathological oxygen supply interference (Leithner & Royl, 2014).
1.4.1.7 Neural activity and correlation with BOLD signal

As the BOLD signal does not directly measure neural activity, it was necessary for studies to show how much the BOLD signal correlates with actual measured neural activity. A summary of the sources and detection methods of neural activity can be found in a review by Buzsáki, Anastassiou, & Koch (2012). Put simply, electrode recordings can detect the “spike” activity related to the action potentials of single or multiple neurons, which occur at a frequency above 300 Hz (Logothetis & Wandell, 2004) or a summation of electrical activity from a wider area called a local field potential (LFP), which occurs at a frequency under 100 Hz (Buzsáki et al., 2012; Katzner et al., 2009). The LFP is thought to reflect an “average” of all neural signals within a small volume of neural tissue surrounding an electrode (Ekstrom, 2008; Herreras, 2016). A study by Logothetis et al. (2001) using simultaneous fMRI, action potential recordings and LFP recordings found the BOLD signal correlates more strongly with lower frequency LFP recordings compared to higher frequency unit recordings or spike activity.

A review by Ekstrom (2008) describes how different cortical regions vary in BOLD signal correlation to either LFP or high-frequency neural spiking activity. In general, the BOLD response has been shown to correlate well with LFP (Brinker et al., 1999; Goldman et al., 2002; Huttunen et al., 2008; Martin et al., 2006), however, the reader is warned that there are exceptions to this, pointing to particular examples such as the hippocampus, where a significant correlation between LFP and BOLD signal was not found (Ekstrom et al., 2009). A number of potential reasons for this were mentioned, including the hippocampus being active in baseline fMRI conditions, giving at times a negative overall activation when compared to an active task. The hippocampus also receives a number of inhibitory connections, which may decrease measured neural activity but still demand metabolic energy processes, giving an increased BOLD signal. For a full table and summary of studies investigating metabolic (using both fMRI and PET) correlations with neural activity measurements, see Table 1 in Ekstrom (2008). In general, it was stated by Ekstrom that particularly within the neocortex, BOLD is often correlated with LFP. However, care must be taken when making direct inferences about underlying neural activity from the BOLD response alone and having correlations with other modalities such as Electroencephalography (EEG) would be beneficial (Ekstrom, 2008).

1.4.1.8 Haemodynamic response

The shape of the BOLD signal during cortical activity is known as the haemodynamic response or haemodynamic response function (HRF). Compared to neural events, which occur
within tens of milliseconds, the haemodynamic response is slow and begins approximately 2 seconds after neural response. This is due to the slower occurrence of vascular events compared to neural signalling. The haemodynamic response has a characteristic shape, known as the canonical HRF (Figure 1-12). Many studies have shown an initial dip in the first 2 seconds following the event (Menon et al., 1995). This is thought to possibly represent a rapid increase in oxygen utilisation before the blood flow changes to compensate (Kim & Ogawa, 2012). This finding, however, remains inconsistent between studies (Hu & Yacoub, 2008). As the subsequent increase in blood flow to neurologically active regions occurs, the BOLD signal begins to increase as the deoxyhaemoglobin is washed out. This signal reaches a peak in about 5 seconds. Single fast events will give such a peak. In response to sustained stimuli, consecutive peaks may form a broad plateau. Following peak activity, the signal decreases and will often have an undershoot period, known as the post-stimulus undershoot, before returning to baseline (Buxton et al., 1998; Chen & Pike, 2009; van Zijl et al., 2012). The cause of this undershoot is still debated (Kim & Ogawa, 2012; van Zijl et al., 2012; Yablonskiy et al., 2013) but may be due to a decrease in blood flow with high venous blood volume maintained, giving a higher concentration of deoxyhaemoglobin (Chen & Pike, 2009) or be due predominantly to a sustained increase in oxygen metabolism, with minor contributions from blood flow changes (van Zijl et al., 2012).
1.4.1.9 Noise
The components of the measured BOLD signal contain other elements such as a drift component, heart rate and respiratory-related signal change and also noise alongside the actual response. It is thought these other components are of a low frequency and so typically a high pass filter is used to help remove these signals as much as possible (Kruggel et al., 1999).

1.4.2 fMRI Experiment design
There are two basic types of fMRI experiment design – the block and event-related design.

1.4.2.1 Block fMRI design
A block design is a type of fMRI procedure where the experimental task is presented multiple times in series e.g. 20 consecutive saccade trials. This is then followed by the control task, e.g. fixation, which is presented for the same duration as the series of saccade trials. These task sequences are known as “blocks”. During analysis, the BOLD signal from the control
task block is subtracted from the experimental task block signal, giving activation specific to the experimental task.

When performing a block design experiment, multiple factors are at play. Many trials within each experimental block are desirable, in order to increase the detectable BOLD response, but timing and minimising patient fatigue must be considered. If the experimental block is too long, fatigue may set in, leading to a drop in performance approaching the last few tasks within the block. Tasks within a blocked design should also be relatively brief, to allow enough task repetitions within a block for meaningful BOLD activity to build up. A blocked design is well suited for experiments using saccades. Saccades by definition occur rapidly and many trials can be carried out within a reasonable timeframe. The blocked design generates a summation of all the BOLD responses for many repetitions of a task. Because of this, it is very good at detecting regions that show task-related activity in the brain. A drawback of a blocked design is that it does not provide information regarding the timing of the task-related activity. Using the memory-guided task as an example, a sequence of events must occur in the brain to process and generate each memory-guided saccade. A blocked design will only capture the summation of all the activity from the memory-guided trials without information on the timing and time course of the activity. An event-related fMRI design is needed to investigate the timing of BOLD activity.

1.4.2.2 **Event-related design**

An event-related design is used to study the haemodynamic response following individual stimuli or “events”. This type of design assumes there will be neural activity occurring for a short and discrete amount of time following the presentation of a stimulus.

The event-related design treats neural activity for each task component as a discrete occurrence or “event” - as opposed to a blocked design that sums up activation from many repetitions of a task. The event-related design can show regions active at specific time points or intervals during the task whereas a block design shows consolidated activation across the task, with no timing discrimination. Each neural impulse has a corresponding haemodynamic response which can be detected by BOLD signal changes. Event-related trials are presented one at a time, and are separated by an inter-trial interval. An inter-trial interval can be set up to be long enough for the haemodynamic response to return to baseline before the next trial, this being known as a slow event-related design. In contrast, for rapid event-related design, events are spaced closer than would allow the haemodynamic response to reach baseline
before the next task. This causes an overlap between two haemodynamic responses. This does not pose a problem as it has been shown that haemodynamic activity summates linearly with repeated trials (Pollmann et al., 2000) and can be taken into account with convolution. An advantage of having a short inter-trial interval is the ability to have a higher number of trials within a set period, giving more power. This results in short inter-trial interval experiments being more powerful at detecting task-related activity. Longer inter-trial intervals allow better depiction of time courses due to trials being adequately spaced to minimise overlap (see time course section in Chapter 6, page 195 for an explanation of how fMRI software accounts for overlap during time course generation). Practical elements such as patient fatigue must be considered when designing event-related studies. The study duration should be kept to a reasonably managed length while having enough repetitions for activation to be significantly detected.

1.4.2.3 **Preprocessing**

Raw MRI data needs to go through a series of adjustments, known as preprocessing, before being able to be used for statistical analysis. fMRI has a number of unique pre-processing steps, of which slice timing usually occurs first. In each repetition time (TR), a scan of the whole brain (or selected scanning area) is made. fMRI acquires this whole-brain image by a series of slices in 2D, which are put together in a stack to form the 3D whole-brain image. Commonly, an interleaved slice acquisition sequence is used. This means the slices are not taken in a consecutive order. Rather, if the image slices were numbered consecutively i.e. 1, 2, 3 and so on, starting from the bottom of the brain to the top, the odd-numbered slices (1, 3, 5, …) are taken first and then once the last odd-numbered slice is acquired, the sequence moves back to the bottom of the brain to then acquire the even-numbered slices. The time interval between the acquisition of spatially neighbouring slices 1 and 2 for example, even though they occur within one TR, are separated temporally by around half the TR duration, or from 1-2 seconds if using a 3 second TR. This can make a significant difference to activations in an event-related design. A process known as temporal interpolation is used to account for this. This estimates the activation difference needed to account for timing differences of a specific slice in relation to a selected reference slice (Henson et al., 1999; Sladky et al., 2011). Temporal interpolation options are usually provided in fMRI analysis software such as *Statistical Parametric Mapping (SPM)* (Friston, 2007).
Head motion has a potentially large impact on data, confounding the relationship between voxel activation and voxel location in fMRI studies (Friston et al., 1996; Maclaren et al., 2013). Excessive head motion can also lead to incorrect measurements of brain structure (Reuter et al., 2015; Savalia et al., 2017). The first step to correcting for motion is to shift all the fMRI scans using a “rigid body” transformation, translating and rotating the scans to a selected reference scan (Jiang et al., 1995). Motion correction parameters generated from this step can be later entered into the statistical model as “nuisance variables”, or covariates of no interest, to help eliminate effects caused by motion and not actual activation (Johnstone et al., 2006). The images are then resampled so the motion-corrected changes are applied to the image by a process called spatial interpolation, available in most fMRI analysis programs (Friman & Westin, 2005; K. Friston, 2007).

Functional scans have a low spatial resolution. In order to make inferences on BOLD activation changes, the active functional areas need to correspond with known structural locations. Structural MRI generally gives higher spatial resolution and functional images are aligned to this in a step called co-registration. This co-registration step is used for all modalities, including DTI and ASL scans, which are all aligned to the reference structural image.

Co-registration occurs firstly at an individual level. For comparing group-level differences, every voxel from all MRI imaging modalities must correspond to the same spatial location for every subject. However, each subject’s brain is of varying size and shape. Normalization is the process by which the scans from each individual are warped and morphed into a standardised shape and size. As our patient sample is from an elderly population, a probabilistic elderly brain template (Lemaître et al., 2005) was used for this study, in order for the normalisation step to reflect age-related changes. Normalization is an automated process within fMRI analysis programs such as SPM (Friston, 2007).

The final preprocessing steps involve removing noise by filtering and smoothing. Low-frequency signals that are unrelated to the underlying haemodynamic response can arise from signal drift and cardiac and respiratory drift (Cordes et al., 2001). Filtering strategies exist in an attempt to remove signals from these confounding sources (Kruggel et al., 1999).

Smoothing combines each voxel point with the weighted average of neighbouring voxels, effectively “blurring” the image. This blurring is a result of the removal of sharp contrasting intensities from neighbouring voxels and helps improve the signal to noise ratio (SNR) by averaging out random noise values. This does have an effect of decreasing effective spatial
resolution but the improvement of SNR helps with the detection of significant cortical activity (Parrish & Gitelmant, 2000).

1.4.2.4 **Group analysis – general linear model**

fMRI analysis uses a general linear model (GLM). Essentially, a GLM is run at every voxel - for example a multiple regression at every voxel. This type of analysis is known as a mass univariate statistical analysis.

The general linear model/multiple regression equation can be written as:

\[ Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_n X_n + \varepsilon \]

Where,

\( Y \) = dependent variable – e.g. actual measured BOLD signal in a voxel.

\( X_i \) = independent variables - as in a multiple regression. These can be age, sex, motion parameter corrections and estimated BOLD response. N represents the total number of independent variables used in the analysis.

\( \beta \) = Beta weight - The beta weight is a regression coefficient of the independent variable. Beta 0 (a constant) models an offset representing all the factors held constant.

\( \varepsilon \) = Error - a measure of the deviation of the actual signal from the expected signal due to noise and other un-modelled factors.

In multiple regression, multiple independent variables are used to predict a single dependent variable. In fMRI analysis, the onsets and durations of the task are convolved with a haemodynamic response – often the canonical HRF. This gives an estimated BOLD signal which is expected to occur with task-related activity and is an independent variable of the regression analysis or the GLM. Other possible confounders such as motion can be added as independent variables into the GLM analysis, which may help explain some of the observed variance in the BOLD signal. The recorded BOLD signal is the dependent variable. In GLM analysis, the model containing each independent variable is fitted to the BOLD signal by minimising residual error e.g. by least squares. This process generates weighting parameter estimates at every voxel (\( B_1 \) to \( B_n \), called beta weights or regression coefficients) for each independent variable. An error value for each voxel is also calculated which represents the deviation between the observed voxel value and the expected voxel value, due to noise and other un-modelled factors. Every beta weight is tested for statistical significance, with the null
hypothesis being that the beta weight $= 0$ (no correlation between BOLD signal and independent variable). The beta weight for each individual independent variable can be selectively displayed in a statistical parametric map. This visually highlights voxels which meet the significance threshold to reject the null hypothesis. In an example of displaying significant beta weight for expected task BOLD activation independent variable, highlighted voxels can be considered regions active for the experimental task.

The GLM equation can be equivalently written in matrix notation as $\mathbf{Y} = \mathbf{B} \mathbf{X} + \mathbf{\varepsilon}$. $\mathbf{Y}$ represents the column of values for the dependent variable, $\mathbf{B}$ the beta weight column, $\mathbf{\varepsilon}$ the error column and $\mathbf{X}$ the design matrix. This is fit at every voxel.

A design matrix (Figure 1-13) can be used to visualise the independent variables in the GLM analysis, with one row per observation and one column per independent variable. The colour intensity of the boxes represents the numerical values of each variable.

Figure 1-13 – Design matrix construction. A design matrix is a convenient way to visualise the independent variables added into the general linear model. In the diagram above, task-related neural events are convolved with the HRF (1) to form an estimated BOLD response for the task (2). The columns in the design matrix can be thought of as flipping BOLD response vertically (3) and converting the BOLD signal change values into a corresponding shade i.e. lighter shades for lower values and darker shades for higher (4). Additional values such as motion parameters or age or sex can be added as additional columns forming the design matrix (5). Each column represents each independent variable and each row
represents a scan image in time in a first level analysis and subject for a second level analysis. Each column represents an X value in the GLM equation and has a corresponding beta weight. Voxels with significant beta weights can be highlighted in a brain map to show regions corresponding to the independent variable, i.e. the expected task-related BOLD activity. Note the figures above are representative only and do not reflect actual experimental values.

The GLM allows us to localise brain activation (Friston et al., 1995). This approach can be applied to a single subject, known as a first-level analysis, and as a group analysis after the individual analysis, known as a second-level analysis.

In a second-level analysis, parameters such as age and sex are typically added as independent variables, while motion parameters are generally added at the first level. In a second level analysis, it is possible to test whether activation differences seen in the first level (i.e. within subjects) are significantly different at a group-level (e.g. between Parkinson’s patients and controls).

A similar process is used in a voxel-based analysis (VBA). Instead of fMRI BOLD activation however, values such as grey matter volume, blood flow perfusion values in ASL and FA and MD values in DTI can be examined. This can be used to detect group differences in those values or assess correlations with variables such as eye movement performance (Ashburner & Friston, 2000).

1.4.2.5 Multiple comparisons problem

A sequence of fMRI scans for one subject can contain over 100,000 voxels, with every voxel undergoing a statistical test of its change over time. Even if no actual activation changes were present, if the usual p-value of 0.05 is used, we could expect 5% of the 100,000 voxels to give a type 1 error (false positive). This equates to 5000 voxels which would be seen as significantly active purely due to chance. This effect was amusingly illustrated by Bennett et al. (2009) by using fMRI to scan a deceased Atlantic salmon. On presenting the fish a series of photographs, “significant” activation was seen in the salmon brain responding to the photo presentation compared to rest, even when using a p-value of 0.001.

This is an example of what is known as the multiple comparisons problem. The higher the number of statistical tests performed, the greater the chance of obtaining a false positive result. This needs to be accounted for and the process is known as correction for multiple comparisons. The correction method used in this study is called family-wise error (FWE). FWE is the probability of obtaining a type 1 error in all (a family) of statistical tests. FWE can be controlled using Bonferroni correction, permutation tests and random field theory. Bonferroni correction is simply dividing the p threshold by the number of tests being done. This assumes all tests to be independent of one another, which is not valid in smoothed fMRI
data and hence typically is not used in fMRI studies. That is Bonferroni correction is overly conservative when applied to fMRI studies and risks type 2 errors (true positive being rejected) (Nichols & Hayasaka, 2003). Permutation testing involves reshuffling the design matrix into different orders. For example, an experimental condition can be switched with the control condition and the statistical analysis re-run. This reshuffling is repeated until all possible permutations of the design matrix have been analysed, giving a distribution of beta weights. The original beta weight is then compared to this distribution and a result is considered significant if above a defined significance level. This requires significant computing power (Nichols & Hayasaka, 2003) and was not used in this study.

Random field theory works by accounting for the relatedness of neighbouring voxels, i.e. it does not apply the crude assumption that all voxels are independent. Random field theory can calculate the likelihood of type 1 errors occurring for a given statistic level in data of a certain smoothness (referring to not the smoothing process, but rather as a measure of how well correlated each voxel is with adjacent voxels). This is an efficient means of calculating family-wise error and is less stringent than Bonferroni correction (Brett, Penny, & Kiebel, 2003).

1.4.2.6 Imager’s fallacy
Another comparisons problem was noted by Henson (2005), who described a certain type of reporting which he termed the “imager’s fallacy”. This occurs by reporting a group difference based only on a visual difference between two cortical activation maps without explicitly testing the statistical difference between the two groups. That is, it is possible for one image to show a difference which does not remain when directly tested with the other image. For example, a region seen to be significant in one map may have only just exceeded the significance threshold, whereas the corresponding region in the other map may have only just failed to reach that threshold. Testing the two directly would show no significant difference between the two. That is, the difference between significant and not significant is not necessarily itself significant (Gelman & Stern, 2006). It is thus insufficient to report group differences using only a visual comparison. If a claim is to be made, there is a need to report the statistical measure used to test the group differences (Poldrack et al., 2008).

1.4.3 Parkinson’s disease and Imaging
MRI techniques have been applied to PD in an attempt to further characterise the disease and understand the process by which it occurs. The latest MRI machines give more contrast and
resolution than previous generations, providing a powerful tool for brain research (Stéphane Lehericy et al., 2017).

The diagnosis of parkinsonian syndromes can prove difficult. Parkinsonian disorders encompass Parkinson’s disease (PD) as well as multiple system atrophy (MSA), progressive supranuclear palsy (PSP) and dementia with Lewy bodies (DLB). Clinicians often make errors in the correct diagnosis of the disease especially in the early stages, with error rates as high as 24% (Hotter et al., 2009). Correct diagnosis is very important due to the different pathogenesis and treatment involved in different parkinsonian syndromes. The use of MRI to provide more objective data in regards to diagnosis is being investigated (Barber et al., 2017; Brooks & Tambasco, 2016; Niethammer et al., 2011; Saeed et al., 2017) but has not yet been incorporated in diagnostic criteria, which remains clinically based.

With new neuroprotective techniques being developed, it is important to be able to reliably track the progression of PD (Stocchi & Olanow, 2003). Current measures for assessing PD severity, such as the MDS-UPDRS (Goetz et al., 2008) are subjective rating scales. Imaging may provide more objective values which can mark disease progression (Tuite, 2017).

1.4.3.1  **Structural imaging in PD**

Studies into structural changes in PD have yielded varying results. However, disease-related changes have consistently been identified with changes in cognitive status in PD and disease state in PD using various MRI modalities.

The basal ganglia are severely affected in PD, resulting in the degeneration of the nigrostriatal system (Jellinger, 2012) which leads to neuron atrophy in the striatum (Stephens et al., 2005; Zaja-Milatovic et al., 2005). Consistent evidence from imaging studies points to atrophy in the putamen and caudate nucleus in PD. A number of studies report atrophy of the caudate head (Apostolova et al., 2010; Lee et al., 2011; Pitcher et al., 2012; Sterling et al., 2013). Likewise, putamen atrophy has been reported (Atasoy et al., 2004; Geng, Li, & Zee, 2006; Krabbe et al., 2005; Lisanby et al., 1993; Melzer et al., 2012; Pitcher et al., 2012; Tinaz, Courtney, & Stern, 2011; Weintraub et al., 2011). It is thought the atrophy of the striatum may be affected early in the disease (Tessa et al., 2014) and “bottoms out” after 5 years (Lewis et al., 2016). However, other studies have reported no change in striatal volume (Almeida et al., 2003; Ghaemi et al., 2002; Messina et al., 2011; Schulz et al., 1999). A possible reason for this may be due to the scanners used by the studies not able to detect a...
group difference being of 1.5T or less. This study uses a 3T scanner, which provides improved spatial resolution compared to a 1.5T scanner.

The thalamus is a major relay station for brain structures, including connections from the basal ganglia to the cortex (Herrero et al., 2002). Shape differences in the thalamus, with no volume changes, have been reported in PD (McKeown et al., 2008), but atrophy has been reported also (Summerfield et al., 2005).

The substantia nigra, a site of cell death in Parkinson’s disease, is difficult to define using structural MRI due to its small size and low contrast (Cho et al., 2011; Lehericy et al., 2014). Likely due to these reasons, the findings of SN volume in PD are conflicting and it has been reported as unchanged (Geng et al., 2006; Oikawa et al., 2002), decreased (Krabbe et al., 2005; Menke et al., 2009; Minati et al., 2007; Ziegler et al., 2013) or even increased (Kwon et al., 2012). More recently, significant SN area and volume differences were found by Aquino et al. (2014) correlating to different stages of the disease. Newer, high field 7T MRI studies have shown “smudging” in the SN and crus cerebri boundary (Cho et al., 2011) and another study has boldly stated that PD is able to be radiologically diagnosed with 7T MRI, using abnormalities seen in SN structure, reporting a 100% sensitivity and 96.2% specificity in group discrimination between 19 PD and 17 controls (Cosottini et al., 2014).

There is a lack of reliable segmentation to define the globus pallidus in MRI, making this area difficult to visualise using non high-field MRI (Iacono et al., 2011). However, studies have reported atrophy (Geng et al., 2006; O’Neill et al., 2002) which is possibly more pronounced with impaired cognition in PD (Tambasco et al., 2011).

The hippocampus and amygdala are structures linked with memory and dementia (Barnes et al., 2006; Eichenbaum, 2004; Phelps, 2004). Both hippocampus and amygdala atrophy have been found to occur with cognitive decline in PD (Beyer et al., 2007; Bouchard et al., 2008; Camicioli et al., 2003; Ibarretxe-Bilbao et al., 2008; Junque et al., 2005; Laakso et al., 1996; Melzer et al., 2012; Nagano-Saito et al., 2005; Riekkinen et al., 1998; Summerfield et al., 2005; Tam et al., 2005; Weintraub et al., 2011). Hippocampal atrophy is less certain in PD without dementia, with some studies reporting atrophy (Brück et al., 2004; Summerfield et al., 2005) while others show no hippocampal atrophy in cognitively normal PD (Burton et al., 2004; Camicioli et al., 2004; Nagano-Saito et al., 2005). Similarly, non-demented PD patients may show amygdala atrophy, thought to be related to depression (Surdhar et al., 2012), or no amygdala atrophy (Huang et al., 2015).
Voxel-based morphometry studies of structural MRI have shown a number of differences in cortical grey matter volume in PD patients, including regions outside of the basal ganglia. Grey matter volume loss was seen in the frontal lobe (Burton et al., 2004), regions of the hippocampus, thalamus, and anterior cingulate (Summerfield et al., 2005), caudate nucleus and putamen (Pitcher et al., 2012). Melzer et al. (2012) found grey matter atrophy in the temporal, parietal and frontal lobe and the caudal hippocampus, correlating with cognitive state.

Other forms of structural imaging such as R2 (inverse of T2) have been investigated for the use for diagnosing and rating PD progression. R2 is a form of structural MRI and, as mentioned, is the inverse of T2 (R2 = 1/T2). T2 was discussed on page 56, but to summarise, T2, also known as spin-spin relaxation, is a relaxation constant referring to the loss of phase between spinning nuclei caused by interactions between spins. Different tissue types have different T2 values, and hence, different R2 values. The differences in value give different signal profiles, which is used in MR imaging to distinguish between tissue types.

Iron accumulations in the brain are thought to reflect oxidative stress, leading to neuronal damage in PD (Napoli et al. & d’Ischia, 2011). Autopsy studies have shown higher iron accumulations in the SN for PD brains (Dexter et al., 1987). Iron accumulations can be imaged using R2, which is thought to reflect non-haem iron concentration (Graham et al., 2000). A number of studies have reported R2 differences in PD, showing increased SN iron content (Graham et al., 2000; Martin et al., 2008). Iron accumulations were even seen in asymptomatic LRRK carriers (Pyatigorskaya et al., 2015), potentially indicating this could be a PD diagnostic tool and biomarker (Hopes et al., 2016). However, other studies have shown conflicting results (Dashtipour et al., 2015; Reimão et al., 2016) and more trials are needed before this can be established as a biomarker, potentially requiring a combined R2 analysis with other MRI modalities, such as DTI (Du et al., 2011).

A recently validated MRI technique known as quantitative susceptibility mapping (QSM) has been shown to provide better detection of iron levels within the brain by compensating for non-homogenous magnetic fields by deconvolution (Langkammer et al., 2012; Wang & Liu, 2015). Studies employing this technique have found a greater significance of iron accumulations within the substantia nigra in PD compared to findings from previous R2 experiments (Du et al., 2016). A preliminary study was able to describe a progressive pattern of iron accumulation in the PD brain (Guan et al., 2017), showing the QSM technique to be a promising new means for detecting progressive iron deposition in PD.
Nigrosomes are groupings of dopaminergic cells within the SN, characterised by specific immunohistochemical staining (Damier et al., 1999a). Five nigroome groups were described by Damier et al., with nigroome-1 being proportionally the most affected in PD (Damier et al., 1999b). Nigroome-1 can be seen using 7T (Blazejewska et al., 2013) and 3T (Schwarz et al., 2014). The appearance of a healthy nigroome-1 has been likened to the shape of a swallow tail by Schwarz. Identifying abnormal nigroome-1 could provide a relatively simple diagnostic means for PD, using relatively common 3T MRI scanners (Schwarz et al., 2014).

Neuromelanin can also be visualised using MRI (Nakamura & Sugaya, 2014). Neuromelanin is a dark pigment contained in SNpc which is typically lost in PD (Zecca et al., 2001). Using MRI sensitive to neuromelanin, differences in SNc volumes have been reported by Castellanos et al. (2015). This has shown promise for the diagnosis and use as a biomarker of PD progression in a number of reports (Hatano et al., 2017; Reimão & Ferreira, 2016; Tuite, 2017). This neuromelanin-sensitive MRI technique was also found to be able to differentiate essential tremor from PD (Reimão et al., 2015) and enhances the robustness of another imaging modality (DTI) when used to identify regions of interest for DTI testing of PD-related changes (Langley et al., 2016).

1.4.3.2 Diffusion tensor imaging (DTI) in PD

DTI MRI can produce a number of metrics: the two most studied are fractional anisotropy (FA) – the direction preference of diffusion, and mean diffusivity (MD) – the rate of diffusion, without a directional component (Soares et al., 2013). Studies, in general, report decreased FA values of the SN in PD (Chan et al., 2007; Peran et al., 2010; Vaillancourt et al., 2009) along with increased MD values the SN in PD (Du et al., 2014; Nagae et al., 2016; Scherfler et al., 2013).

Findings have been mixed for differences in DTI values in other subcortical nuclei. Shape analysis has found altered right pallidum shape, with fibre tracts arising from the altered pallidus region having lower FA and higher MD in PD vs controls (Menke et al., 2014). MD has been found increased in globus pallidus itself (Nagae et al., 2016), with FA and MD differences in the thalamus (Peran et al., 2010) and putamen as well (Jiang, Shi, Niu, Xie, & Yu, 2015). Other studies have shown no difference in DTI values of non-nigral subcortical nuclei (Gattellaro et al., 2009; Loane et al., 2016).

A number of factors may contribute to the variability in subcortical nuclei DTI findings, including scanner protocol, strength and experimental analysis type. Subcortical nuclei are also subject to increased iron deposition with age. These iron deposits can affect DTI
measures, making it difficult to differentiate group changes (Pfefferbaum et al., 2010; Schwarz et al., 2013). A systematic review by Cochrane & Ebmeier (2013) stated that larger studies are needed in order to develop a valid repeatable biomarker using DTI, and that it will likely need to be used in conjunction with other imaging modalities.

Free water, e.g. cerebrospinal fluid or oedema - water molecules not restricted by the cellular environment, can be detected using DTI (Pasternak et al., 2009). Free water shows an MD value many times greater than water within cellular structures and was found to be elevated in the SN in PD. Free water also increased with PD progression (Ofori et al., 2015). A technique called fibre tracking, which reconstructs the direction of white matter tracts using DTI (Mori & Van Zijl, 2002) has been used to map the nigrostriatal tract connecting the SN to the striatum (putamen and caudate) (Lehéricy et al., 2004). Using fibre tracking, Menke et al. (2009) found decreased connectivity while Sharman et al. (2013) found no anatomical nigrostriatal connectivity difference in PD (but did find functional connectivity differences using resting-state fMRI).

1.4.3.3  

**Arterial spin labelling (ASL) in PD**

Studies using ASL have found a number of perfusion deficits in PD. An established disease-related perfusion pattern has been seen in PET with decreased metabolic activity in the posterior parietal cortex, lateral premotor cortex and supplementary motor area with increases seen in the pontine, pallidothalamic and cerebellar regions (Ma et al., 2007). An ASL perfusion network has been found to correlate closely with this network (Ma et al., 2010). Kamagata et al. (2011) found hypoperfusion in the posterior cortex. Melzer et al. (2011) described an ASL PD-related perfusion network which correlated with cognition, as assessed using the Montreal cognitive assessment (MoCA), and motor status as assessed using the UPDRS part III. This network score showed absolute perfusion deficits in the parietal-occipital cortex, cuneus, precuneus and middle frontal gyrus. Intact perfusion was seen in the globus pallidus, putamen, anterior cingulate and postcentral gyri. Al-Bachari et al. (2014) found a diffuse widespread increase in arterial arrival time (time taken for tagged blood to reach the imaged areas), suggesting a compromised neurovascular status in PD. Lin et al. (2016) found widespread hypoperfusion in PD, similar to the previous ASL PD studies, but also found further decreases in the frontal lobe in non-dementia PD patients (PD-N) and cerebellum in PD dementia (PD-D) patients on dopaminergic (levodopa or equivalent) medications. This difference in perfusion was thought to relate to cognition or disease severity and could potentially be used for disease management planning. Lin et al. (2017) found ASL perfusion deficits in the middle frontal gyrus that correlated with clinical severity (UPDRS

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part I score) and perfusion deficits in autonomic control regions (within the frontal lobe and insula) correlated with impaired autonomic function scores (score described by Low (2003)).

### 1.4.3.4 Functional MRI

The human brain functions as a collection of multiple networks, with individual cortical regions continuously sending and receiving information as an integrative network - even at rest (van den Heuvel & Hulshoff Pol, 2010). Resting-state fMRI is used to investigate functional connectivity, or the temporal correlation of neural activity in anatomically separate cortical regions (Barkhof et al., 2014; van den Heuvel & Hulshoff Pol, 2010). This is done using fMRI at rest, with no explicit task. A number of such studies in PD have found functional connectivity disruptions in a large number of networks. The motor network showed decreased connectivity in the supplementary motor area, DLPFC and striatum (Hacker et al., 2012; Wu et al., 2009, 2011) and increased connectivity of the left cerebellum, left primary motor cortex, left parietal cortex (Wu et al., 2009). Increased functional connectivity was also seen from STN to cortical motor regions and posterior putamen (Baudrexel et al., 2011; Rolinski et al., 2015). Studies into pre-symptomatic LRRK2 mutation patients found altered resting-state connectivity in striatocortical and nigro cortical circuits compared to controls (Vilas et al., 2016), which are similar to changes seen in PD (Helmich et al., 2015). Resting-state fMRI also shows potential as a biomarker and has been able to differentiate PD from controls (Szewczyk-Krolikowski et al., 2014) and PD from AD (Rolinski et al., 2015).

Overall, these studies have identified a large number of potentially affected cortical regions in PD during rest, including regions such as the fronto-parietal visual area (Prodoehl et al., 2014) which could suggest the activity of these regions may also be altered during task performance.

Movement abnormalities have been investigated in PD using functional imaging techniques. A consistent picture of cortical motor differences in PD has not yet been described, with studies reporting varying locations and sizes of PD motor activation changes (Grafton, 2004; Rowe & Siebner, 2012). A meta-analysis of 24 functional imaging movement studies by Herz et al. (2014) found the changes converged into a fronto-parietal region, lateralized to the left, containing the pre-supplementary motor area, primary motor cortex, inferior parietal cortex, and superior parietal lobule. However, both increases and decreases in activity were seen in this region depending on motor task timing and movement selection. Within the basal ganglia, posterior motor putamen activation was found to be decreased in PD. This decrease in motor putamen activity was more likely detected in PD patients with more significant motor impairment measured by mean UPDRS III scores (Herz et al., 2014).
Functional MRI studies of saccade performance in PD are scarce. While no consensus exists on the cortical basis of saccadic eye movement abnormalities in PD, fMRI studies have reported activation differences in PD in the FEF and parietal cortex during saccades. Using blood oxygen level dependent (BOLD) activation and functional MRI, Rieger et al. (2008) observed marked hypoactivity in frontal eye fields and supplementary eye fields during a simple self-paced saccadic task. The activation pattern was also different in PD patients, showing more above-threshold voxels in general, despite the frontal hypoactivity, with greater BOLD activity in posterior parietal, temporal and occipital lobe regions. Cameron et al. (2012) found altered preparatory FEF and DLPFC activity in PD compared to controls in the antisaccade task. Lemos et al. (2016) found hypoactivity in the left FEF during prosaccades and hyperactivity in the right parietal cortex for both prosaccades and antisaccades.

The overall goal of this study is to add to the understanding of the basis of eye movement performance deficits in PD. This thesis will use MRI as a means to assess the extent of which PD-related changes within the brain contribute to saccade deficits. I will first be using a voxel-based analysis on structural, ASL and DTI scans to make comparisons with eye movement performance measures. The second part of this thesis will focus on fMRI of eye movements in PD. Reflexive and predictive tasks are used in the first part of the fMRI section, followed by an investigation into memory-guided saccades in PD. No study has yet investigated predictive and memory-guided saccades in PD using fMRI. Memory-guided saccades encompass activation in frontal and parietal eye fields as well as DLPFC activation. The contribution of functional brain imaging with eye movement performance changes from ongoing longitudinal studies could be used to develop biomarkers of PD progression. The findings in this thesis will provide increased understanding of the disease-related brain changes contributing to eye movement deficits in PD.

1.5 Overall thesis aims

The overarching aim of this thesis is to determine if saccadic eye movement abnormalities can reflect brain health in PD and controls. Certain saccade performance measures show correlation to PD severity as measured by cognitive impairment and motor impairment. Similarly, brain changes in PD have been detected with a number of MRI modalities, which are more severe in later disease states. These correlations, in turn, support the idea that saccade measures may correlate with disease-related brain changes and thus reflect brain health in PD. As covered previously, certain saccadic eye movement measures show
characteristic deficits in PD. However, the changes in the brain leading to these deficits remains unknown. Perfusion and white matter tract changes in the frontal and parietal cortices have been reported in PD, even early in disease (Melzer et al., 2011; Melzer et al., 2013). fMRI studies have revealed possible altered FEF and parietal lobe activity during the antisaccade task in PD but, as previously discussed (page 76), no consensus over characteristic functional changes in PD have been made. It is known that the frontal and parietal cortices contain key eye fields involved in saccade control. It would be then feasible that disease-related brain changes involving the eye fields in the disease could contribute to PD-related saccade abnormalities. However, to date, no study has compared structural, ASL and DTI measures to saccadic eye movement performance in PD using a voxel-based analysis to determine if this is true. In this thesis, I aim to determine if eye movement performance in PD is reflected by corresponding disease-related changes in eye fields in the brain, by comparing saccade performance to a number of MRI measures. Secondly, due to the comparatively large numbers of participants used in this study, I also aim to provide better understanding of general eye movement control of non-PD people to expand our present knowledge of saccade control. In regards to this aim, I emphasise that every study in this thesis contains a control group, used as a comparison to the PD group. Should novel findings be detected in controls, this would allow comment on saccade control for the general non-PD population.

Each chapter has a specific section dedicated to outlining the aims of its study and how these contribute to answering my overall questions. In summary, Chapter 3 will compare saccadic eye movement performance to structural, ASL and DTI modalities using a voxel based analysis. This will determine if differences in correlation exist between eye movement performance and MRI measures, e.g. volume or perfusion, in the PD brain compared to controls. Chapter 4 will compare eye movement performance to expression of a characteristic PD perfusion network, aiming to determine if saccade performance may be a reflection of a higher expression of a PD-related perfusion pattern seen across multiple regions. Chapter 5 will investigate reflexive and predictive saccades in PD and control groups using fMRI and will determine if functional activity in eye fields is altered in PD for these tasks. This study also allows comparisons of brain activity between reflexive and predictive tasks and allow comment upon saccade control for these tasks in PD and the general population. Lastly, Chapter 6 will investigate memory-guided saccades, using the same technique as Chapter 5 at first, but, in addition, will use an event-related fMRI design. This will allow a timecourse to be generated of BOLD activity during task performance and would determine if altered
BOLD activity during task performance is present in the diseased group compared to controls. Furthermore, I plan on applying a principal component analysis (PCA) to extract common patterns from the BOLD timecourses, which may not be immediately visually obvious. The technique is only used in Chapter 6 and will be further discussed within that Chapter. The use of PCA would allow comment upon the use of this technique in the fMRI investigation of eye movements and may provide further insights into eye movement control for both PD patients and the general population.
2 Study methods – General

2.1.1 Patient diagnosis and recruitment
All participants were recruited through the New Zealand Brain Research Institute, where this study took place. The diagnosis of PD for study participants was made by a movement disorders specialist (TJA) using the UK Parkinson’s Disease Society Brain Bank diagnostic criteria for PD (Hughes et al., 1992). Patient exclusion criteria were the presence of atypical PD, past moderate to severe head injury, previous stroke, major depression, past history of neurosurgery, learning disabilities, cardiovascular disease, insulin-dependent diabetes, the use of medications known to affect the central nervous system and uncorrected visual acuity worse than 6/12 in the best eye. Consent was provided by all participants with families/caregivers providing consent for cognitively impaired patients. This study was approved by the Upper South A Ethics Committee of the New Zealand Ministry of Health.

2.1.2 Cognitive testing
All participants underwent a series of cognitive tests covering five cognitive domains (executive function; attention; learning and memory; visuospatial/visuoperceptual and language), as part of the level II criteria for PD-MCI (Litvan et al., 2012). MCI participants had unimpaired functional activities of daily living, as verified by interview with a significant other, and scored 1.5 standard deviations or below normative values on at least two measures within at least 1 of the five MDS cognitive domains. PD dementia was determined using the Movement Disorder Society (MDS) Task Force criteria, as set out by Dubois et al. (2007). Additionally, general cognitive testing was performed using the Montreal Cognitive Assessment (MoCA). Within each cognitive domain, standardized scores from the constituent neuropsychological tests were averaged to provide individual cognitive domain scores; global cognition for each participant was expressed as an aggregate z-score, obtained by averaging four domain scores (language was excluded due to low variability), reflecting overall cognitive status.

2.1.3 Parkinson’s motor symptoms testing
Many participants involved in this study were recruited over time and are part of a longitudinal study of PD. The UPDRS (Fahn & Elton, 1987) was initially used to assess motor function. A portion of the subjects in this study were originally recruited and assessed before the introduction of the revised MDS-UPDRS (Goetz et al., 2008). The UPDRS part III score of these participants was converted using the process described by Goetz, Stebbins, &
Tilley (2012) to be equivalent to the revised MDS-UPDRS III. To note, for convenience, all mentions of UPDRS collected for the studies of this thesis refers to the MDS-UPDRS.

2.1.4 Eye movement laboratory

All eye movements in the laboratory were recorded using an iView X high-speed infrared pupil and corneal tracking system (SMI Berlin), generating monocular (left) video eye recordings at 1250 Hz. Participants were instructed in the process around eye movement recordings, which involved sitting behind the eye tracker, with head resting on an adjustable chin rest. Patients were instructed to minimise head movements, with seating and eye tracker heights adjusted to patient comfort before commencing the task. Task stimuli were presented on a screen located 160.5 cm in front of the participant.

For Chapters 3 and 4, task stimuli were projected onto the screen with a resolution of 800 × 600 pixels and 100 Hz refresh rate. Red squares of 12 × 12 pixels, subtending 0.75 degrees of visual angle, were used as fixation and target stimuli, appearing on a grey background. The display was situated 160.1 cm away from the participant. The screen measured 109.2 cm × 82.9 cm. A second green stimulus of the same size and shape was used for the antisaccade task. iView calibration took place using a 13 point system before each trial.

Eye movements for Chapters 5 and 6 were recorded and tracked using the same eye-tracking setup, however, a display of 1280 × 720 resolution was used. The display was situated 164.9 cm away from the participant’s eye and measured 111.4 × 85.3 cm. Red square targets subtending 1.25 degrees were used as stimuli and were projected onto a grey background. Calibration was carried out using the iView (SMI) software before each task, using 13 points covering the entire task area.

The fMRI studies used the Real Eye imaging system (Avotec, Inc). This comprised four subsystems: eye illumination, eye imaging, camera interface and the tracker interface. This allowed the eye movement task to be displayed to the participant whilst in the MRI scanner using a goggle setup, with the tracker interface allowing iView to be used by the experimenter to record eye movements. Unlike the eye movement tasks performed in the laboratory, the field of view and visual angles have not yet been determined by the goggle manufacturer. A 9 point eye calibration was carried out before each task. An important point to acknowledge early is that, this system proved unreliable in maintaining tracking of gaze in the scanner and was not able to reliably track saccades. This proved a limitation, as in-bore eye movement performance was not able to be factored into the study analysis. The eye tracker did not allow accurate discernment of error trials from correct and hence all trials, correct and error trials,
were included in the final analyses and timecourses for the event-related memory guided task (Chapter 6). This is a noted study limitation. Thus study results are carefully interpreted acknowledging this limitation.

Ideally, trials with error anticipatory saccades should be excluded as these are likely to arise from a different mechanism from the main memory-guided task. One effect from this is the potential for error saccades to influence activity measured during what is named the “memorisation” event, or the event when the flash appears but the participant is instructed to maintain fixation on the original stimuli. Should an error reflexive saccade be made at this stage, this would activate a separate reflexive saccade mechanism, rather than the expected fixation/spatial memory mechanism for this event.

2.2 MRI modalities

No single MRI modality is able to provide all the information regarding the nature of the brain tissue changes which may affect eye movement performance. Hence various MRI modalities are used to provide an insight into the various potential changes in the brain, be it structural or functional, which could lead to saccade performance changes in PD and controls. Pathological processes in PD develop at different rates over time e.g. cortical atrophy is most clearly demonstrated only late in the disease with dementia (Melzer et al., 2012; Nagano-Saito et al., 2005; Ramirez-Ruiz et al., 2005; Sanchez-Castaneda et al., 2009; Summerfield et al., 2005; Weintraub et al., 2011), however blood flow changes have been demonstrated in earlier, non-demented PD patients (Fernandez-Seara et al., 2012; Melzer et al., 2011). QSM (briefly summarised on page 72 of introduction) has been used to detect very early nigrostriatal changes in PD and is a potential development in an imaging-based diagnosis of PD (Kim et al., 2018). There is therefore a need for multiple measures to capture these pathologies. Each subsequent chapter deals with a different imaging technique. Information detailing each imaging technique is discussed within each chapter. The sections below contain a critical analysis of the advantages and disadvantages of each scan type:

2.2.1 Structural MRI

Structural MRI gives the advantage of high spatial resolution, providing an excellent picture of structure and tissue type within the brain. As previously discussed, structural MRI has the advantage over other types of 3D brain imaging, such as CT, in that it provides high contrast between fat-containing structures such as white matter over grey matter. MRI also does not use ionising radiation (e.g. X-ray, SPECT and PET imaging), which is harmful with prolonged or repeated exposure. A disadvantage of structural scanning is that it does not give
any temporal information or information regarding neural processes during task activity. However, structural imaging is necessary to provide the structural information for scan preprocessing for other MRI modalities, such as functional MRI. The use of structural MRI is therefore an essential modality for a high quality MRI study, including functional experiments.

2.2.2 Arterial spin labelling (ASL)

ASL has the advantage of being a non-invasive method to measure bloodflow within the brain, as opposed to PET. This scan type was chosen as this offers significant advantages in convenience and safety for patients, who are able to be scanned and re-scanned using the ASL technique in a standard MRI machine, without the need to order radioactively-tagged compounds from a cyclotron, of which there are very few operating in New Zealand. In addition, local expertise in the use of ASL was available. Comparison of saccade performance to ASL data is novel, both in PD and the general population. The use of ASL would satisfy the aim of providing new insights into eye movement control of these two populations. The advantages of ASL have been previously covered in section 1.4.1.4 on page 57 in the introduction chapter. For example, ASL provides a non-invasive means of determining a functional measure, perfusion. However, this can also be a disadvantage, as a recent review identified 58 potential non-pharmacological perfusion modifiers, including a number of modifiers with large impact (caffeine, aging, blood gases, and drowsiness) on perfusion values (Clement et al., 2018). Another disadvantage of ASL is the relatively low resolution (voxels used are roughly 4 × 4 × 4 mm), compared to other MRI types. Another disadvantage is the relatively long repetition time between individual volumes, giving poor temporal resolution. This limits the use of ASL as a functional scan during live task performance, however long duration or slowly changing tasks (e.g. sleep) may still be investigated using functional ASL. For this reason, ASL was not used for functional imaging in my studies and instead was employed to determine blood flow in resting state only. For active task performance, BOLD fMRI was used instead.

2.2.3 Diffusion tensor imaging (DTI)

DTI detects white matter tract change and this sequence is conveniently able to be completed in the same MRI scanner used for other experiments in this thesis. As with ASL, the investigation of DTI in eye movements is novel for PD. DTI has revealed pathological
diffusion metric changes (decreased FA) in the substantia nigra in PD, as well as similar, albeit less consistent findings in other subcortical nuclei (summarised in section 1.4.3.2 in the introduction chapter regarding DTI in PD). For these reasons, DTI scans were used in my study to test if diffusion changes within the brain could relate to eye movement performance deficits in PD and also in the general population.

The use of DTI is limited in my thesis, with only one chapter (Chapter 3) containing a DTI study. This study is intended to be an initial comparison of MRI modalities to eye movement performance and thus a simple whole brain voxel-based method was used. More advanced DTI techniques can be used to map out fibre tracts through the brain using a process called probabilistic tractography. This however comes with its own complexities and was hence not used in my study. DTI resolution is low and is sensitive to normalization, with individual fibre tracts running very closely together. There is significant individual variability which needs to be accounted for when running tractography analyses. In addition, the DTI acquisition we used is now relatively old. At the time, it was a simple and robust sequence to estimate DTI metrics in the brain, however, with the evolution of both acquisition and modelling techniques, there are many more options available that attempt to overcome limitations of the DTI model. For example, HARDI acquisitions and associated models (constrained spherical deconvolution and diffusion kurtosis imaging) now allow more accurate treatment of multidirectional, crossing fibres by allowing detection of diffusion in multiple directions and by using more sophisticated models of water diffusion (Tournier et al., 2008; Tuch et al., 2002). These techniques have been suggested for future studies, particularly in conjunction with findings from other imaging modalities such as fMRI.

2.2.4 Functional MRI

fMRI is used extensively in this thesis. The fMRI technique employed in this study is a widely-used and validated method of investigating brain activity during task performance by contrasting BOLD activity between a baseline state (fixation) and task activity (saccade tasks). fMRI offers the advantage of providing temporal information but is restricted in the amount of spatial resolution it provides compared to structural MRI. Compared to PET, fMRI offers faster detection time (seconds compared to tens of second in PET) and generates images which can be overlayed on the high resolution structural images. The timing of BOLD fMRI detection also allows a timecourse of BOLD activity changes to be generated in an event-related fMRI design. fMRI is also non-invasive compared to PET, and is able to be
carried out in the same MRI scanner as the other imaging modalities and repeated safely. For these reasons, I chose to use fMRI to investigate functional activity during saccades for my thesis.

A disadvantage of fMRI compared to PET is that the detected BOLD response itself tells us very little about the components which make up the BOLD signal, which is affected by a combination of cerebral blood flow (CBF), cerebral metabolic rate of oxygen (CMRO₂) and cerebral blood volume (CBV). This has been previously discussed in depth, along with known issues in correlating the BOLD signal to neural activity in the introductory chapter section 1.4.1.6 page 58 - 59. This is opposed to PET, which, using directly tagged compounds, can directly measure the uptake of glucose and oxygen. ASL can also directly quantify bloodflow, which fMRI is unable to. These disadvantages can be minimised using information from a combination of each MRI modality. In addition, BOLD fMRI volumes acquired with an echo planar imaging sequence are particularly susceptible to geometric distortion as a consequence of the image acquisition (Poustchi-Amin et al. 2001). There are methods to minimize the impact of these distortions, but such extreme distortions are not generally present in functional PET data. This thesis contains studies using structural, ASL, DTI and fMRI allowing possible cross-comparison, should similar regions are found to be affected across multiple modalities. A further issue when using fMRI, though common to all functional imaging, is the need for patients to remain still for scanning during task performance. This was minimised by using a head brace and foam. This requirement for minimal movement did mean that I was limited to testing relatively mildly-impaired PD patients (noted in discussion section 6.5.1 page 257).

2.3 Other techniques

2.3.1 PET

PET can also be used for functional imaging of the brain. This was not employed in this study as it would involve a separate scan in addition to the MRI, which would be difficult given resources (PET is approximately twice as expensive as an MRI session in New Zealand) and time constraints. In addition, PET has an added invasive element - leading to possibly more difficult recruiting, and subjecting participants to an increased risk of complications from the scan. As mentioned previously, PET can directly measure the uptake of glucose and oxygen, which gives different information on the nature of the brain changes compared to MRI. Logistically, PET also requires the ordering of specialist radioactively tagged compounds from a cyclotron; currently radioactive tracers are sourced from either Wellington, New
Zealand, or Melbourne, Australia, making logistics more complicated. PET tasks need to be in long blocks of tens of seconds, as the tracer needs this time to reach and be utilised by the brain. This would make it more challenging to create an event related timecourse, as opposed to using fMRI. PET in general also gives comparatively poorer resolution without the ability to overlay findings on a high quality structural MRI image (unless a separate MRI session was completed) – only CT is currently available in Christchurch to provide concurrent structural imaging to PET. An advantage is that the PET scanner is quieter, which may be a consideration should a planned experiment contain an auditory component, but this was not needed for my studies. For these reasons, fMRI was chosen over PET for the functional tasks of my thesis.

2.3.2 MEG/EEG

Other functional brain analysis techniques include EEG and MEG (Babiloni et al., 2009). EEG detects neural activity measured directly on the scalp. MEG detects the same activity from the electromagnetic signal generated by neural activity just inside the scalp. The advantage of EEG and MEG are that they have no haemodynamic response lag as opposed to the BOLD signal detected by fMRI, providing superb temporal resolution, and potentially a more direct measure of neuronal activity. EEG is also relatively inexpensive. The disadvantage of EEG is poor spatial resolution, of several centimetres, with neural signals having to go through multiple layers of tissue and the skull to be detected, compared to the millimetre resolution of fMRI. EEG is also unable to detect activity within deeper brain structures such as the basal ganglia. MEG is more able to detect activity within the sulci and offers slightly better resolution. MEG however requires an extremely specialised scanner, costing several millions, to be able to detect the miniscule magnetic fields generated by the neurons in the brain and is not currently available in New Zealand. It is possible, although technically challenging, to combine fMRI and EEG, using the strengths of each technique to provide both improved spatial and temporal resolution (Huster et al., 2012). This may be an option for future studies to consider by building from the results of this thesis, investigating specific regions of interest.

2.4 Magnetic resonance imaging protocol

All MR images were obtained from a 3.0 Tesla General Electric HDx scanner (GE Healthcare, Milwaukee, WI, USA) with an eight-channel head coil.
Structural images used a 3D T1 weighted spoiled gradient recalled echo acquisition or SPGR. Repetition time (TR) = 6.6 s, echo time (TE) = 2.8 s, inversion time (TI) = 400 ms, flip angle = 15 deg, FOV = 250 mm, acquisition matrix = 256 × 256 × 170, slice thickness = 1 mm, voxel size 0.98 × 0.98 × 1.0 mm³), bandwidth = 32 kHz, scan time = 5 min 3 s.

ASL scans were obtained using a stacked spiral, fast spin echo acquisition with pseudo-continuous ASL and background suppression: TR = 6 s, echo spacing 9.2 ms, post-labelling delay = 1.5 s, labelling duration 1.5 s, 8 interleaved spiral arms with 512 samples at 62.5 kHz bandwidth and 30 phase coded 5 mm thick slices, NEX = 5, voxel size = 3.75 × 3.75 × 5.0 mm³, scan time 8 min 11 s.

DTI scans were obtained using a 2D diffusion-weighted spin echo, echo planar imaging sequence, with diffusion weighting in 28 uniformly distributed directions ( b = 1000 s/mm²) and 4 acquisitions without diffusion weighting ( b = 0 s/mm²): TE/TR 84.4/13000 ms, flip angle = 90 deg, acquisition matrix = 128 × 128 × 48, reconstruction matrix = 256 × 256 × 48, FOC = 240 mm, slice thickness = 3 mm, reconstructed voxel size = 1.07 × 1.07 × 3 mm³, scan time = 7 min 9 s.

fMRI scans took place over four separate runs, with the first run being the block design task (the subsequent three runs being memory-guided tasks, to be covered in Chapters 5 and 6). Each run followed the same scanning protocol, differing only in length: TR = 3000 ms, TE = 35 ms, flip angle = 90 deg, FOV =220 mm, acquisition matrix = 64 × 64, 44 slices per volume, slice thickness = 3 mm, voxel size = 3.4 × 3.4 × 3mm³. Scan time = block/event durations (See Chapters 5 and 6).

2.5 SPM8

For MRI analysis, I used the program SPM8. SPM or statistical parametric mapping, is a MATLAB-based program widely used for imaging analysis, which allows a whole-brain voxel-based comparison of various MRI values to another measurement, such as eye movement performance. SPM also allows contrasting of an active task BOLD signal to a baseline signal to detect functional brain activity specific to task performance.

This section focuses on detailing potential sources of error when using SPM. The use of SPM for preprocessing has been previously described in the introductory chapter (section 1.4.2.3 page 64). The first preprocessing step in SPM is an attempt to minimise the effects of movement. Excessive head movement leads to errors and motion artefacts during recording. SPM applies motion correction through the use of a “rigid body” transformation applied to all
scans. In addition, motion parameters generated during MRI pre-processing were used in the statistical model for all first-level (individual) models as a way to attempt to account for gross motion. Head movement was monitored during live scanning. Scans showing significant movement were repeated. The head was semi-immobilised in our study, using foam padding within a plastic head brace.

Segmentation is a preprocessing step in which the types of brain tissue - white matter, grey matter and CSF are divided and analysed separately. This is an automated process and the potential for error exists (Despotović et al., 2015). SPM employs a unified segmentation algorithm and a tissue probability template (Ashburner & Friston, 2003). This technique has been continually refined in later versions, with recalculated tissue probability maps and more refined algorithms. To minimize error, it is recommended to use the latest software version (Ashburner et al., 2013; Kazemi & Noorizadeh, 2014) - which was SPM8 at the time of my studies.

Normalization or the warping of an individual scan to a standardised brain template presents as another opportunity for error within the preprocessing steps in SPM. The normalization process is an automated process of spatial transformation, where the differing brains of all participants are stretched and warped to fit a standardised template (Ashburner et al., 2013). To assist the normalization process, it is important to ensure the images are aligned as much as possible prior to the normalization process. For this, the co-registration step is employed (Ashburner et al., 2013). This ensures the 0 0 0 co-ordinate (placed at the anterior commissure) is placed in the same location for all scans prior to the normalization process. Visual inspection after this process is important, to ensure the co-registration process has correctly occurred for all scan types. To minimize error, it is again recommended to use the up-to-date software. The later SPM versions allow the use of further improved normalization techniques - a newer method has been developed named “Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra”, or DARTEL (Ashburner, 2007). DARTEL uses an updated non-linear warping algorithm, giving improved localisation and registration between individuals of different brain shapes, compared to the warping algorithm used by SPM8 by default (Ashburner & Friston, 1999). This requires the use of an additional toolbox package for SPM8 and was not employed in this thesis. Shoot is an even newer toolbox made for SPM12 and is considered a further improvement over DARTEL (Ashburner & Friston, 2011). The Shoot toolbox is intended to supersede DARTEL in the near future (The FIL
Methods Group, 2013). Future studies will undoubtedly make use of these ongoing improvements to further minimise error.

Another source of error in the normalisation process is encountered with abnormal brain pathology. The automated image matching process used in normalization can mismatch abnormal or absent structures to the template, leading to decreased sensitivity or the attribution of false positives to lesion-containing regions (Crinion et al., 2007). Scans were inspected by a specialist radiologist and any scans with significant pathology were excluded from the study. Another effect important to account for is the effect of aging on the brain. Brain atrophy is a commonly seen in the elderly population. Should the template brain not closely match the expected age-related changes of participant brains, normalization errors may occur. An elderly person template was used in my experiment to minimize potential age-related mismatches compared to using a template constructed from a younger population (Lemaître et al., 2005).

Despite our best efforts in terms of realignment, segmentation and normalization, there could still be some residual misalignment or real individual anatomical differences still present. In order to meet assumptions for the random field theory, and as an attempt to account for any residual misalignment, smoothing was used as part of the preprocessing steps (See preprocessing 1.4.2.3) (Ashburner & Friston, 2000). As previously covered, smoothing is also used to suppress noise and increase signal to noise ratio as well as accounting for residual variability. Smoothing is done through a convolution of voxel values with the averages of its surrounding voxels using a gaussian kernel, specified as a full width at half maximum (FWHM) value. This value is specified by the experimenter. The choice of the FWHM value needs to be carefully considered, as selecting a too-small or too-large value can lead to errors. Investigations have found that a FWHM value that is too high gives an overestimation of the spatial extent of the activations, i.e. clusters appear larger, and as noted, too small a value gives poor ability to suppress noise and individual variability (Liu et al., 2017; Mikl et al., 2008). Mikl et al. (2008) had suggested approximately 8 mm as a good optimum for fMRI and reiterated the importance of testing for multiple comparisons.

2.6 Principal component analysis

Principal component analysis (PCA) is a type of analysis used to determine patterns or “components” which make up complex datasets which may not be immediately visually obvious. This can be used for example to determine patterns within BOLD activity during task
performance, giving further insight into the functions of each region (Sugiura et al., 2004). This analysis technique is only used in Chapter 6 so a detailed discussion of this technique is located within that chapter.
3 Saccadic eye movements and multi-modal MRI in PD – A voxel-based analysis

3.1 Introduction
PD has been classically described as a disease involving the basal ganglia, but widespread cortical abnormalities are also evident, particularly in later stages of the disease (Braak et al., 2003). Neuroprotective therapies are the subject of ongoing clinical trials and a reliable and objective marker of PD progression is needed for the development and implementation of these future treatments. Aside from clinical rating scales such as the MDS-UPDRS, there is currently no accepted objective measure of PD progression or biomarker. Eye movements form one potential avenue for the development of PD biomarkers. Saccadic eye movement control is modulated by a number of cortical regions known as eye fields (Müri & Nyffeler, 2008; Pierrot-Deseilligny et al., 2003; Pierrot-Deseilligny et al., 2004), which show connections to much of the same basal ganglia circuit known to be impaired in PD (Hikosaka et al., 2000). Characteristic eye movement abnormalities have been found to be related to motor severity and cognitive impairment in PD (MacAskill et al., 2012). Using fMRI, frontal lobe hypoactivity with parietal lobe hyperactivity has been reported in the self-paced saccade task (Rieger et al., 2008) and during pro and antisaccade tasks (Lemos et al., 2016) in PD. Saccadic eye movement impairments such as hypometria and antisaccade errors were associated with decreases in functional resting-state connectivity within the default mode network in PD compared to controls (Gorges et al., 2013, 2016). To date, no study has compared structural, ASL and DTI scans to saccadic eye movement performance in PD using a voxel-based analysis.

The use of MRI modalities such as structural MRI, perfusion MRI and DTI may reveal abnormalities not able to be detected with functional MRI, therefore, complementing functional imaging findings. As opposed to functional MRI, structural imaging provides excellent information about the anatomy of the brain. Structural MRI provides a much higher spatial resolution, allowing detection of small anatomical changes. The BOLD signal detected in fMRI is influenced by a combination of cerebral blood flow (CBF), cerebral metabolic rate of oxygen (CMRO₂) and cerebral blood volume (CBV) (Buxton, 2013; Ekstrom, 2010). It is not possible to tell, from measured BOLD signal alone, which parameter is responsible for driving BOLD signal change. Perfusion MRI is able to measure absolute blood flow values which can aid fMRI interpretation, as well as providing information on perfusion value.
changes in PD directly. DTI can detect microstructural cellular damage, which may, in turn, contribute to the task-related or connectivity fMRI abnormalities seen in the prior studies in the literature.

3.1.1 Voxel-based analysis of eye movement performance

A number of MRI analysis methods can be used to investigate changes in the brain. A voxel-based analysis (VBA) is one such technique which performs a statistical test at every voxel in the brain (or within a large selected masked population, such as the grey or white matter or cortex). This is opposed to a region of interest (ROI) analysis, where selected voxels in specific regions are compared. VBA is able to compare voxels within the whole brain despite different brain sizes/shapes/orientations using unified segmentation and spatial normalization, where every participant’s brain image is spatially warped into standardised stereotactic space (Ashburner & Friston, 2000). The advantage of the VBA technique is that it requires no a priori hypotheses or assumptions on regions involved with a particular measure, whereas manually identifying regions of interest can lead to subjectivity and bias. A disadvantage is the number of voxel calculations being performed at a whole-brain level, leading to the multiple corrections problem, where a large number of voxels are bound to be found as false positives. Correction for multiple comparisons needs to be applied, although should a too stringent correction threshold be used, actual difference may not survive.

3.1.2 Summary of eye movement findings in PD

I have previously discussed eye movement findings in the introduction (see Introduction chapter page 49). To summarise, the most widely reported finding in saccadic eye movements is hypometria, or undershooting, of saccades. This is particularly evident in voluntary saccades, however, reflexive saccades show a similar undershoot, which correlates mainly with motor impairment. Reaction times for reflexive saccades are impaired in later disease stages and increase with cognitive decline (MacAskill et al., 2012). PD patients also make fast express saccades more often and have difficulty inhibiting unintended reflexive saccades when instructed not to in voluntary tasks (Chan et al., 2005). An undershooting initial saccade with a number of corrective step-like saccades is typically seen in memory-guided saccades in PD. Reaction time is slower in antisaccade tasks and more error prosaccades are made by PD patients compared to controls (Briand et al., 1999).
3.1.3 Summary of voxel-based MRI studies in PD

3.1.3.1 Structural imaging

As discussed in the introduction, reports of structural disease-related change in PD are variable, with individual differences such as disease duration or clinical subtype (i.e. tremor dominant vs impaired postural gait subtype) thought to contribute to cortical structural differences (Benninger et al., 2009; Rosenberg-Katz et al., 2013). One consistent finding is cortical atrophy/thinning in the presence of cognitive impairment and dementia. A number of studies have consistently reported temporal, frontal and parietal, along with limbic and paralimbic atrophy in PD with dementia (Burton et al., 2004; Melzer et al., 2012; Nagano-Saito et al., 2005; Ramirez-Ruiz et al., 2005; Sanchez-Castaneda et al., 2009; Summerfield et al., 2005; Weintraub et al., 2011). Cortical thickness has been found to be reduced in frontal, parietal, temporal and occipital lobes in PD with cognitive impairment (Hwang et al., 2013; Pagonabarraga et al., 2013; Pereira et al., 2014). In PD patients with mild cognitive impairment (MCI), cortical atrophy was seen in the temporal, parietal and frontal cortices, in a less extensive pattern compared to PD-dementia patients, (Beyer et al., 2007; Melzer et al., 2012). While patients with cognitive impairments (either MCI or dementia) show consistent atrophy, the evidence for cortical changes in non-dementia PD patients is inconsistent. Atrophy in non-dementia PD patients has been reported in the prefrontal and frontal lobe (Brück et al., 2004; Burton et al., 2004), temporal lobe (Beyer et al., 2007; Joana Braga Pereira et al., 2012; Summerfield et al., 2005), parietal and occipital lobe (Nishio et al., 2010). Other studies have reported no cortical volume changes in non-dementia PD patients, possibly due to non-dementia classification criteria varying between studies (Ellfolk et al., 2013; Melzer et al., 2012; Weintraub et al., 2011).

Eye movements have been shown to correlate with aspects of disease state and cognition in PD (MacAskill et al., 2012; Terao et al., 2011). Eye movements, therefore, may be a reflection of general disease and cognitive state In this study, I compare eye movement task performance to structural imaging in PD to investigate the cortical changes related to eye movement deficits in PD. This will add to our understanding of the sources of saccadic eye movement performance deficits in PD, with the overall aim of assessing and validating the relationship between eye movements and brain health.

3.1.3.2 ASL in PD

As mentioned in the introduction (page 74), ASL studies have described extensive perfusion changes in PD and support the idea that ASL changes correlate with disease severity and
cognition. A PD-related perfusion pattern has been identified, describing hypoperfusion of the parieto-occipital cortex, precuneus/cuneus and middle frontal gyri (Melzer et al., 2011). This pattern was found to correlate with scores of cognitive impairment and PD motor impairment. PD-related perfusion deficits are also seen early in the disease, with hypoperfusion seen in frontal, parietal and occipital cortices along with the caudate nucleus in non-demented PD patients (Fernandez-Seara et al., 2012).

Using manually-defined ROI around the caudate nucleus, ASL perfusion differences between opposite sides of the caudate nucleus were seen to be associated with PD disease status as measured by the Hoehn and Yahr scale (Yamashita et al., 2017). A dual analysis of ASL and cortical atrophy in PD showed a pattern of hypoperfusion in the precuneus and thinning of the parietal cortex in PD. There was, however, preserved blood flow and cortical thickness in the cingulate and frontal lobe. This pattern was found to correlate with motor symptoms in PD (Madhyastha et al., 2015).

Saccadic eye movement measures have been found to reflect elements of motor or cognitive impairment. In a number of studies, ASL changes have been related to motor symptoms and cognitive impairment. I suggest that ASL changes may relate to saccadic eye movement measures. In this chapter, I compared ASL perfusion values to eye movement performance in a voxel-wide approach in grey matter regions. This may provide insight into the relationship between functional blood flow deficits and their effects on eye movement performance abnormalities and disease severity.

3.1.3.3 DTI in PD

DTI has not been used to compare to eye movement performance, but has been compared to other metrics such as cognition and clinical severity scores. FA and MD values of the substantia nigra have been shown to correlate with disease severity as measured by the H&Y and UPDRS in some studies (Chan et al., 2007; Scherfler et al., 2013; Zhan et al., 2012), but not all (Du et al., 2011). Zhang et al., (2016) found the DTI properties (FA, along with radial and axial diffusivity) of the nigrostriatal tract to be abnormal in PD and to correspond with motor deficits assessed using UPDRS-III.

DTI studies of cortical regions have found changes in the temporal lobe and cingulate cortex, which show decreased FA and increased MD in PD (Deng et al., 2013; Price et al., 2016; Zhan et al., 2012). These changes may be related to cognitive decline (Auning et al., 2014; Kamagata et al., 2012; Rae et al., 2012), however, frontal lobe and anterior cingulate FA changes have been found in non-demented PD patients in other studies (Kamagata et al., 2012).
These inconsistencies may be attributable to the differences in populations investigated. For example, the inclusion of MCI patients within a non-dementia group may make it more likely to show abnormal DTI metrics, while samples comprising mostly PD with normal cognition, may show less severe DTI differences, or none at all. Furthermore, differing criteria for classifying mild cognitive impairment may contribute to the differences in findings (Melzer et al., 2013).

Extensive white matter changes appear to be associated with cognitive impairment in PD, with DTI measures of the corpus callosum (and the adjoining region of the anterior cingulate) in particular having been noted to correlate with cognitive state (Deng et al., 2013; Kamagata, Motoi, et al., 2013). Widespread changes in MD value throughout white matter fibre tracts have been found in even mild cognitive impairment in PD patients (Melzer et al., 2013). Using an ROI analysis of over 40 fibre tracts, both FA and MD values of numerous tracts showed correlation with cognitive measures, such as attention and executive/visual function (Zheng et al., 2014). Interestingly, a number of tracts such as the corticospinal tract and thalamus-motor cortex tract have been found to show an FA increase. This may represent some compensatory reorganisation of certain white matter tracts in PD or preferential loss of a single fibre population in an area of crossing fibres (Mole et al., 2016).

Following the Braak hypothesis (Braak et al., 2003), smell is noted to be significantly affected in PD, and changes in olfactory regions (and oddly the cerebellum) have been found using DTI, relating to olfactory dysfunction (Ibarretxe-Bilbao et al., 2010; Rolheiser et al., 2011; Scherfler et al., 2013; Zhang et al., 2011). Zhang et al. (2011) were one of the few studies to use a whole-brain voxel-wise approach to determine a correlation between a score (olfactory detection and discrimination scores) and DTI value. Other studies use tract-based analyses (Ibarretxe-Bilbao et al., 2010) or DTI values from ROIs (Scherfler et al., 2013). While it is possible the cerebellum may indirectly influence olfactory function through an as-yet-unknown mechanism, the study by Zhang and colleagues may highlight an issue with doing a whole-brain analysis, being the multiple comparisons problem. While Zhang et al. corrected for multiple comparisons (using a calculated random distribution of cluster size for $p < 0.001$ using Monte Carlo simulation), a possibility exists, due to the sheer number of statistical tests performed at a whole-brain voxel-level, that a portion of the results may be false positives, particularly for small unilateral effects as reported in the study, with no known neurological mechanism to explain the correlation between altered DTI values of the cerebellum and olfactory function. Therefore, careful interpretation must be applied when using a whole-brain voxel-based analysis.
3.1.3.4 **Voxel-based comparisons**

Previous studies have used a voxel-based group comparison to determine grey matter changes with discrete cognitive impairment category in PD (Melzer et al., 2012). In studying the cognitively-impaired PD population, the definition of groups can cause differences in results, e.g. if MCI participants are not analysed as a separate group but are included in a general “PD non-dementia” group. This may cause the “non-dementia” group to show changes due to contributions largely driven by the MCI sub-group, which would not be evident if the PD-MCI were separated out. Another voxel-based analysis method exists without the need to categorise groups. This approach compares a covariate of interest to whole-brain voxel values. This is in effect a multiple regression, with the values at every voxel tested with a regressor or covariate of interest, for example, a continuous cognitive score or measure of eye movement performance. This allows an association to be established between voxel values and the covariate, with regions showing significant association able to be displayed on a map of the brain. An advantage of this method is there is no need to define and categorise separate groups based on regressor values, e.g. classifying unimpaired, MCI and dementia groups from cognitive scoring, eliminating the variability due to different methods of group categorisation.

The patient group used in this study is from the same sample as in the study by Melzer et al. (2012). Previously, this group of patients was analysed using a group-based comparison. Here, using the same pool of PD patients, I investigated voxel-wise correlations of grey matter volume and cognitive scores using a *continuous* regressor of interest. For example, instead of using discrete categories such as PD-N, PD-MCI and PD-D, I used a continuous measure of cognition (global cognitive Z-score). I would expect to see similar regions to correlate between grey matter volume and cognitive impairment severity as those regions described as varying between cognitive groups in a voxel-based group comparison by Melzer et al. (2012).

But more importantly, this voxel-wise comparison will also be performed with eye movement measures. There is currently no established categorisation of patients for degree of saccadic eye movement impairment severity. Characteristic deficits are noted in PD, with reflexive latency and gain shown to relate to cognitive and motor components of PD. Studies using structural MRI have found correlations between cerebellar and frontal lobe grey matter volume with pro and antisaccade performance in healthy controls (Ettinger et al., 2005). No study has yet used eye movement performance measures to correlate with grey matter/ASL/DTI measures in PD. Using this VBA technique, we can compare the association between multiple MRI measures (grey matter volume, ASL, and DTI) and measures of eye
movement performance. This will assess if eye movement measures directly reflect cortical change as detected by MRI.

3.1.4 Aims of study
This initial study allows us to test the idea that eye movement performance reflects brain health in PD which may be detected using various MRI modalities. My hypothesis is that these eye movement performance changes in PD will also manifest as meaningful structural, fibre tract and perfusion changes, as assessed by structural, ASL and DTI modalities. I hypothesise these changes will be in regions known to be involved in eye movement control, namely the eye fields, showing less volume, reduced bloodflow and lower measures of intact white matter structure in PDs with impaired eye movement performance, compared to controls.

Secondly, I aim to use each MRI modality to gain a better understanding of the types of changes which may be occurring in PD that are related to eye movement deficits. Each modality provides a different type of information regarding brain structure - structural scans give insight into atrophy, ASL into perfusion and DTI into white matter fibre tract integrity. My question is which of these changes that occur in PD relate to eye movement deficits. I hypothesise any could contribute, but it may be a combination. A stronger finding would be having concordant regions show changes across various modalities. This would indicate several changes have occurred in a given area resulting in eye movement deficits, and therefore could indicate a region of interest which could be targeted in future studies. Chapter 3 is a cross-sectional study using voxel based morphometry.

3.2 Methods
3.2.1.1 Participants
A convenience sample of 96 PD patients (68 male, 28 female) and 33 controls (22 male, 11 female) were recruited for this study at the New Zealand Brain Institute matched for age and sex ratio. The average age of PD patients was 67 (range 46 to 84) with an average MDS-UPDRS-III score of 41 (range 6 to 97), average global cognitive Z-score of -0.3 (range -2.7 to 1.1) and average MoCA of 24 (range 10 to 30). The average age of controls was 69 (range 45 to 83) with an average global cognitive Z-score of 0.5 (range -0.4 to 1.5) and average MoCA of 27 (range 23 to 30).
3.2.1.2  **Cognitive and PD motor symptom testing**

Cognitive and motor symptom testing are described in Chapter 2. To briefly summarise, all participants underwent a series of cognitive tests covering cognitive domains as part of the level II criteria for PD-MCI (Litvan et al., 2012). General cognitive testing was performed using the Montreal Cognitive Assessment (MoCA). The MDS UPDRS part III (motor component) was used to assess the majority of our participants, with a scaling factor applied to scores of earlier patients scored using the previous UPDRS system, to make scores comparable MDS-UPDRS III (Goetz et al., 2008).

3.2.1.3  **Eye movements**

Eye movements were recorded using an infrared eye-tracking system as described in Chapter 2 and were previously gathered as part of an ongoing longitudinal eye-movement trial. Four eye movement tasks were analysed in this study: the reflexive, predictive, memory-guided and antisaccade tasks. These represent a spectrum from reflexive saccades (saccadic eye movements made to new unanticipated targets that appear in the environment) to volitional saccades (deliberately-made saccades to a pre-determined location).

The accuracy of the first saccade (primary gain) and reaction times (latency) were measured. Saccade gain was calculated as the ratio of the angular amplitude of the primary saccade to the angular amplitude of the target. Saccade latencies were defined as the time from stimulus occurrence until the onset of the corresponding saccade towards it.

3.2.1.4  **Reflexive task**

The reflexive task was presented via a red square target that jumped horizontally in 5, 10, 15 or 20 degree steps at an unpredictable, pseudorandom interval varying between 550 ms and 1800 ms. Participants were instructed to look at and follow this stimulus as quickly and accurately as possible. This was presented in a block of 108 trials. Within these trials, variants of the reflexive task were present, namely, the step task, gap-reflexive task and the overlap-reflexive task. The step task presents one stimulus immediately after another, with no delay in-between stimulus presentation. The gap reflexive task had a period in-between successive stimulus presentations, with a temporal “gap” of 200 ms, before the next stimulus appears. The overlap task had a period of 200 ms when the previous stimulus continued to be presented, along with the new stimulus, before the previous stimulus was extinguished. The gap and overlap variants were designed to investigate the effects of fixation disengagement on reflexive saccades which are not investigated in this current study. Consequently these
reflexive saccade variants were not included, with only the step reflexive trials used in this study.

3.2.1.5  **Predictive task**
The predictive task used an alternating red square target, which jumped at a regular intertrial interval between two fixed positions at -10 to 10 degrees visual angle from the screen centre. Three durations of inter-trial interval were used: 750 ms, 1400 ms and 2050 ms in separate blocks. This study analysed only 750 ms trials. Longer predictive inter-trial intervals become more difficult to accurately predict and become closer to visually-guided reflexive saccades performance wise. Each inter-trial interval was presented as a block of 20 trials. Only predictive saccade trials employing 750 ms duration were used in this study.

3.2.1.6  **Memory-guided task**
The memory-guided task began with an initial red square fixation stimulus. After two seconds, a peripheral “flash” was presented for 400 ms at 5, 10, 15, or 20 degrees left or right of center in the horizontal plane. Participants were instructed to maintain fixation during the flash appearance but to memorise the spatial location of the flash. At 2.5 seconds after the disappearance of the flash, the fixation stimulus was extinguished, with a simultaneous tone. This cued the participants to make a saccade to the remembered location of the flash. After 3 seconds, the position of the target “flash” stimulus was re-illuminated and formed the fixation stimulus for the next trial. The first trial began with the fixation stimulus in the centre. The next trial begins from last stimulus location (the flash location of its preceding trial). A total of 2 runs of 17 trials each were used. A 10 trial practice block was used for task familiarisation before the actual trial commenced.

3.2.1.7  **Antisaccade task**
The antisaccade trial began with a central green square fixation stimulus. After periods of 1.5 s, 2.0 s or 2.5 s, the fixation stimulus was extinguished. Simultaneously, a red square peripheral stimulus appeared at 5, 10 or 15 degrees left or right of the fixation stimulus. Participants were instructed to make a saccade to the mirror opposite position of this peripheral stimulus as quickly as possible. The reappearance of the central fixation stimulus then commenced the next trial. Participants were instructed to correct erroneous antisaccades (reflexive saccades made to the red stimulus target, instead of in the mirror opposite location) as quickly as possible. As with the memory-guided task, a 10 trial practice block was used for task familiarisation before the actual experimental task commenced. Gain was measured from the initial central fixation to the mirror opposite of the stimulus, i.e. positive gain values were
in the correct direction, away from the visible target. Error antisaccades were excluded from the analysis.

3.2.1.8 **Error exclusion**
Saccades performed less than 70 ms were classed as anticipatory saccades and were excluded from reflexive, memory-guided and antisaccade task analysis. To note, for the predictive task, saccades are often made in anticipation of the stimulus before the stimulus actually shifts, resulting in a negative latency. Hence, saccades with negative latency were not excluded from the analysis of the predictive task. Primary gain values of under 0.2 were excluded from analysis as these may represent small fixational movements, such as square wave jerks, which were not true target-related saccades. In the memory-guided task, trials with saccades which occurred during the fixation or “flash” period were considered error trials and were excluded from analysis. In the antisaccade task, saccades made to the peripheral stimulus, as opposed to the mirror opposite location, were classed as errors. Comparisons using eye movement measures were limited to primary gain and latency only. This study is a preliminary study using eye movement measures to compare to MRI measures in PD and I chose to only use these two primary measures of eye movement performance. Error saccade measurements i.e. error gain and latency were not used and all error saccades were excluded from analysis.

3.2.1.9 **MRI acquisition**
MRI scans were acquired using a 3 Tesla General Electric MRI scanner. Structural, ASL and DTI scans were acquired using the protocol described in Chapter 2.

3.2.1.10 **MRI preprocessing**
Before analysis of MRI data can take place, the raw scans must be pre-processed. This is a series of steps needed to adjust the raw data from the MRI scanner into a workable standardised form, necessary for analysis. This process is done through the program SPM8 (Wellcome Department of Cognitive Neurology, University College London, UK) – a MATLAB (7.10.0 R2010a, Mathworks, MA, USA) based toolbox specifically designed for the analysis of brain imaging data. Scans were converted to NIFTI format and reoriented using the anterior commissure as the 0,0,0 co-ordinate.

Structural: The T1 image was segmented using unified segmentation and normalization, outlined in Ashburner & Friston (2000). This process segments the white matter, grey matter and CSF using a tissue probability map from an older person atlas (Lemaître et al., 2005). This was done in order to minimise possible bias associated with age. The segmentation process produced separate grey matter, white matter and CSF files, which were normalized (to 100
Montreal Neurological Institute (MNI) space) and modulated. Modulation preserves the actual volume of a given voxel by adjusting the signal proportional to the amount of warping.

ASL: Quantified cerebral blood perfusion images (ASL) were coregistered to the T1 image, and normalized using the structurally-derived normalization parameters.

DTI: Image processing employed both FSL 4.1.6 (www.fmrib.ox.ac.uk/fsl) and SPM8. Diffusion-weighted images were motion- and eddy current distortion-corrected. The diffusion tensor was then calculated at each voxel using DTIFIT, producing fractional anisotropy (FA) and mean diffusivity (MD) images, and then brain-extracted using BET. The b0 image (as well as the FA and MD) was then coregistered to the high resolution, T1-weighted SPGR image using mutual information in SPM8. Normalization parameters produced during unified segmentation of the T1-weighted image were applied to the FA and MD images to warp them into standard space and resliced to 1 mm³ isotropic.

All normalised images were then smoothed using a Gaussian kernel with a full width at half maximum (FWHM) value of 8 × 8 × 8 mm for structural and DTI images and 10 × 10 × 10 mm for ASL images.

3.2.1.11 MRI statistical analysis
Statistical analyses of structural, ASL and DTI scans were performed using SPM8. A general linear model (GLM) was used to assess voxel-based associations between eye movement metrics (saccade latency and primary gain) and MRI metric (grey matter volume, perfusion, FA, and MD). Separate models were constructed to investigate each MRI metric with 1) latency and 2) primary gain. Patient age, sex, group (Control/PD) and intracranial volume (calculated as the summation of GM, WM, and CSF segments, included only in the T1 comparisons) were entered as covariates in each model.

Additional models were constructed to investigate cognitive Z-scores and MDS-UPDRS part III, with patient age, sex, group and intracranial volume as covariates (latency and primary gain were not included in these models). Every model was analysed for within-group and between-group differences.

3.3 Results
In this chapter, results tables from all analysis carried out are provided in the tables below, listing the findings of every comparison. Only corrected values can be considered valid results, however, it can often be useful to visualise the uncorrected (p < 0.001 in this study) results of correlation in some cases. Corrections for multiple comparisons establish a
threshold which is considered significant at a family-wise error (FWE) of $p < 0.05$. This correction, in effect, shows only the voxels having the most significant correlation, which can be mentally visualised as a “tip of the iceberg”, arising from uncorrected voxels not visible below the significance threshold. The uncorrected results can often give insight into the overall pattern of any significant correlation, allowing us to determine if significant clusters arise from randomly distributed voxels, or from a symmetrical distribution, of which only one side remains above threshold to be seen once corrected. Uncorrected results are only shown for findings where significant voxels showed a bilateral appearance and located within functionally relevant regions. These are only provided for illustrative comparison with corrected results. Result images from all the analyses cannot be reasonably displayed in the results section. In this chapter, images are only shown of voxels which were significant across a number of correlations, within areas known to be involved in eye movement control, known to be affected in PD or which occurred in a bilateral pattern. Figures of all other detected significant results, such as those seen isolated to one single correlation analysis, or located in undefined regions (often subgyral, deep within the brain, outside of known BAs and of any defined cortical/subcortical structure), can be found in the Appendix.

3.3.1 **Grey matter volume associations with eye movement measures**

Table 3-1 and Table 3-2 show regions having a significant association between grey matter volume and eye movement performance measures. In addition, Figure 3-1 and Figure 3-2 visually display voxels showing significant association as glass brain images in three planes. The significant areas refer to the presence of voxels which show correlation for that comparison which are significant after correction for multiple comparisons using family wise error at $p < 0.05$. 
Table 3-1 – Grey matter (GM) volume association with saccade latency. Dash (-) indicates no significant regions showing correlation seen.

<table>
<thead>
<tr>
<th></th>
<th>GM volume and reflexive latency</th>
<th>GM volume and predictive latency</th>
<th>GM volume and memory-guided latency</th>
<th>GM volume and antisaccade latency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive association - PD group</td>
<td>-</td>
<td>-</td>
<td>Subgyral regions (Appendix Figure 9-1)</td>
<td>-</td>
</tr>
<tr>
<td>Positive association - Control group</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Negative association - PD group</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Negative association - Control group</td>
<td>-</td>
<td>-</td>
<td>Left temporal pole (Figure 3-1 A)</td>
<td>-</td>
</tr>
<tr>
<td>PD group association more positive than control group</td>
<td>-</td>
<td>-</td>
<td>Left temporal pole (Figure 3-1 B)</td>
<td>-</td>
</tr>
<tr>
<td>Control group association more positive than PD group</td>
<td>-</td>
<td>-</td>
<td>Subgyral regions (Appendix Figure 9-2)</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 3-2 – Grey matter (GM) volume association with saccade primary gain. Dash (-) indicates no significant regions showing correlation seen.

<table>
<thead>
<tr>
<th></th>
<th>GM volume and reflexive primary gain</th>
<th>GM volume and predictive primary gain</th>
<th>GM volume and memory-guided primary gain</th>
<th>GM volume and antisaccade primary gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive association - PD group</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Positive association - Control group</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Left temporal pole (Figure 3-2 A)</td>
</tr>
<tr>
<td>Negative association - PD group</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Negative association - Control group</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PD group association more positive than control group</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Control group association more positive than PD group</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Left Brodmann area (BA) 6 (Figure 3-2 B)</td>
</tr>
</tbody>
</table>
Figure 3-1 (A) shows voxels with a negative association (i.e. decreased volume with increasing saccade latency) for the memory-guided task in the control group. Those voxels were clustered in the left temporal pole, significant at FWE p < 0.05. The same analysis within the PD group showed no significant voxels.

Figure 3-1 (B) shows voxels with significantly more negative association (i.e. decreased volume with increasing saccade latency) in the control group compared to PD for the memory-guided task. A cluster in the same region in the left temporal pole showed a significantly more negative grey matter volume association with increasing memory-guided saccade latency in the control group compared to PDs.

**Voxels showing decreases in grey matter volume relative to increasing memory-guided saccade latency in controls - within-group analysis**

(A)  

**Voxels showing decreases in grey matter volume relative to increasing memory-guided latency that were greater in controls compared to PD – between-group analysis**

(B)  

Left temporal lobe pole

Left temporal lobe pole

Figure 3-1 – Significant voxels showing association, corrected for multiple comparisons (FWE p < 0.05) for the described saccade task and group.
Figure 3-2 (A) shows voxels with significant positive grey matter volume association with increasing antisaccade gain (i.e. increased volume with increasing antisaccade gain) in the control group. Again, the left temporal pole is significant. Within-group PD correlation showed no significant voxels and a between-group comparison did not show significant group correlation differences in the temporal lobe pole.

Figure 3-2 (B) shows voxels which have a more positive association (i.e. increasing grey matter volume with increasing antisaccade gain) in the control group compared to the PD group. An alternative interpretation is that the images show voxels in the PD group showing a more negative association (decreased grey matter volume associated with increasing antisaccade gain) compared to the control group. An extremely small cluster in Brodmann area (BA) 6 was significant. In within-group analyses, no voxels in BA6 were seen to significantly correlate with antisaccade gain in PD or control groups.

Figure 3-2 – Significant voxels showing association, corrected for multiple comparisons (FWE p < 0.05) for the described saccade task and group.
3.3.2 ASL associations with eye movement measures

Table 3-3 and Table 3-4 show regions having a significant association between ASL perfusion values and saccadic eye movement performance measures. In addition, Figure 3-3 and Figure 3-4 visually display voxels showing significant association as glass brain images in three planes.

Table 3-3 – ASL perfusion association with saccade latency.

<table>
<thead>
<tr>
<th></th>
<th>ASL perfusion and reflexive latency</th>
<th>ASL perfusion and predictive latency</th>
<th>ASL perfusion and memory-guided latency</th>
<th>ASL perfusion and antisaccade latency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive association - PD group</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Positive association - Control group</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Negative association - PD group</td>
<td>-</td>
<td>-</td>
<td>Precuneus (Figure 3-3 A)</td>
<td>-</td>
</tr>
<tr>
<td>Negative association - Control group</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PD group association more positive than control group</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Control group association more positive than PD group</td>
<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
</tbody>
</table>

Table 3-4 – ASL perfusion association with saccade primary gain.

<table>
<thead>
<tr>
<th></th>
<th>ASL perfusion and reflexive primary gain</th>
<th>ASL perfusion and predictive primary gain</th>
<th>ASL perfusion and memory-guided primary gain</th>
<th>ASL perfusion and antisaccade primary gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive association - PD group</td>
<td>-</td>
<td>-</td>
<td>Bilateral caudate (Figure 3-4)</td>
<td>Precuneus (Figure 3-3 B)</td>
</tr>
<tr>
<td>Positive association - Control group</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Negative association - PD group</td>
<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>Negative association - Control group</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>PD group association more positive than control group</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Control group association more positive than PD group</td>
<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
</tbody>
</table>
Figure 3-3 (A) shows voxels which have a negative association of perfusion with memory-guided saccade latency in the PD group (i.e. decreasing perfusion associated with increasing memory-guided saccade latency). Once corrected for multiple comparisons, a cluster in the precuneus remained significant. In contrast, within-group correlation in controls showed no significant voxels and a between-group comparison did not show significant group-level correlation differences between PD and controls in the precuneus.

Figure 3-3 (B) shows voxels with positive associations of perfusion value with antisaccade gain (i.e. increasing perfusion associated with increasing antisaccade gain) in the PD group. Once corrected for multiple comparisons, a cluster in the precuneus remained significant. In contrast, within-group correlation in controls showed no significant voxels and a between-group comparison did not show significant group-level correlation differences between PD and controls in the precuneus.

*Voxels showing decreases in ASL perfusion with increasing memory-guided latency in PDs - within-group analysis*

*Voxels showing increases in ASL perfusion with increasing antisaccade gain in PDs – within-group analysis*

![Precuneus](image1)

![Precuneus](image2)

Figure 3-3 – Significant voxels showing association, corrected for multiple comparisons (FWE p < 0.05) for the described saccade task and group.
Figure 3-4 shows voxels where perfusion correlated positively with memory-guided saccade gain (i.e. increasing perfusion associated with increasing memory-guided saccade gain) for the PD group. Clusters in the caudate nucleus were seen bilaterally. No caudate voxel clusters were seen to be significantly correlating with memory-guided saccade gain within the control group and a between-group comparison did not show significant group correlation differences in the caudate nucleus.

**Voxels showing increases in ASL perfusion relative to increasing memory-guided saccade gain in PDs - within-group analysis**

![Caudate nucleus (Uncorrected)](image)

![Caudate nucleus (FWE p < 0.05)](image)

Figure 3-4 – Significant voxels showing association, corrected for multiple comparisons (FWE p < 0.05) for memory-guided saccade gain and ASL values for the PD group.
### 3.3.3 DTI - FA values associations with eye movement measures

Table 3-5 and Table 3-6 show regions having a significant association between FA values and eye movement performance measures.

**Table 3-5 – FA values association with saccade latency.**

<table>
<thead>
<tr>
<th></th>
<th>FA and reflexive latency</th>
<th>FA and predictive latency</th>
<th>FA and memory-guided latency</th>
<th>FA and antisaccade latency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive association - PD group</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Positive association - Control group</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>Negative association - PD group</td>
<td>-</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Negative association - Control group</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PD group association more positive than control group</td>
<td>-</td>
<td>-</td>
<td>Right BA 6 (Figure 3-5)</td>
<td>-</td>
</tr>
<tr>
<td>Control group association more positive than PD group</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Table 3-6 – FA values association with saccade primary gain.**

<table>
<thead>
<tr>
<th></th>
<th>FA and reflexive primary gain</th>
<th>FA and predictive primary gain</th>
<th>FA and memory-guided primary gain</th>
<th>FA and antisaccade primary gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive association - PD group</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Positive association - Control group</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Right BA 8 (Appendix Figure 9-3)</td>
</tr>
<tr>
<td>Negative association - PD group</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Negative association - Control group</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PD group association more positive than control group</td>
<td>-</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Control group association more positive than PD group</td>
<td>-</td>
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</tr>
</tbody>
</table>
Figure 3-5 shows voxels having a more positive association (i.e. increasing FA value with increasing antisaccade gain) in the PD group compared to the control group. This is equivalent to the alternative interpretation, that it shows voxels where the control group had a more negative association (decreased FA value associated with increasing memory-guided saccade latency) compared to the PD group. Once corrected for multiple comparisons, a cluster in BA6 remained significant. No within-group correlation between FA and memory-guided latency in BA6 was seen for both PD and control groups.

Voxels showing increases in FA value with increasing memory-guided saccade latency seen greater in PD compared to controls – between-group analysis

Figure 3-5 – Voxels showing significantly more positive correlation (increasing FA value with increasing memory-guided saccade latency) in the PD compared to the control group – equivalent to voxels showing a more negative correlation (decreasing FA value correlated with increasing memory-guided saccade latency) in the control group compared to the PD group. An area in Brodmann area 6 is significant for this comparison. Corrected for multiple comparisons (FWE p < 0.05).
### 3.3.4 DTI - MD values associations with eye movement measures

Table 3-7 – MD values association with saccade latency.

<table>
<thead>
<tr>
<th>Positive association - PD group</th>
<th>MD and reflexive latency</th>
<th>MD and predictive latency</th>
<th>MD and memory-guided latency</th>
<th>MD and antisaccade latency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left superior temporal gyrus</td>
<td>Left superior temporal gyrus</td>
<td>Left middle frontal gyrus</td>
<td>Left insula</td>
<td>Anterior cingulate</td>
</tr>
<tr>
<td>(Appendix Figure 9-4)</td>
<td>(Figure 3-9 A)</td>
<td>(Appendix Figure 9-5)</td>
<td></td>
<td>(Figure 3-10 B)</td>
</tr>
<tr>
<td>Positive association - Control group</td>
<td>-</td>
<td>-</td>
<td>Left temporal pole</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td></td>
<td>(Figure 3-6 A)</td>
<td>-</td>
</tr>
<tr>
<td>Negative association - PD group</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Negative association - Control group</td>
<td>-</td>
<td>-</td>
<td>Precuneus</td>
<td>-</td>
</tr>
<tr>
<td>PD group association more positive than control group</td>
<td>-</td>
<td>-</td>
<td>Precuneus</td>
<td>-</td>
</tr>
<tr>
<td>Control group association more positive than PD group</td>
<td>-</td>
<td>-</td>
<td>Left temporal pole</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(Figure 3-6 B)</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 3-8 – DTI MD values association with saccade primary gain.

<table>
<thead>
<tr>
<th>Positive association - PD group</th>
<th>MD and reflexive primary gain</th>
<th>MD and predictive primary gain</th>
<th>MD and memory-guided primary gain</th>
<th>MD and antisaccade primary gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undefined interhemispheric region</td>
<td>Left temporal pole (Figure 3-9 B)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Appendix Figure 9-6)</td>
<td></td>
<td></td>
<td>Left temporal pole</td>
<td>Left BA 6 (Figure 3-10 A)</td>
</tr>
<tr>
<td>Positive association - Control group</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Left BA 6 (Figure 3-10 A)</td>
</tr>
<tr>
<td>Negative association - PD group</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Left BA 6 (Figure 3-10 A)</td>
</tr>
<tr>
<td>Negative association - Control group</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Left BA 6 (Figure 3-10 A)</td>
</tr>
<tr>
<td>PD group association more positive than control group</td>
<td>-</td>
<td>-</td>
<td>Left temporal pole</td>
<td>Left temporal pole (Figure 3-7 B)</td>
</tr>
<tr>
<td>Control group association more positive than PD group</td>
<td>-</td>
<td>-</td>
<td>Left temporal pole</td>
<td>Left temporal pole (Figure 3-7 B)</td>
</tr>
<tr>
<td></td>
<td>Left BA 17 (Appendix Figure 9-8)</td>
<td>-</td>
<td></td>
<td>Left BA 6 (Figure 3-10 A)</td>
</tr>
</tbody>
</table>
Table 3-7 and Table 3-8 show regions having a significant association between MD values and saccadic eye movement performance measures. Figure 3-6 to Figure 3-16 display voxels showing significant association as glass brain images in three planes.

Figure 3-6 (A) shows voxels having a positive association between MD value and memory-guided saccade latency (i.e. increased MD value with increasing memory-guided saccade latency) in the control group. Once corrected for multiple comparisons, a cluster in the left temporal pole remained significant. Additionally, smaller clusters showing correlation are visible in the right superior temporal gyrus, right middle temporal gyrus and right superior frontal. The same analysis within the PD group showed correlation in the left insula, with no correlation in the left temporal pole (see Appendix Figure 9-5).

Figure 3-6 (B) - shows a group comparison between PD and controls. This figure shows where there was significantly more positive association (i.e. increased MD value with increasing memory-guided saccade latency) in the control group compared to the PD group. Once corrected for multiple comparisons, a cluster in the left temporal pole remained significant. Less prominent clusters showing the same correlation group difference are visible in the right superior temporal gyrus, right middle temporal gyrus and right superior frontal gyrus.

Voxels showing increases in MD value with increasing memory-guided saccade latency in controls - within group analysis

Voxels showing increases in MD value with increasing memory-guided saccade latency seen greater in controls compared to PD – between-group analysis

Figure 3-6 – Significant voxels showing association, corrected for multiple comparisons (FWE p < 0.05) for the described saccade task and group.
Figure 3-7 (A) shows the voxels having a significant negative association between MD value and antisaccade gain (i.e. decreasing MD associated with increasing antisaccade gain) in the control group. Once corrected for multiple comparisons, a cluster in the left temporal pole remained significant. The same analysis within the PD group showed no significant clusters for this correlation.

Figure 3-7 (B) shows a group comparison between PD and controls. These images show where there was a more negative association of MD value with antisaccade gain (i.e. decreased MD value with increasing antisaccade gain) in the control group compared to the PD group. Once corrected for multiple comparisons, a cluster in the left temporal pole remained significant.

**Voxels showing decreases in MD value with increasing antisaccade gain in controls – within-group analysis**

(A)

**Voxels showing decreases in MD value with increasing antisaccade gain seen greater in controls compared to PD – between-group analysis**

(B)

Left temporal lobe pole

Left temporal lobe pole

Figure 3-7 – Significant voxels showing association, corrected for multiple comparisons (FWE p < 0.05) for the described saccade task and group.
Figure 3-8 (A) shows voxels having a significant negative association between MD values and memory-guided saccade latency (i.e. decreasing MD with increasing memory-guided saccade latency) in the control group. Once corrected for multiple comparisons, a cluster in the precuneus remained significant. The same analysis within the PD group showed no significant voxels for this correlation.

Figure 3-8 (B) shows a group comparison between PD and controls. These images show where there was a more negative association with memory-guided saccade latency (i.e. decreasing MD value with increasing memory-guided saccade latency) in the control group compared to the PD group. Once corrected for multiple comparisons, a cluster in the precuneus remained significant.

**Figure 3-8 – Significant voxels showing association, corrected for multiple comparisons (FWE p < 0.05) for the described saccade task and group.**
Figure 3-9 (A) shows voxels having a significant positive MD value correlation with predictive saccade latency (i.e. increasing MD with increasing predictive latency) for the PD group. Once corrected for multiple comparisons, a cluster in the left middle frontal gyrus remained significant. The same analysis within the control group showed no significant voxels for the same correlation and similarly, a between-group comparison did not show significant group-level correlation differences.

Figure 3-9 (B) shows voxels having a positive association with antisaccade gain (i.e. increasing MD with increasing antisaccade gain) in the control group. Once corrected for multiple comparisons, a cluster in the left BA6 remained significant. The same analysis within the PD group showed no significant voxels for this correlation.

Figure 3-9 – Significant voxels showing association, corrected for multiple comparisons (FWE p < 0.05) for the described saccade task and group.
Figure 3-10 (A) shows a group comparison between PD and controls. These images show voxels having a more positive association with antisaccade gain (i.e. increasing MD value associated with increasing antisaccade gain) in the control group compared to the PD group. Once corrected for multiple comparisons, a cluster in the left BA 6 remained significant.

Figure 3-10 (B) shows voxels having a positive association with antisaccade latency (i.e. increasing MD with increasing antisaccade latency) in the PD group. Once corrected for multiple comparisons, a cluster in the anterior cingulate remained significant. The same analysis within the control group showed no significant clusters and a between-group comparison did not show significant group-level correlation differences for this correlation.

Figure 3-10 – Significant voxels showing association, corrected for multiple comparisons (FWE p < 0.05) for the described saccade task and group.
Figure 3-11 shows uncorrected (A) and corrected (B) voxels which have a negative association with predictive saccade gain (i.e. decreasing MD with increasing predictive saccade gain) in the control group. Uncorrected images are shown to illustrate the bilateral appearance with the more lenient threshold criteria. In the uncorrected images, bilateral cerebellum clusters show correlation between MD values and predictive gain. Once corrected for multiple comparisons, only the left cerebellum cluster remained significant. The same analysis within in the control group showed no significant voxels. A group comparison showed a significant difference in the cerebellum cluster correlation between PD and control groups (See Figure 3-12).

Voxels showing decreases in MD value with increasing predictive saccade gain in controls – within-group analysis

Figure 3-11 – Significant voxels showing a negative association (decreasing MD value with increasing predictive saccade gain) in the control group. Left set of images (A) show a glass brain representative of uncorrected voxel of correlation with right set of images (B) show voxels for the same correlation corrected for multiple comparisons (FWE p < 0.05).
Figure 3-12 shows uncorrected (left) and corrected (right) group comparisons between PD and controls. Uncorrected images are again shown to illustrate the bilateral cluster appearance with the more lenient threshold criterion. These images show voxels having a more negative association with predictive saccade gain (i.e. decreased MD value associated with increasing predictive saccade gain) in the control group compared to the PD group. Once corrected for multiple comparisons, only a cluster in the left cerebellum remained significant.

*Voxels showing decreases in MD values with increasing predictive saccade gain seen greater in controls compared to PD – Between-group analysis*

![Voxels showing decreases in MD values with increasing predictive saccade gain seen greater in controls compared to PD – Between-group analysis](image)

Figure 3-12 – Voxels showing significantly more negative association (decreasing DTI MD value with increasing predictive saccade gain) in the control group compared to the PD group. Left set of images (A) show a glass brain with uncorrected voxels. Right images (B) show voxels for the same correlation corrected for multiple comparisons (FWE p < 0.05).
3.3.5 Imaging measure associations with cognitive Z score

Table 3-9 and Figure 3-13 to Figure 3-15 display results of voxel-based analyses of cognitive Z-score correlation with imaging measures. Findings of correlation from undefined subgyral regions are provided in the Appendix.

Table 3-9 – Grey matter, ASL and DTI voxel value association with cognitive Z-score.

<table>
<thead>
<tr>
<th>Positive association - PD group</th>
<th>Grey matter volume and cognitive Z-score</th>
<th>ASL perfusion and cognitive Z-score</th>
<th>DTI FA and cognitive Z-score</th>
<th>DTI MD and cognitive Z-score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left BA 6 and Right BA 44 (Figure 3-13)</td>
<td>Precuneus (Figure 3-14)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Positive association - Control group</th>
<th>Grey matter volume and cognitive Z-score</th>
<th>ASL perfusion and cognitive Z-score</th>
<th>DTI FA and cognitive Z-score</th>
<th>DTI MD and cognitive Z-score</th>
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<th>Negative association - PD group</th>
<th>Grey matter volume and cognitive Z-score</th>
<th>ASL perfusion and cognitive Z-score</th>
<th>DTI FA and cognitive Z-score</th>
<th>DTI MD and cognitive Z-score</th>
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<td>Subgyral region (Appendix Figure 9-9)</td>
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<td>Scattered frontal and temporal regions (Figure 3-15)</td>
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<th>Negative association - Control group</th>
<th>Grey matter volume and cognitive Z-score</th>
<th>ASL perfusion and cognitive Z-score</th>
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<th>PD group association more positive than control group</th>
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<th>Control group association more positive than PD group</th>
<th>Grey matter volume and cognitive Z-score</th>
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Figure 3-13 shows voxels which have a positive association with cognitive Z-score (increasing grey matter volume with increasing cognitive Z-score) in the PD group. Once corrected for multiple comparisons, clusters within the left BA 6 and right BA 44 remained significant. The same analysis in the control group showed no significant clusters. No group difference was found in a between-group comparison between PD and controls.

_Voxels showing increases in grey matter volume with increasing cognitive Z-score in PDs – within-group analysis_

Figure 3-13 – Significant voxels showing a positive association (increasing grey matter volume with increasing cognitive Z-score) in the PD group. Images corrected for multiple comparisons (FWE p < 0.05).
Figure 3-14 shows voxels having a positive association with cognitive Z-score (i.e. increasing ASL perfusion with increasing cognitive Z-score) in the PD group. A cluster within the precuneus, along a much smaller cluster in BA 19, were seen to be significant. The same analysis in the control group showed no significant voxels. No group difference was found in a between-group comparison between PD and controls.

Voxels showing increases in ASL perfusion with increasing cognitive Z-score in PDs – within-group analysis

![Images of significant voxels showing positive association](image)

Precuneus
Brodmann area 19

Figure 3-14 – Significant voxels showing a positive association (increasing ASL perfusion correlated with increasing cognitive Z-score) in the PD group. Images corrected for multiple comparisons (FWE p < 0.05).
Figure 3-15 shows voxels having a negative association with cognitive Z-score (i.e. decreasing MD values with increasing cognitive Z-score) in the PD group. Small, scattered clusters in the temporal and frontal lobe were seen to be significant. The same analysis in the control group showed no significant voxels. No group difference was found in a between-group comparison between PD and controls.

*Voxels showing decreases in MD value with increasing cognitive Z-score in PDs – within-group analysis*

![Images](image)

Superior temporal gyrus

Brodmann areas 1, 21, 26 and 37

Figure 3-15 – Significant voxels of negative association (decreasing DTI MD values correlated with increasing cognitive Z-score) in the PD group. Images corrected for multiple comparisons (FWE p < 0.05). A number of clusters within the temporal and frontal lobes were seen to show this significant negative correlation.
3.3.6 Imaging measure associations with MDS-UPDRS part III score

Table 3-10 shows the results of a voxel-based analysis of MDS-UPDRS part III with structural and ASL and DTI scanning data. Figures of voxels showing significant correlation in undefined subgyral regions are provided in the Appendix.

Table 3-10 – Grey matter, ASL and DTI voxel value association with MDS-UPDRS part III.

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<th></th>
<th>Grey matter volume and MDS-UPDRS part III</th>
<th>ASL perfusion and MDS-UPDRS part III</th>
<th>DTI FA and MDS-UPDRS part III</th>
<th>DTI MD and MDS-UPDRS part III</th>
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<tr>
<td>Positive association - PD group</td>
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<tr>
<td>Negative association - PD group</td>
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<td>Undefined subgyral regions (Appendix Figure 9-10)</td>
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Aside from a single cluster in subgyral regions deep within the cortex, not within defined BAs or cortical/subcortical structures (see Appendix Figure 9-10), no voxels were seen to show correlation between imaging measures and MDS-UPDRS part III score.

3.4 Discussion

Significant correlation was seen between multiple MRI modalities and saccadic eye movement measures in both PD and controls. These regions included the left temporal pole, caudate nucleus, precuneus, anterior cingulate and cerebellum. Other smaller regions throughout the cortex and in subgyral regions (not within defined BAs or defined cortical/subcortical regions) were also seen to correlate in isolated, single analyses (see Appendix for results from all comparisons). What makes findings from the mentioned regions more likely indicative of true correlation are:

- A bilateral appearance where relevant.
- The repeated finding of correlation across multiple MRI scan types or comparisons.
- Findings in regions known to be affected in PD or involved in eye movement control.
- Associations in an expected direction e.g. lower perfusion correlating with poorer eye movement performance measures.
Conversely, individual regions showing correlation in a single result only, may raise suspicions that they result from the multiple comparisons problem. The voxel-based analysis technique used in this study carried out large numbers of statistical tests at an individual voxel level (see multiple comparisons problem in the Introduction chapter (page 68)). In addition, as previously mentioned, nearly 200 eye movement and imaging measure comparisons were carried out in total in this study. Despite our efforts to control this with correction for multiple comparisons, results must be carefully interpreted to avoid ascribing function to single small clusters, which are significant, but may be false positives. However, we must also look at the wider picture and look for consistent patterns across the multiple analyses.

3.4.1 Left temporal lobe pole

The left temporal pole showed an association between memory-guided latency in both grey matter volume and MD for the control group but not in PD. Similarly, both grey matter and MD value correlation was seen with antisaccade gain within the control group but not in PD. The left temporal pole is not classically considered an eye movement control region, but has been described to have an integrative function, with connectivity studies revealing left temporal pole connections to widespread cortical and subcortical regions, including regions involving working memory and executive functions, such as the DLPFC, parahippocampal cortex and hippocampus (Pascual et al., 2015). This may explain temporal pole volume and diffusion correlations with the saccade tasks that require some degree of executive function as in the memory-guided and antisaccade tasks. The results show only the control patients, not PD patients, having an association between temporal pole MRI values with eye movement performance. As the correlation found does not indicate impairment, rather only that an association exists between recorded MRI values and saccade performance in one group, which is significantly different from the other, we cannot directly infer that a particular group has temporal pole dysfunction from these results. However, this may be tested with a voxel-based morphology (VBM) analysis, using the same participant sample in a future study. We can speculate, however, that from impairments in eye movement performance recorded in PD for both memory-guided and antisaccade tasks, that regions involved in these eye movement tasks may be impaired in PD. Supporting the idea of impaired temporal lobe function in PD are studies showing grey matter atrophy in the temporal lobe (Burton et al., 2004; Melzer et al., 2012; Nagano-Saito et al., 2005; Summerfield et al., 2005) and temporal lobe deficits in DTI in PD (Auning et al., 2014; Deng et al., 2013; Rae et al., 2012). Particularly relevant is the study by Melzer and colleagues, who showed large areas of temporal lobe atrophy in PD-
dementia patients using a similar patient sample as this study. Should cortical degenerative or cortical reorganisation processes in PD lead to less temporal pole involvement in the memory and antisaccade tasks, this may show as a non-significant association between temporal pole MRI values and saccade measures compared to unaffected controls as seen in this study. It should be noted this region lies on the boundary of brain at the very tip of the temporal lobe. A potential possibility is that inaccuracies in segmentation may have some contribution to this finding (see sections 2.5 and also 3.4.9). The discussion above is speculative and I stress again that the temporal lobe pole currently is not known to contribute to saccade function. Furthermore, this finding is seen only unilaterally, whereas most saccade regions are bilateral. Despite left temporal lobe pole findings in multiple modalities, these issues do raise the possibility that left temporal pole findings are artefact driven.

3.4.2 Precuneus

ASL blood flow values to the precuneus were seen to negatively correlate with memory-guided latency within the PD group, i.e. lower blood flow was associated with higher latency. This was not observed in the control group, and a between-group comparison showed no significant group difference. Higher ASL blood flow to the precuneus was also seen to correlate with increasing gain in the antisaccade task in the PD group. This correlation was again not observed in the control group with a between-group comparison showing no significant group difference.

The precuneus is commonly activated in functional imaging and is thought to be integrated with a wide range of activities, including visuospatial processing and episodic memory recall (Cavanna & Trimble, 2006). The precuneus is also involved with a number of networks including the resting-state default-mode network and fronto-parietal attention network (Utevsky, Smith, & Huettel, 2014). Either directly or indirectly through network connections, the precuneus is likely to be involved in some form with memory-guided saccades and antisaccades, with both tasks containing visuospatial processing and attention elements. Memory-guided saccades in PD will be examined in Chapter 6 later in this thesis using fMRI. The ASL-derived PD-related perfusion pattern was characterised by precuneus hypoperfusion in PD (Melzer et al., 2011). The correlation detected in precuneus perfusion with memory-guided and antisaccade performance in PD, but not in controls, could potentially reflect a broader range of precuneus perfusion values in PD, which is not seen in controls. The ASL network score states the expression of this perfusion pattern correlates with disease severity. Hence, with a spread of PD disease severity of the patients in this study, we would expect
precuneus perfusion values to span a greater range in the PD group than controls. Having this range, along with a greater range of impairments in saccade performance may allow a correlation to be detected in the PD group with eye movement performance more easily, whereas a tighter perfusion range and performance in controls may be too narrow to detect the same correlation. However, between-group differences in precuneus and eye movement correlation were not significant so this remains speculation.

Conversely, a relationship between MD values and memory-guided saccade latency was seen in the precuneus in controls, so performance variability across subjects is sufficient to detect relationships. This relationship was negative (i.e. increasing MD with decreasing latency) and did remain significant in a between-group comparison. Increasing MD values indicate a higher level of diffusion, which indicates that more water diffusion in the precuneus is related to lower latencies in the control group. This is an unusual result, as higher MD values usually indicate cell damage, which should not correspond with decreased saccade latency, or a better reaction time. This result cannot be easily explained, however, it is known that DTI values are highly sensitive to preprocessing steps such as normalisation. This will be discussed in more detail in a section below, but DTI values can vary greatly between different tissue types, e.g. white matter and CSF. If normalisation is not accurate or distorted by the presence of atrophy, a small misalignment, particularly around tissue transition zones, can give large changes in diffusion values. The precuneus borders the CSF between the two brain hemispheres and has been shown to atrophy in patients with early Alzheimer’s (Karas et al., 2007). Should cognitively-impaired controls show precuneus atrophy, this may affect normalization, potentially giving falsely positive results. The interpretation of MD value is also not necessarily straightforward. A higher MD, for example, may not necessarily indicate cell damage - a study into cortical lesions in multiple sclerosis demonstrated that acute lesions showed higher MD whereas chronic lesions, in fact, showed mildly reduced MD values (Tievsky et al., 1999). Hence increased MD values do not always imply detrimental microstructure changes. Seeing MD correlation in controls with eye movement measures and not PD patients could indicate the precuneus is more involved in saccadic eye movements in the control group compared to the PD group. However, without being sure of the pathological microstructure changes in PD and how this relates to MD value changes, it is difficult to deduce further conclusions. This result, showing a counterintuitive direction of correlation, raises suspicion this result may have arisen by chance or noise i.e. the multiple comparisons problem.
3.4.3 Caudate

Focal perfusion changes to bilateral regions in the head of the caudate nucleus were seen to be positively associated with memory-guided saccade gain in the PD group - that is, increased perfusion to the caudate head corresponded to an increase in memory-guided saccade amplitude. The caudate nucleus has been reported to be involved in memory-guided saccades from a previous lesion study (Vermersch et al., 1999), which showed a patient with a caudate lesion generated memory-guided saccades of reduced gain. This lesion was mainly located in the body of the caudate and it was suggested the body of the caudate is involved in a network controlling visuospatial memory. Supporting this are studies showing connections of the caudate to regions thought to be involved in visuospatial memory – the DLPFC has connections with the body and head of the caudate nucleus (Yeterian & Pandya, 1991) and FEF has connections to the body of caudate, rostral to the genu (Stanton et al., 1988). Caudate neurons have been seen to discharge for memory-guided saccades (Hikosaka et al., 1989), which is thought to indicate that the caudate is part of a mechanism for initiating saccades under a memory or learnt context (Hikosaka et al., 1989).

As noted previously, the correlation seen in our study does not directly indicate impairment, only that the perfusion values of a region significantly correlated with a saccade measure. However, if the correlation were to reflect a range of caudate function ranging from aberrant to normal in PD, providing the spread in perfusion values needed to see a significant correlation with an eye movement measure, this may potentially indicate abnormal caudate function in PD. This effect was not seen in control groups and a between-group comparison between controls and PD yielded no significant difference. As a result, the discussion of potential caudate dysfunction in PD compared to controls remains speculative at this stage. Future studies investigating differences in the caudate nucleus between PD and controls may consider a more targeted method, such as an ROI analysis, to further investigate.

3.4.4 Anterior cingulate

Higher MD diffusion values in the anterior cingulate were seen to correlate with longer antisaccade latency in the PD group. This was not seen in the control group and a between-group analysis found no group difference. Cingulate cortex correlation between predictive latency and MD values can also be seen in the PD group, but only in uncorrected data. Anterior cingulate DTI value changes have been previously reported in other studies with the progression of cognitive impairment in PD (Deng et al., 2013) and the cingulate gyrus was found to be associated with the attention domain in cognitive testing (Zheng et al., 2014).
Impaired attention processing in PD could possibly prolong antisaccade latency, and is a possible mechanism by which higher MD values, should increasing MD values indicate cell damage, correlate with prolonged antisaccade latency. No significant group difference in cingulate and eye movement performance was found, so this is speculative.

### 3.4.5 Cerebellum

Once corrected for multiple comparisons, unilateral (left) cerebellar tonsil MD values negatively correlated with predictive saccade gain in the control group. That is, a higher MD value was associated with a decreased gain value. The uncorrected findings, however, show a bilateral correlation pattern in the cerebellum. The PD group did not show this correlation in the same task and a between-group comparison showed a significant group difference. Cerebellum volume association has been reported in VBA of prosaccade eye movements in healthy controls (Ulrich Ettinger et al., 2005). The cerebellum itself is thought to be involved in predictive movements (Miall, 1998) including predictive saccades (Lee et al., 2016). A possible explanation for these findings is that PD-related neural changes lead to decreased involvement of the cerebellum in predictive saccades. This, in turn, may lead to no significant correlation in DTI MD values with predictive saccade performance, whereas in unaffected controls, changes in cerebellar cellular structures may negatively influence predictive saccade performance, which are reflected by increasing MD values correlating with impaired predictive saccade gain.

### 3.4.6 BA 6 and middle frontal gyrus

DTI MD and FA diffusion values were seen to correlate with saccade performance measures in small unilateral clusters within BA 6 in a number of comparisons between MD values within-groups. Between-group association differences (but not within-group correlation) with eye movement performance were detected using FA and grey-matter volume - also in small unilateral FEF clusters These results may be expected, as BA 6 contains the human FEF (Vernet et al., 2014), which is known to be involved with nearly all saccadic eye movement tasks. In fact, it is surprising to note that these areas were not seen to correlate to a greater extent, or in a more bilateral pattern. If these small correlated regions were to represent a subtle subthreshold correlation, we may expect the uncorrected data, which has a lower threshold (but as a result, shows more noise), to show a pattern involving more widespread and bilateral FEF correlation with eye movement measures. This was not observed, with the significant regions arising from single unilateral clusters, even in the uncorrected data. Findings of small clusters in isolated, single correlations make it conceivable these findings are from noise or are false positives rather from true correlations. Should these findings reflect
actual correlation however, a mechanism for this may be that compromised cellular integrity from disease processes in PD results in increased MD, decreased FA or decreased grey matter volume. These altered metrics may in turn correlate with impaired saccade performance measures.

For example, increased MD values are usually thought to indicate decreased cellular integrity, which should correlate with more impaired saccade performance, however, as previously mentioned, MD values may not always indicate cellular structure deficits. Increasing MD correlated with increasing antisaccade gain in controls. From eye movement measures in the laboratory, the average antisaccade gain was 0.96 for the control group, indicating the control group did not tend to overshoot, and a higher gain (up to 1.0) indicates more accurate saccades. Therefore, it is unlikely that an increased MD value is associated with increased cellular damage, as this should not correlate with increased antisaccade accuracy. A remote possibility remains that microstructure changes, not necessarily detrimental, causes an increased MD value, and also correlates with improved antisaccade accuracy within a small unilateral cluster in BA 6. More plausibly however, this result may represent a false positive finding. The small and unilateral nature of this finding supports this conclusion, and the location of the cluster, even in uncorrected images, show that this result arises from a single unilateral cluster in BA 6, more posterior than where we would expect the BA 6 eye control region (the FEF) to be located.

3.4.7 Other areas
Other comparisons did yield small areas of statistically significant voxel value correlation with eye movement performance. In one-off comparisons, significant regions were seen in BA 27, BA 17, superior temporal gyrus and insula (see Appendix). A number of comparisons showed voxels associated with eye movement performance located in undefined subgyral regions, which are uninterpretable using the current VBA method. These results possibly arise from inaccuracies in computer automated segmentation in grey matter structural scans, normalisation inaccuracies in DTI scans, or could be false positives as a result of multiple comparisons. Scattered subgyral DTI value changes could also represent white matter microstructure changes in PD, commonly reported with cognitive impairment (Auning et al., 2014; Rae et al., 2012), which would require a separate tract-based analysis to detect - this could be the focus of future studies. We have only discussed regions which have been observed in more than one comparison, show in a bilateral pattern, are from regions previously described to be affected in PD, or known to be involved in eye movement control. These regions, seen in in isolated, single correlations, were of small unilateral clusters, and
When observing the uncorrected results, appeared to arise from clusters of an asymmetrical distribution from no known regions of eye movement control. Hence we must be cautious in our interpretation of significant results seen in one-off comparisons as these findings are likely to represent error/noise or a false positive.

**3.4.8 Regions of significant association with Z score and MDS-UPDRS measures**

We tested the sensitivity of the VBA method by using the cognitive Z-score and MDS-UPDRS part III score as a covariate of interest. This acted as a sort of reference level to compare to the results of our oculomotor measure analysis. Past studies have shown extensive grey matter volume and DTI changes in VBA group comparisons once patient groups were classed into separate cognitive groups, e.g. PD- unaffected, PD-MCI and PD-dementia (Burton et al., 2004; Hattori et al., 2012; Melzer et al., 2012; Nagano-Saito et al., 2005).

We would, therefore, expect regions seen to be affected in cognitive decline in PD to be correlated with the cognitive Z-score. Results from this current VBA correlational approach found only small scattered clusters of voxel correlation with both cognitive Z-score and MDS-UPDRS part III score. In a grey matter volume comparison with cognitive Z-score, the PD group showed small clusters in BA6 and BA44 which showed positive correlation between grey matter volume and cognitive Z-score. No regions of association were seen in controls and no group difference was detected. ASL scans showed a significant positive correlation between precuneus blood flow and cognitive Z-score within the PD group, with no significant correlation in the control group and no significant between-group difference. DTI MD scans showed scattered clusters negatively correlating with cognitive Z-score within the frontal and temporal lobes. These DTI MD correlations were not seen in the control group and no significant group difference was detected. Using a similar group of patients, widespread frontal and temporal lobe atrophy was found by (Melzer et al., 2012) once the patients were classified into PD-unaffected, PD-MCI and PD-dementia groups and compared between groups. The ASL network score, also using a similar patient group, developed by Melzer and colleagues describes precuneus hypoperfusion in PD (Melzer et al., 2011). What was not observed using a VBA in this study was the extent of the regions described as being affected in PD by the ASL network score (Melzer et al., 2011) or from cognitive group comparisons in PD (Melzer et al., 2012). Instead, comparisons between voxel values and global cognitive Z-score yielded low numbers of small clusters showing significant correlation in single groups and with no significant group difference. Similarly, voxel-based analysis of the MDS-UPDRS part III score with structural ASL and DTI scans found one small subgyral cluster showing correlation, located outside of defined regions or BAs, likely a false positive. Not being able
to detect the same extent of atrophy or blood flow changes with a voxel-based correlational approach suggests that this method is a less sensitive technique for detecting all changes when compared to a VBA group-based comparison. This can potentially be addressed in future studies. Should saccade performance measures be further developed into a group classification system for PD patients based on eye movement impairment (similar to cognitive scoring and MCI/dementia groups), future studies could compare eye movement impairment groups using a VBA group-based comparison, which could, in turn, be compared with our current results.

3.4.9 VBA limitations

VBA has a number of known limitations. The normalization process or the spatial warping of brains of different size can lead to analysis errors in regions where variance is high. A disease state may further increase the chance of normalisation errors. For example, small regions such as the hippocampus have high variability in disease and dementia groups, which may decrease sensitivity to changes in these regions (Burton et al., 2004). Segmentation is another pre-processing step which can potentially induce errors. Segmentation is an automated process which separates grey matter, white matter and CSF-based on tissue probability maps. In the elderly population, grey matter volume declines while CSF increases. This may be accounted for by using an elderly-specific tissue probability template for the segmentation step, however, it may still be possible for incorrect tissue types to be segmented for a severely atrophied or diseased population. Burton et al. (2004) noted in the PD dementia population, even when using elderly specific templates, grey matter “atrophy” was detected in regions in the occipital lobe that lay very close to the skull, making it possible that other tissue types on the inner skull surface were included during segmentation for this group. Other anatomical factors, such as large ventricle size, were also thought to lead to possible CSF inclusion after segmentation in deep subcortical nuclei (Good et al., 2001).

Other types of analyses, such as using ROI, may be more suited for detecting subtle changes in smaller regions. Schwarz et al. (2013) have found a number of studies that detected significant changes in subcortical nuclei using only an ROI approach, and not with a whole-brain VBA analysis. ROI analysis involves defining a specific region rather than running a statistical test on every voxel in the whole-brain. The advantage to the ROI method is a reduction in the multiple comparisons problem, due to fewer statistical tests being run. The counter-argument, however, is that if an actual significant difference exists, they should be expected to show in the whole-brain analysis without potentially biasing results by pre-selecting regions. Previous studies using VBA have, however, failed to detect PD group
changes in smaller subcortical structures such as the basal ganglia in relatively unimpaired PD patients, but these were able to be detected with manual ROI tracing methods (Lee et al., 2011; Menke et al., 2014). Likewise, Rae et al. (2012) reported ROI analyses using tract-based statistics was able to detect DTI changes that VBA was unable to detect, unless having to resort to a more liberal threshold criteria. In this current study, using a relatively large number of participants, we were able to detect differences in voxel value and eye movement performance correlation in a number of regions using a voxel-based analysis approach and a strict family-wise error correction. An ROI approach could be used in the future to further investigate the regions found in this current study in an independent sample.

3.4.9.1 DTI limitations

The pattern of correlation in DTI FA data was seen to form an unusual pattern in uncorrected results, often just on the very edges of the brain (Figure 3-16). These did not fit into any defined cortical regions and were not significant after adjusting for multiple comparisons. I am highly suspicions the findings shown in Figure 3-16 arise from artefacts and use this figure to demonstrate the possibility of error.

*Regions showing correlation between FA and reflexive latency*

*Regions showing correlation between FA and predictive latency*

![Image showing correlation patterns](image)

Figure 3-16 – Example of the artefact pattern seen in the uncorrected DTI FA images. “Significant” regions are all located on the very edges of the grey matter and in CSF transition zones.

DTI has particular issues regarding normalisation and analysis. The nature of the diffusion of water within the brain, being directed by cellular structures, means that on the boundaries between tissue types, there can be large variations in diffusion measures over very small distances. This may explain the peculiar patterns formed from regions of “correlation”
between DTI measures and eye movement performance. Due to the issues mentioned above, DTI imaging is sensitive to normalisation. If normalization is not accurately achieved, large changes can be observed due to misalignment of close regions, with large signal changes. Cortical atrophy in the diseased population could lead to distortions during computer driven normalisation. This could potentially cause a misalignment of areas of high diffusivity and low diffusivity, particularly on the boundaries of tissue transition, which may give rise to the patterns as “significant” regions, but do not actually correspond with eye movement control.

Due to these issues, techniques such as tract-based spatial statistics (Smith et al., 2006) have been developed to improve the sensitivity of group DTI analysis, by using a mean fibre tract skeleton of all patients, onto which FA values are projected. The advantage of this technique is that it reduces the impact of misalignment. This may be an option to explore for future DTI-related analysis.

### 3.4.10 Discussion - Study aims

These findings address the first aim of this investigation, detecting areas showing correlation between voxel value and eye movement performance. These correlations however occur in areas not explicitly known to be involved in eye movement control and were located in regions such as the temporal lobe and precuneus. It may be possible these areas make indirect contributions to eye movements, however the possibility of these being a type 1 error (i.e. a false positive) is also discussed. The lack of significant changes in the eye fields, and a lack of significant group difference between PD and controls, argue against my initial hypothesis that adverse changes in eye fields will be greater in PD compared to controls. With the relatively large numbers of participants used in this study, it is likely that any disease-related structural or perfusion changes in the brain within the eye fields are small and unable to be detected using the current voxel-based analysis technique. Suggestions for improvements for future studies are discussed in section 3.4.9, entitled “VBA limitations”.

The areas found in these results are also able to satisfy the second question of allowing comparison of different imaging modalities to gain more insight into affected areas. The use of multiple modalities gives a more complete picture of possible sources of dysfunction within the brain, which a single modality is not able to. The use of multiple modalities in current imaging research allows measure of different aspects of a sequential disease process. For example, we have reason to believe that white matter fibre tract changes would precede cortical atrophy - studies using very similar patient groups have found microstructural white
matter changes in early non-dementia PD (Melzer et al., 2013) and structural changes manifesting most clearly in late, demented PD states.

In this study, aside from the temporal lobe, there was little crossover in findings between the different MRI modalities used. This demonstrates that findings from one modality which correlate with eye movement performance may not necessarily be demonstrated in another. For example, areas showing perfusion deficits with impaired eye movement performance did not consistently show atrophy or fibre tract changes in the same regions. This does not necessarily mean a conflicting result, as each scan type gives different information. Overall, as mentioned previously, from the limited areas found to correlate with eye movement performance within our large PD sample, it is likely that any disease related brain changes which can be reflected by eye movement performance are small and poorly-detected using the current VBA technique.

3.5 Conclusions
Significant areas of correlation in the left temporal pole, caudate nucleus, precuneus, anterior cingulate and cerebellum were seen in PD and control groups for a number of saccade tasks. Group differences in correlation were detected in the left temporal pole, precuneus and cerebellum. From the large number of participants in our study, findings of relatively few areas of correlation and group differences of imaging and eye movement performance measures, particularly in cortical regions known to be involved in eye movement control, suggests that differences between PD and controls in eye movement regions, while present, are subtle when detected using a voxel-based approach. Future studies could consider an ROI approach or more refined DTI techniques, such as tractography, to compare eye movement performance and voxel values using regions found in this study. Overall, this study shows that using a voxel-based analysis of structural, ASL and DTI scans, we are unable to detect significant brain changes in PD reflecting saccade performance deficits within known regions of eye movement control. This does not however exclude the possibility of functional changes during task performance, which can be present despite normal structural anatomy. The brain is also known to operate not as isolated regions but in interconnected networks. Therefore a network-based measure may be a better reflection of brain function than an individual voxel based measure. For these reasons the next chapter will investigate the possibility of correlation between a PD-based perfusion network score. Chapters 5 and 6 will use functional MRI to investigate saccadic eye movement related activity during active task performance.
4 Arterial spin labelling cerebral perfusion network correlation with saccadic eye movement performance in PD.

4.1 Introduction

Using ASL MRI, a PD-related cerebral perfusion network has been identified, using the same patient group as the participants in Chapter 3 (Melzer et al., 2011). This network describes a global decrease in perfusion in PD, with several areas of characteristic hypoperfusion in the posterior parieto-occipital cortex, precuneus, cuneus and middle frontal gyri compared to healthy controls. Meanwhile, relatively preserved perfusion was found in globus pallidus, putamen, anterior cingulate, and post and precentral gyri. This pattern is depicted in Figure 4-1. Each individual can be assigned a single score indicating the extent to which this individual expresses the characteristic pattern, with a higher score corresponding to a more “Parkinson’s-like” perfusion pattern in the brain. The development of this ASL-derived network score brings the potential of a new tool to track PD progression with an objective and repeatable measure. Using a similar approach, previous studies using radiotracer methods such as positron emission tomography (PET) have reported changes in brain metabolism/perfusion specific to motor or cognitive aspects of the disease (Eidelberg, 2009). In contrast, the study by Melzer and colleagues (2011) identified only one PD-specific perfusion pattern, with multiple regression analysis finding significant correlation of this overall PD perfusion pattern with both the Montreal Cognitive Assessment (MoCA) and the motor component of the UPDRS part III).

Figure 4-1 – PD-related ASL perfusion pattern (Melzer et al., 2011). Blue areas indicate markedly reduced perfusion, grey areas indicate mildly reduced perfusion, red areas indicate preserved perfusion.
The PD perfusion network describes characteristic hypoperfusion in the middle frontal gyrus and posterior parietal-occipital cortex, regions containing the human FEF and PPC. Therefore, should the expression of the PD-related perfusion network correlate with saccade performance, it may indicate that hypoperfusion within these regions described by the PD perfusion pattern relate to saccadic eye movement performance deficits in PD.

4.1.1 Saccadic eye movements in PD

Saccade performance in PD has been previously covered in the Introduction chapter (page 49). To summarise, the classical finding for saccades in PD is hypometria – that is, the initial saccade made to a target falls short and is compensated by subsequent corrective saccades (Chan et al., 2005; White Saint-Cyr, & Sharpe, 1983; White et al., 1983). It has also been shown that the extent of the performance degradation is correlated with PD severity and cognitive status, as assessed by the UPDRS and MoCA (MacAskill et al., 2012). Complex volitional eye movement tasks such as the antisaccade task are more affected than simple visual guided tasks (Anderson & MacAskill, 2013). The sources of PD eye movement deficits are unclear, with imaging studies so far managing to find frontal eye field and parietal cortex BOLD activation changes in functional imaging during self-paced and antisaccades in PD (Lemos et al., 2016; Rieger et al., 2008).

4.1.2 Aims of study

This study will test if eye movements correlate with another objective measure of PD progression, namely an ASL-derived perfusion pattern, which has been previously shown to reflect PD motor status and cognition (reference network score paper). The aim of this comparison is to see if two objective measurements can be shown to correlate in PD. This would allow us to test the hypothesis that impaired eye movement performance reflects an abnormal disease-related perfusion pattern in PD, giving more insight into the nature of the causes of eye movement deficits in PD. The network score is not developed enough at this stage to be used as a gold standard of PD progression, thus the aim of this investigation is not to develop a functional biomarker from eye movements alone, but is limited to an initial test to investigate if these two objective markers of PD status show any association. Chapter 4 is a cross-sectional study using multiple regression.
4.2 Methods

4.2.1 Participants

MRI and eye movement data were collected from a convenience sample of 68 PD (49 male and 19 female) and 24 controls (15 male and 9 female) at the New Zealand Brain Research Institute. The average age of the PD participants was 68 years (range 46 to 84), with average years of education of 13.5 years (range 8 to 19) and an average MoCA of 23.4 (range 10 to 30). The average age of controls was 69 (range 50 to 79), with 13.3 years of education (range 10 to 19) and average MoCA of 27.1 (range 23 to 30). Cognitive and PD motor symptom testing was carried out for every participant using the methods described in Chapter 2.

4.2.2 Imaging procedure and ASL-derived network score

A PD-related ASL perfusion network was identified in a previous study by Melzer et al. (2011). A voxel-based principal component analysis (PCA), as described by Spetsieris et al. (2009) was used to derive a perfusion pattern in the study participants from ASL data (Melzer et al., 2011). A grey matter mask was applied to the ASL perfusion images which were then log transformed, de-meaned and entered into a PCA. Principal component images were calculated and a backward stepwise binomial logistic regression was performed to identify disease-related principal components. The model expressing the smallest Akaike information criterion was chosen to best distinguish PD from control.

The disease-related principal components were used in linear combination (based on the parameters derived from the logistic regression) to form a single characteristic PD-related perfusion pattern, which was Z-scored. The network score, indicating the expression of the PD-related perfusion pattern in each individual (or the similarity between each individual’s ASL scan and the characteristic PD-related pattern), was determined by applying the same linear combination of model parameters to PD-related principal component scores. This network score was standardised, with control mean set to 0. Reliability of the spatial extent of the derived PD-related perfusion pattern was estimated using a bootstrap estimation procedure (Efron & Tibshirani, 1986). Since the original study by Melzer and colleagues (2011), an updated network, incorporating additional participant scans enrolled in the longitudinal PD study, has been devised. Network scores ranged from -0.77 to 4.01 for PD and -0.81 to 1.48 for controls.
4.2.3 Eye movement recordings

We chose four standard eye movement tasks to compare with the network score: The reflexive, predictive, memory-guided and antisaccade tasks. The saccade paradigms and measures in this study were the same as employed by Chapter 3 (see page 98).

4.2.4 Data Analysis

All data was analysed using R version 3.2.0 (R Development Core Team, 2008). Eye movement parameters from each participant were averaged for each task and plotted against their individual network score. The network score was also plotted against MoCA and MDS-UPDRS III scores. The lm function was used to calculate regression models. Graphs were generated using ggplot2 (Wickham, 2009). The first regressions performed were simple linear regressions between eye movements and network score. Additional comparisons were performed between eye movement measures and UPDRS III scores and cognitive Z-score. Multiple regressions were then performed with the network score along with the cognitive Z-score and UPDRS III to assess the relationship of other PD measures on eye movement performance. This is important to assess the potential contribution of other regressors (independent variables), not limited to the network score, on eye movement performance (dependent variable).

4.3 Results

The results of simple linear regressions between eye movement performance measures and network score are provided in Table 4-1, Figure 4-2 and Figure 4-3. In the PD group, there was a significant relationship, in all tasks, between the gain of the primary saccade and the network score, with a higher network score being associated with a smaller gain. Significant regressions between the network score and saccadic latency were also seen in the PD group, in all tasks, with a higher network score being associated with a longer reaction time to initiate a saccade. The controls showed a smaller network score (mean score = 0) compared to PD patients and did not show this relationship with eye movement performance, which perhaps is expected due to controls showing a much-reduced range of network scores. Additional figures of regressions between eye movement measures and UPDRS III scores and cognitive Z-score are provided in the Appendix (Figure 9-12 to Figure 9-13).
Figure 4-2 – Linear regressions between network score and saccadic eye movement gain from the reflexive, predictive, memory and antisaccade tasks. The x-axis represents the network score of each participant. The y-axis represents primary gain in each task. Straight lines indicate least squares regressions for each measure. The grey intervals around each regression line indicate the 95% confidence interval of the regression. The cognitive category of PD participants (PDN - normal, PD-MCI - PD mild cognitive impairment, PDD - PD dementia) is indicated by the colour of the plotted points.
Figure 4-3 – Linear regressions between network score and saccadic eye movement latency from reflexive, predictive, memory and antisaccade tasks. The x-axis represents the network score of each participant. The y-axis represents latency. Straight lines indicate least squares regressions for each measure. The grey intervals around each regression line indicates the 95% confidence interval of the regression. The cognitive category of PD participants (PDN - normal, PD-MCI - PD mild cognitive impairment, PDD - PD dementia) is indicated by the colour of the plotted points.
Table 4-1 – P and R squared values for each eye movement and network score comparison.

<table>
<thead>
<tr>
<th></th>
<th>Estimate (gain)</th>
<th>Std. Error</th>
<th>t value</th>
<th>p-value</th>
<th>r squared</th>
</tr>
</thead>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>0.93</td>
<td>0.02</td>
<td>59</td>
<td>&lt; 0.001</td>
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<tr>
<td>Network score</td>
<td>-0.04 per point</td>
<td>0.01</td>
<td>-4.1</td>
<td>&lt; 0.001</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>Predictive gain</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>0.80</td>
<td>0.02</td>
<td>32</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>Network score</td>
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<td>-3</td>
<td>0.004</td>
<td>0.1</td>
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<tr>
<td><strong>Memory-guided gain</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Intercept</td>
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<td>0.03</td>
<td>27</td>
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<td></td>
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<td>Network score</td>
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<td>-3</td>
<td>0.003</td>
<td>0.11</td>
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<tr>
<td><strong>Antisaccade gain</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>Intercept</td>
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<td>0.04</td>
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<table>
<thead>
<tr>
<th></th>
<th>Estimate (ms)</th>
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<th>t value</th>
<th>p-value</th>
<th>r squared</th>
</tr>
</thead>
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<td></td>
<td></td>
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<tr>
<td>Intercept</td>
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<td>8.6</td>
<td>25</td>
<td>&lt; 0.001</td>
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<tr>
<td>Network score</td>
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<td>2.3</td>
<td>0.021</td>
<td>0.06</td>
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<td></td>
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</tr>
<tr>
<td>Intercept</td>
<td>71</td>
<td>19</td>
<td>3.8</td>
<td>&lt; 0.001</td>
<td></td>
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<tr>
<td>Network score</td>
<td>37 per point</td>
<td>13</td>
<td>2.9</td>
<td>0.004</td>
<td>0.11</td>
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<tr>
<td><strong>Memory-guided latency</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Intercept</td>
<td>297</td>
<td>34</td>
<td>8.7</td>
<td>&lt; 0.001</td>
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<td>Network score</td>
<td>132 per point</td>
<td>24</td>
<td>5.6</td>
<td>&lt; 0.001</td>
<td>0.32</td>
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<tr>
<td><strong>Antisaccade latency</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
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<td>27</td>
<td>16</td>
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<td>Network score</td>
<td>56 per point</td>
<td>19</td>
<td>2.9</td>
<td>0.005</td>
<td>0.11</td>
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</tbody>
</table>
Multiple regression tables

Table 4-2 and Table 4-3 show the results of the multiple regression analyses. The significant linear regressions in individual comparisons between network score and eye movements do not remain in a multiple regression, likely due to association between the “independent” variables.

Table 4-2 – Table of multiple regression between saccade gain values, with network score, cognitive Z-score and MDS UPDRS part III score used as predictors.

<table>
<thead>
<tr>
<th>Reflexive gain</th>
<th>Estimate</th>
<th>Std. Error</th>
<th>t value</th>
<th>p-value</th>
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</thead>
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<tr>
<td>Intercept</td>
<td>0.93</td>
<td>0.03</td>
<td>36.9</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Network score</td>
<td>-0.02 per point</td>
<td>0.01</td>
<td>-1.6</td>
<td>0.12</td>
</tr>
<tr>
<td>Cognitive Z-score</td>
<td>0.03 per point</td>
<td>0.02</td>
<td>1.8</td>
<td>0.08</td>
</tr>
<tr>
<td>MDS-UPDRS III</td>
<td>-0.0002 per point</td>
<td>0.0006</td>
<td>-0.4</td>
<td>0.73</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Predictive gain</th>
<th>Estimate</th>
<th>Std. Error</th>
<th>t value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>0.86</td>
<td>0.04</td>
<td>22</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Network score</td>
<td>-0.03 per point</td>
<td>0.02</td>
<td>-1.5</td>
<td>0.14</td>
</tr>
<tr>
<td>Cognitive Z-score</td>
<td>-0.01 per point</td>
<td>0.03</td>
<td>-0.4</td>
<td>0.72</td>
</tr>
<tr>
<td>MDS-UPDRS III</td>
<td>-0.002 per point</td>
<td>0.001</td>
<td>-2.1</td>
<td>0.04</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Memory gain</th>
<th>Estimate</th>
<th>Std. Error</th>
<th>t value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>0.86</td>
<td>0.04</td>
<td>20.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Network score</td>
<td>0.003 per point</td>
<td>0.02</td>
<td>0.1</td>
<td>0.89</td>
</tr>
<tr>
<td>Cognitive Z-score</td>
<td>0.05 per point</td>
<td>0.03</td>
<td>1.7</td>
<td>0.08</td>
</tr>
<tr>
<td>MDS-UPDRS III</td>
<td>-0.003 per point</td>
<td>0.001</td>
<td>-2.7</td>
<td>0.01</td>
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</table>

<table>
<thead>
<tr>
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<th>Estimate</th>
<th>Std. Error</th>
<th>t value</th>
<th>p-value</th>
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<tr>
<td>Intercept</td>
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<td>Network score</td>
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<td>-1.6</td>
<td>0.12</td>
</tr>
<tr>
<td>Cognitive Z-score</td>
<td>0.04 per point</td>
<td>0.05</td>
<td>0.7</td>
<td>0.47</td>
</tr>
<tr>
<td>MDS-UPDRS III</td>
<td>-0.001 per point</td>
<td>0.002</td>
<td>-0.8</td>
<td>0.45</td>
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</table>
Table 4-3 – Table of multiple regression between saccade latency values, with network score, cognitive Z-score and MDS UPDRS part III score used as predictors.

<table>
<thead>
<tr>
<th>Reflexive latency</th>
<th>Estimate (ms)</th>
<th>Std. Error</th>
<th>t value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>200</td>
<td>11.2</td>
<td>17.8</td>
<td>&lt; 0.001</td>
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<tr>
<td>Network score</td>
<td>-11 per point</td>
<td>6.3</td>
<td>-1.7</td>
<td>0.09</td>
</tr>
<tr>
<td>Cognitive Z-score</td>
<td>-25 per point</td>
<td>8.1</td>
<td>-3.1</td>
<td>0.003</td>
</tr>
<tr>
<td>MDS-UPDRS III</td>
<td>0.9 per point</td>
<td>0.3</td>
<td>3.3</td>
<td>0.002</td>
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</table>

<table>
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<th>Std. Error</th>
<th>t value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>22</td>
<td>28.9</td>
<td>0.8</td>
<td>0.45</td>
</tr>
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<td>Network score</td>
<td>16 per point</td>
<td>16.8</td>
<td>1</td>
<td>0.34</td>
</tr>
<tr>
<td>Cognitive Z-score</td>
<td>-1.4 per point</td>
<td>20.7</td>
<td>-0.07</td>
<td>0.95</td>
</tr>
<tr>
<td>MDS-UPDRS III</td>
<td>1.9 per point</td>
<td>0.7</td>
<td>2.6</td>
<td>0.01</td>
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<table>
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<th>t value</th>
<th>p-value</th>
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<td>&lt; 0.001</td>
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<td>1.1</td>
<td>0.26</td>
</tr>
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<td>Cognitive Z-score</td>
<td>-81 per point</td>
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<td>0.01</td>
</tr>
<tr>
<td>MDS-UPDRS III</td>
<td>5.1 per point</td>
<td>1.1</td>
<td>4.5</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Anti latency</th>
<th>Estimate (ms)</th>
<th>Std. Error</th>
<th>t value</th>
<th>p-value</th>
</tr>
</thead>
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<td>&lt; 0.001</td>
</tr>
<tr>
<td>Network score</td>
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<td>24.1</td>
<td>0.03</td>
<td>0.98</td>
</tr>
<tr>
<td>Cognitive Z-score</td>
<td>-39 per point</td>
<td>29.5</td>
<td>-1.3</td>
<td>0.2</td>
</tr>
<tr>
<td>MDS-UPDRS III</td>
<td>3.0 per point</td>
<td>1</td>
<td>2.9</td>
<td>0.005</td>
</tr>
</tbody>
</table>

4.4 Discussion

For the PD-related ASL network score to be a viable biomarker, we would expect it to be associated with the established measures of PD status. Melzer et al. (2011) demonstrated that the network score shows significant correlation with the MoCA, which itself is associated with PD progression (Dalrymple-Alford et al., 2011; Emre, 2003; Janvin et al., 2006). The network score was found to correlate with motor symptom severity as assessed by the MDS-UPDRS part III. This current study demonstrates that consistent correlations are present in comparisons between the network score and saccadic eye movement measures in PD. While this correlation does not imply causation, the consistent correlations with eye movement measures, seen to be characteristically impaired in PD, helps to validate that an ASL-derived perfusion network may be a viable biomarker for disease status and progression. Significant correlation with eye movement performance also shows that the perfusion impairments
described by the PD network may contribute partly to the causes of saccadic eye movement deficits in PD.

From Figure 4-2 and Figure 4-3, the control group showed a smaller network score expression range compared to the PD group – this is expected as a PD-like perfusion pattern should be expressed only weakly in controls. PD-N (non-cognitively impaired) patients tended to show lower network score expression and better eye movement performance, with PD-MCI and PD-D groups showing worse eye movement performance and higher network score expression. This is, again, expected due to the known correlation of both network score and eye movements with cognitive impairment. A control subject was noted to have a very low antisaccade gain value. On further investigation, this subject had only made one “correct” antisaccade at a gain value of 0.2 - all other saccades made were direction errors and were excluded from analysis. A weakness of this study was that a minimum number of correct saccades wasn’t specified to account for performance anomalies demonstrated by this patient. The presence of the gain measurement of this participant does not change the study conclusions as the correlation between antisaccade gain and network score did not reach significance for controls. However future studies would benefit by specifying a minimum number of correctly performed saccades to include for study analysis.

In simple linear regressions between network score and eye movement measures, the spread of eye movement values around the predicted regression line, indicated by the R squared value (with higher values indicating lower spread) ranged from approximately 0.1 to 0.2 for all eye movement task comparisons, except for memory-guided latency, which showed an R squared value of 0.3, indicating a tighter spread. Visual evaluation of the memory-guided latency and network score comparison (Figure 4-3) suggests a non-linear relationship between memory-guided saccade latency and network score. Below a network score of approximately 1.0, latency values tend to remain low. Above network scores of 1, latency values were seen to steeply increase and spread. This non-linear effect was seen also in comparisons of UPDRS III scores and cognitive Z-scores with memory-guided latency (see Appendix). Latency values appeared to abruptly increase above a UPDRS-III score of approximately 40, and with global cognitive Z-scores below -0.75. In contrast, memory-guided gain was seen to correlate in a roughly linear pattern. Other graphs also appear to show this linear trend of saccade performance values. This could potentially indicate that as the disease progresses, a point is reached, specifically in memory-guided saccade latency values, where values fall “off a cliff”,
with non-linear deterioration. If this drop-off point can be determined, this could potentially form a categorical marker of PD progression for the future. This could be further tested using a segmented “broken stick” regression to see if two linear functions are a better description for the data versus one linear function.

Multiple regressions were also performed with network score, cognitive Z-score and MDS-UPDRS-III score as predictors of saccade performance in order to test if the association seen between network score and eye movements may be explained by other measures of PD progression. It is known that the network score correlates with cognitive impairment (as measured by the MoCA) and motor status (Melzer et al., 2011). This, therefore, makes multiple regression potentially difficult to interpret, due to multicollinearity (i.e. having correlated predictors). Multiple regressions involving the mentioned predictors with reflexive latency and memory-guided latency showed significant correlation with cognitive Z-score and UPDRS, but the correlations seen in simple regression between network score and eye movement measures did not remain significant. Multiple regression of predictive latency, predictive gain and memory-guided gain showed only significant correlation with the UPDRS. The network score and MoCA, both showing correlation with the listed eye movement measures in simple regression, did not show significant correlation within multiple regression. Antisaccade latency, antisaccade latency and reflexive gain showed the most marked demonstration of the effect of multicollinearity, with no significant correlation with any predictor value, despite each predictor showing significant correlation individually. The ASL network is known to relate to both cognition and motor status. Previous perfusion studies using PET have found separate metabolic patterns in PD linked to motor and cognition (Eidelberg, 2009). The motor pattern, described as the “Parkinson’s disease-related pattern” or PDRP, is characterised by increased pallidothalamic and pontine metabolic activity, with reduced activity seen in the frontal premotor cortex, inferior parietal and parietal-occipital areas (Eidelberg et al., 1994). The metabolic cognitive pattern, described as the “PD-related cognitive pattern” or PDCP, showed increased metabolic activity in the cerebellar cortex and dentate nuclei with reduced activity in the frontal lobe premotor cortex, SMA, precuneus and inferior parietal lobule (Huang et al., 2007). The network as described by Melzer et al. did not produce a separate perfusion pattern distinct for motor and cognitive symptoms, but was formed from principal components relating to both aspects. The effect of multicollinearity seen in the multiple regressions in this study suggests that the network score reflects both motor status and cognition in PD, which supports the premise that the ASL network score is a measure of general disease status, including both cognitive and motor components in PD.
The ASL-derived network describes widespread cortical hypoperfusion in PD, including the posterior parieto-occipital cortex, precuneus/cuneus and middle frontal gyri, with unchanged perfusion in the globus pallidus, putamen, anterior cingulate and post- and precentral gyri. The Eidelberg PD pattern was interpreted as showing regions of decreased perfusion and surprisingly, regions of increased perfusion in the pallidothalamic and pontine regions. Using absolute perfusion values possible with ASL, it was determined that PD patients showed no areas of increased perfusion, with an overall reduction in perfusion, with particular regions of severe hypoperfusion. Regions interpreted as having increased perfusion by Eidelberg et al. were found to have, instead, preserved perfusion using ASL (Melzer et al., 2011). Additional evidence suggests that this subcortical hypermetabolism in PD could be explained as an artefact of biased global mean normalization (Borghammer et al., 2010; Borghammer et al., 2009).

The ASL-derived network shows hypoperfusion in many areas known to be involved in eye movement control. Areas of saccadic control have been investigated using functional MRI techniques and transcranial magnetic stimulation (TMS). Pierrot-Deseilligny et al. (1995, 2003) and Milea et al. (2005) identified the frontal eye field (middle frontal gyrus), supplementary eye field (supplementary motor area), parietal eye field (posterior parietal cortex), dorsolateral prefrontal cortex (prefrontal cortex), basal ganglia and superior colliculus as involved in saccadic eye movement control. In general, FEF is thought to be involved in triggering voluntary saccades, with the parietal eye field thought to be more involved in reflexive saccades (Müri & Nyffeler, 2008; Pierrot-Deseilligny et al., 2004). The SEF is thought to have a role in memorised saccade sequencing (Pierrot-Deseilligny, Müri, Ploner, Gaymard, & Rivaud-Péchoux, 2003) and the DLPFC to be important in saccade tasks involving working memory (Pierrot-Deseilligny et al., 2003).

The circuits controlling saccadic eye movements share paths in parallel with the skeletal movement control pathway in the basal ganglia. Saccadic eye movements are thought to reflect aspects of motor dysfunction. Characteristic changes in saccadic eye movement performance are seen in PD. In the voluntary (antisaccade) task, PD patients initiate saccades later, make smaller, undershooting saccades and make more errors compared to controls (Briand et al., 1999). PD patients make undershooting initial saccades to memory-guided targets, necessitating multiple step-like corrective saccades (Crawford et al., 1989) and show an increase in unsuppressed reflexive saccades towards the target “flash” in memory-guided or delayed response saccade tasks (Chan et al., 2005). Terao et al. (2011) showed that memory-guided saccades were impaired, even early in the disease. Previous studies have
found mixed results regarding visually-guided reflexive saccades impairment in PD, with several studies finding no difference compared to controls (Briand et al., 1999; Crawford et al., 1989), a subtly prolonged latency (Chambers & Prescott, 2010) or prolonged latency and gain-related to PD motor status and cognitive impairment respectively (MacAskill et al., 2012). With knowledge of widespread cortical pathology in PD (Braak et al., 2003), it is likely that disease-related change, not limited to the basal ganglia, play a role in causing saccadic eye movement deficits in PD.

Imaging studies have supported the idea that cortical structural and functional abnormalities in PD affect eye movement performance. Using structural MRI, Perneczky et al. (2011) found a negative correlation between grey matter volume in PD patients and the variability of saccade latency in the frontal and parietal eye fields. This suggests that PD-related saccadic changes may be associated with grey matter atrophy in saccade-generating cortical regions. A study by Rieger et al. (2008) using functional imaging, found decreased BOLD activity in the frontal cortex during self-paced saccades. ASL has not previously been used to characterise perfusion deficits related to saccadic eye movement measures. However, the correlation of the ASL network score with eye movement measures suggests that this perfusion pattern could reflect abnormalities which may be part of the causes behind saccadic eye movement performance deficits in PD, particularly changes in the posterior parieto(-occipital) cortex and middle frontal gyri.

### 4.4.1 Discussion - Study aims

The results show general correlation between every analysed measure of eye movement performance and ASL network score expression. As the results do show a general pattern, this supports the hypothesis of the study that eye movement performance is able to reflect a PD-specific perfusion pattern, a pattern which has been previously shown to correlate with motor and cognitive impairment in PD. The results however do not suggest that eye movements are sufficiently discriminating at an individual level to be used in a patient-specific measure of disease progression. A large spread in correlation data is evident. From this, the results of this study suggest it is unlikely that eye movements alone can be used as a biomarker at an individual level.
In summary, the relationships between the network score and measures of eye movement performance may indicate that decreased perfusion values in the posterior parietal-occipital cortex, cuneus, precuneus and middle frontal gyrus, as described by the ASL PD network, could reflect part of the cause of saccadic eye movement performance impairments in PD. The ASL derived network score was also seen to correlate with both cognitive and motor status, along with all eye movement measures, supporting the idea that the network score may represent a general measure of overall disease status. An ongoing longitudinal study, with follow-up imaging, is currently being undertaken to further refine and validate this network for use as a potential PD biomarker.
5 Reflexive and predictive saccade tasks in PD and controls using blocked fMRI design

5.1 Introduction

Visually-guided saccades are saccades directed in response to a simple external visual stimulus, while voluntary saccades are saccades made by more deliberate, conscious control. Reflexive saccade paradigms, consisting of following an unpredictable jumping visual stimulus, are used to investigate visually-guided saccades. For voluntary saccades, a type of experimental paradigm such as the predictive saccade task can be used. In this task, the participant is instructed to follow a visual stimulus which alternates between two fixed positions at regular intervals. Instead of making saccades only in response to the stimulus movement, participants performing the predictive task will eventually learn the set pattern of the stimulus movements and can make anticipatory or early saccades to the predicted location of the next stimulus.

Our experimental aim is to investigate the pathophysiological processes underpinning the differences in saccadic eye movement performance between PD and controls using fMRI. Eye movement laboratory-based studies of reflexive saccades have found reflexive saccade latency to be affected by cognition late in the disease and saccade amplitude by motor impairment (MacAskill et al., 2012). Studies of predictive saccades have found a mixed picture of performance deficits in PD. Studies such as by Crawford et al. (1989), Ventre et al. (1992), Duval, Beuter, & Gauthier (1997) and O’Sullivan et al. (1997) reported PD patients having normal saccade reaction times (normal latency) but undershooting amplitudes (reduced primary gain). Others (Bronstein & Kennard, 1985; Ying et al., 2008) have found longer predictive latency in PD while Ventre-Dominey et al., (2001) reported longer latency with normal gain in PD. These variations in findings could be due to variation in motor and cognitive status of PD patients participating in these studies (MacAskill et al., 2012; van Stockum et al, 2008). Our study comprised a PD group with relatively unimpaired cognition to minimise this effect.

Despite the reported saccade performance deficits in PD, fMRI studies investigating eye movements and the cortical function underlying these deficits in PD have been scarce. No study to date has investigated predictive saccades in PD compared to controls using fMRI. Rieger et al. (2008) used a “self-paced” saccade task, consisting of internally generated alternating saccades between two targets left and right of centre. Reiger and colleagues
reported that BOLD-related activity was reduced in the FEF and SEF in PD compared to controls, but increased in the posterior cingulate, parahippocampal gyrus, inferior parietal lobule, precuneus and the middle temporal gyrus. The PEF and occipital regions showed no activation differences. This study was limited, however, by the nature of the task. The self-paced saccade task is an entirely volitional task with no cue to indicate when the participant should make a saccade. Instead, participants make saccades between two fixed points at their own pace. This introduces the frequency of saccades made by each participant as an uncontrolled variable. Saccade frequency is known to correlate with BOLD activity (Kimmig et al., 2001). Secondly, the results reported were not a result of a direct voxel-wise comparison between the PD and control groups. Rather, the active areas reported were generated within the control and PD groups separately. As a result, the apparent differences might not remain if a direct voxel-based comparison was done between the two groups. This can result in an effect known as the “imager’s fallacy”, where a visually-identified activation difference between two separate group analysis does not imply an actual difference when the groups are compared directly within a single model (Henson, 2005; Poldrack et al., 2008).

Reflexive saccades have been investigated using a prosaccade task contrasted to an antisaccade task in PD (Cameron et al., 2012; Lemos et al., 2016). The prosaccade task used by Cameron et al. had only saccade direction as an unpredictable component, with timing and amplitude kept the same for every trial and thus able to be predicted. This may lead to preparatory cortical activity and so may not completely reflect true reflexive saccade activity. A study by Cameron et al. (2012) used fMRI to investigate differences in antisaccade performance in PD and controls. Cameron et al. found the FEF in the PD group showed reduced activity immediately before antisaccade generation. They describe this hypoactivity due to PD patients being less able to establish preparatory activity for saccade execution. A recent paper by Lemos et al. (2016) used a block design fMRI task to investigate the differences between PD prosaccades and antisaccades (in horizontal and vertical saccades – we focus on horizontal). For saccades in the horizontal direction, this study found unilateral hypoactivity in the left FEF for prosaccades and hyperactivity in the right parietal cortex for prosaccades and antisaccades for the PD group. This was thought to represent disease-related hypoactivity in the left frontal cortex, with compensatory changes in the parietal lobe during saccade tasks in PD. It is unclear how a unilateral FEF deficit develops in PD but this does remain somewhat consistent with the idea that FEF hypoactivity is present in PD.

In this study, I investigated cortical activity involved in reflexive and predictive saccades in PD. This study will be a novel fMRI investigation into predictive saccades in PD. We will
control for the number of saccade cues, ensuring each participant is cued to make the same number of saccades - a component which was missing in the self-paced task used by Reiger et al. (2008). For this study, the reflexive task used unpredictable timing, amplitude and direction components, as opposed to past studies using a direction unpredictable component only, with predictable amplitude and timing. Potential group differences in task activity would add significantly to our knowledge of cortical changes in PD. Imaging has long been investigated as a potential tool for diagnosing and tracking Parkinson’s disease progression (Brooks, 2010; Pavese & Brooks, 2009; Weingarten et al., 2015; Politis, 2014). Having group differences able to be detected using fMRI could prove to be a useful tool in the future. In combination with studies investigating eye movement abnormalities over the course of PD, novel biomarkers could be developed to help assess PD severity and progression.

As discussed previously in the Introduction chapter (see page 29), knowledge of the cortical regions involved in saccadic eye movement control was initially derived from primate studies (Bizzi, 1968; Robinson & Fuchs, 1969; Barash et al., 1991; Schlag & Schlag-Rey, 1987; Lynch & McLaren, 1989; Thier & Andersen, 1996). Later, human lesion, stimulation and imaging studies sought to clarify and further investigate the regions of eye movement control first identified in primate trials (Pierrot-Deseilligny et al., 1987; Anderson et al., 1994; Paus, 1996; Gaymard et al., 1998; Grosbras & Paus, 2002; Müri & Nyffeler, 2008). Today, many imaging studies have been carried out, indicating the frontal eye field (FEF) and parietal cortex, containing the posterior parietal cortex (PPC) and the parietal eye field (PEF), are involved in saccadic eye movement control (Mort et al., 2003; Sugiura et al., 2004; Grefkes & Fink, 2005; Parton et al., 2007; Jamadar, Fielding, & Egan, 2013; Vernet et al., 2014). A number of findings remain unclear, however. The FEF has been implicated in nearly every type of eye movement: the preparation and execution of saccades (Bizzi, 1968; Bruce & Goldberg, 1985; Everling & Munoz, 2000; Hanes & Schall, 1996), optokinetic nystagmus, smooth pursuit (Bizzi, 1968; MacAvoy et al., 1991) and fixation (Yoshiko Izawa et al., 2004a, 2004b) but the location of FEF activity found in different experiments varies. Additionally, there are a number of studies that indicate the FEF isn’t a single homogenous region but rather is split into multiple subsections, each with different connections and roles. A medial/lateral split has been described (Gagnon et al., 2002; Simo, 2005) while others have, instead, reported inferior-superior FEF regions (Heide et al., 2001; Luna et al., 1998; Petit et al, 1997). A number of interpretations have been made of the functional differences between these FEF subregions. Petit et al. (1997) reported specific smooth pursuit and saccade subregions within the FEF, with the smooth pursuit region more lateral and inferior than the
saccade region. Both Gagnon et al. (2002) and Heide et al. (2001) interpreted medial and lateral FEF activity to correspond to the generation of small and large size saccades, with larger saccades thought to involve the ventro-lateral FEF. The saccade size theory, however, is in conflict with other sources. Monkey neuro-stimulation studies have shown that large saccades involve the ventro-medial portion of the FEF as opposed to the ventro-lateral (Leigh & Zee, 1999; Bruce & Goldberg, 1985). There does not, therefore, appear to be a consensus on the functional subdivision of the FEF. Our experiment used a block fMRI design, making it possible to compare reflexive and predictive task activity. As a result, in addition to the primary study aim of comparing PD and control activity differences, I am also able to form a discussion regarding the potential subdivision and function of the FEF for the reflexive and predictive task.

5.1.1 Aims of study
Using functional MRI, I ask if performance changes in reflexive and predictive task saccadic eye movement performance PD are reflected in a corresponding functional change, detectable using fMRI in known eye movement control regions. This is linked to the overall aim of the thesis in testing the feasibility of using saccadic eye movement performance as a marker of brain health. My hypothesis is that PD patients will show a functional difference, namely diminished BOLD levels during saccade performance, within regions known to be involved in eye movements, namely the eye fields. The overall number of study participants used in this study (32 total including PD and controls) is larger than many of the present studies of fMRI-based PD studies (Herz et al., 2014). From this, we are also able to compare our findings of regions involved in general eye movement control with previous findings in the eye fields. As will become evident in later sections, findings involving frontal eye field subregions were found in both the PD and control groups, which allowed new interpretations of FEF subregion function. I pose the pre-experimental question broadly in asking if the regions known to be involved in general saccade control are firstly, able to be detected within our relatively large group of study participants, validating our methods; and secondly, if any novel activation differences can be found specific to the study tasks which may help broaden our knowledge of general eye movement control. Chapter 5 is a cross-sectional study using a block design fMRI design.
5.2 Methods

5.2.1 Subjects
Thirty five participants were initially recruited for this study, comprising nineteen PD patients meeting the UK Parkinson’s Disease Society Brain Bank Clinical Diagnosis criteria (Hughes et al, 1992) and sixteen healthy controls matched for sex ratio, mean age and years of education (see Table 5-1 and Table 5-2 for patient demographics). Patients were recruited from a database of participants who had previously undertaken eye movement experiments in our laboratory. Participants who had stated an interest in participating in future PD research were sent an information sheet and cover letter regarding the project. This initial phase was followed up by a phone call confirming interest and setting an appointment. The Montreal cognitive assessment (MoCA) was assessed for all participants and the MDS-UPDRS part III (Goetz et al., 2008) for PD patients.

Three of the nineteen PD patients recruited were excluded from this study with fatigue affecting their ability to correctly perform the eye movement tasks. Controls were healthy volunteers matched to the PD patients by age, sex and years of education.
Table 5-1 – Clinical and demographic characteristics of the PD and Control groups.

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<th>SD</th>
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<tr>
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Table 5-2 – Individual study participant demographics and characteristics.

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5.2.2 Eye movements

The first part of this study involved a single session at the New Zealand Brain Research Institute eye movement laboratory. This visit consisted of participants undergoing the same saccadic tasks that would be used subsequently in the MRI machine. Eye movements were recorded and tracked using the eye-tracking system detailed in Chapter 2 (see page 80).

Eye movements tasks carried out in the MRI scanner were presented and recorded using the Real Eye imaging system (Avotec, Inc). As mentioned previously, this setup proved to be unreliable for the most part and prone to losing tracking of gaze position. Hence in-bore eye movement data was not used in this study, although eye video was able to be used to confirm task compliance within the scanner.

5.2.3 Eye movement task types

Three eye movement tasks were used: reflexive, predictive and fixation. These represent reflexive and volitional eye movements, with fixation representing a control task with no eye movements.

The reflexive task involved the participant following a square stimulus, which jumped left and right at pseudorandom amplitudes and intervals. The target for the reflexive task was randomised to jump left or right horizontally in 10, 15 and 20 degree increments in the horizontal plane at one of 7 locations, each separated by 5 degrees visual angle (7 possible target locations at -15, -10, -5, 0, 5, 10 and 15 degrees of visual angle). The time between each jump was pseudo-randomised in 500, 750 or 1000 ms interval balanced across trials so that the mean inter-trial interval was 750 ms. Hence, 36 trials occurred in each 27 second block. The total stimulus amplitude for each block was kept equal for leftward and rightward directions i.e. each stimulus jump was pseudo-randomised left or right, moving either 10, 15 or 20 degrees each time so that the total amplitude of rightward stimuli shifts were kept equal to total leftward shifts.

The predictive task involved the participant following a stimulus as it jumped back and forth between two fixed locations at a constant interval. The stimulus moved back and forth horizontally at regular intervals between two positions at -10 degrees and +10 degrees horizontally from centre at 750 ms intervals. 36 predictive trials occurred in each 27 second block. Thus the number of trials per block (36) matched the reflexive trials.

For fixation, a square stimulus simply remained stationary at the centre of the screen for 27 seconds, upon which participants were instructed to maintain gaze.
The eye movement tasks were arranged as a block design for fMRI scanning. Each block was a set time period in which the participants performed one of the eye movement tasks continuously. A block was made for each eye movement task - reflexive, predictive and fixation. A pilot study was used to determine the number of trials within each block needed to give a reliable task contrast. The resulting stimulus sequences were found to provide significant activation in the known areas of eye movement control, while keeping the overall task duration within reasonable time limits to minimise patient fatigue. A reflexive block was presented first, followed by the predictive block followed by fixation. Each task block lasted 27 seconds for each task. This three-block sequence was repeated four times, giving a total task duration of 324 seconds ((27 s × 3 block repetitions) × 4 = 324 s), or 5 min 24 seconds.

5.2.4 Magnetic resonance imaging protocol
Images were obtained from a 3.0 Tesla General Electric HDx scanner (GE Healthcare, Milwaukee, WI, USA) with an eight-channel head coil as detailed in Chapter 2 (see page 85). The scanning protocol included T1 structural MRI, arterial spin labelling (ASL), diffusion tensor imaging (DTI) and functional MRI (fMRI). The scans took place within one session, with a total scanning time of approximately 1 hour per participant. Participants lay supine on the scanner bed and were instructed to remain as still as possible for the scanning duration. To further minimize movement, foam padding was placed around the head. Headphones were provided to allow instructions to be conveyed to the patient and to protect against scanner noise. Scanning parameters can be found in Chapter 2 (see page 85).

5.2.5 Data processing
Scans were pre-processed using the software SPM8 using the technique described in section 1.4.2.3 page 64.

Functional images were then realigned to minimise the impact of head movement. The mean functional image was co-registered to the T1 weighted image, shifting all scans from all tasks to be aligned with the structural scan using the anterior commissure as the [0 0 0] co-ordinate. The normalization parameters produced during the unified segmentation and normalization step were then used to warp the slice timing-corrected, co-registered functional images into MNI space. The final step was to smooth the pre-processed images using an isotropic Gaussian kernel with a full width at half maximum of $8 \times 8 \times 8$ mm.

5.2.6 Statistical analysis
A first-level analysis was performed for every subject. Scans were entered into a model with the onsets and duration of each block specified in SPM8. The reflexive task and the predictive...
task were entered as two conditions. Fixation, being the third condition, was not entered into the model as it was the baseline condition, a value which is calculated by SPM8 as the constant. Having both a fixation and constant would mean a partial correlation between the two data sets which produces inefficient statistical estimation of voxel activation (Pernet, 2014). Motion correction parameters in 6 axes produced during pre-processing were added into the model as regressors. Contrast files between the reflexive task and baseline (equivalent to fixation), the predictive task and baseline, reflexive activity greater than predictive activity and predictive activity over reflexive activity were generated for every participant (see Figure 5-1 for an example of the design matrix for a first level or single subject analysis). These contrast files were entered into a second level comparison between the two experimental groups – PD and controls. Age, Sex and MoCA were entered into the second level analysis as covariates. Figure 5-2 shows the design matrix for this second level or group analysis. A display of the regions of BOLD activation was generated using SPM8. 2D slice images of BOLD activation were generated using the program xjView and were superimposed over a normalised structural MRI scan from an elderly brain template. Locations for each brain region were determined using xjView. Second level comparisons were corrected for multiple comparisons using voxel-wise FWE with \( p < 0.05 \).

![Figure 5-1](image)

**Figure 5-1** – Graphical representation of the first-level design matrix for a single participant. Columns A and B represent the conditions of the block trial – “A” represents reflexive BOLD activity, “B” for predictive activity. Columns C to H represent motion correction adjustment parameters generated during the pre-processing step. Column I is the constant or baseline activity of the fMRI scans. The brightness or intensity of each area represents the numeric values of each parameter. Moving from the top to the bottom of the diagram represents the modelled BOLD activity or motion adjustments over time for the entire task.
Figure 5-2 – Second-level design matrix for the block design task. The brightness of each area represents a numerical value. The first two columns are formed from the average of the contrast files for PD in group 1 and controls in group 2. Columns further to the right represent covariate values for age and sex.

5.3 Results

5.3.1 Eyelab results

Saccade performance measures of task latency and primary gain are reported in Table 5-3. There was a significant difference in the primary gain between PD and controls in both the reflexive and predictive task. In both reflexive and predictive tasks, the PD group made shorter saccades, initially undershooting the target compared to controls. The PD group also initiated saccades significantly earlier than controls in the predictive task, indicated by a lower latency. In comparison, for the reflexive task, there was no statistical difference between PD and controls in latency.

Table 5-3 – Group mean and standard deviation (SD) values for saccade performance values as measured in the eyelab. The PD group made undershooting saccades (i.e. significantly reduced in gain) compared to controls and initiated saccades earlier in the predictive task (i.e. with a significantly lower latency).

<table>
<thead>
<tr>
<th>Task parameter</th>
<th>Control (SD)</th>
<th>PD (SD)</th>
<th>t-Test p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reflexive latency (ms)</td>
<td>201 (-19)</td>
<td>194 (-33)</td>
<td>0.54</td>
</tr>
<tr>
<td>Reflexive primary gain</td>
<td>0.91 (-0.04)</td>
<td>0.81 (-0.09)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Predictive latency (ms)</td>
<td>88 (-80)</td>
<td>-41 (-146)</td>
<td>0.005</td>
</tr>
<tr>
<td>Predictive primary gain</td>
<td>0.86 (-0.12)</td>
<td>0.59 (-0.17)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>
5.3.2 fMRI Results

The fMRI findings are presented either as a transparent "glass brain" view of the activations, in which the active areas are seen as grey-black regions, or transverse slices of the 3D fMRI brain image, in which BOLD activity is coloured yellow-red and is overlaid on axial slices of a structural elderly brain template.

5.3.2.1 Reflexive vs Fixation

In the control group, significant areas of activation were seen in the FEF, SEF, PPC, PEF, precuneus, cuneus, middle temporal gyrus and occipital lobe. FEF activity was bilateral and formed a wide area in Brodmann’s area 6 in the middle frontal gyrus and the precentral gyrus (For a review of FEF location, see Vernet et al. 2014). From this, the FEF cluster visually appears as an adjoining medial and lateral clusters, within the total FEF activation (see Figure 5-3). I term these areas the medial FEF and lateral FEF.

Figure 5-3 – Medial and lateral FEF regions labelled using a sample fMRI image. Activations are seen in orange. The two slices pictured are the same image. The rectangle highlights the FEF regions in the left image. In the right image, the region labelled “L” represents the lateral FEF and “M” represents the medial FEF.

SEF activation was seen centrally in the medial frontal gyrus. Parietal lobule and Brodmann area 7 activations were seen, corresponding to the PPC/PEF (as described in Muri et al., 1996). Parietal activation also appeared as two adjoining clusters, with one cluster located in the superior parietal lobule and the other in the inferior. For the PD group, the active areas were very similar to what was seen for the controls, with significantly active areas in the FEF, SEF, PEF, PPC, middle temporal gyrus, precuneus and cuneus, cingulate cortex and occipital cortex. These imaging results for the control and PD group can be seen in Figure 5-4 and Figure 5-5 respectively. Table 5-4 and Table 5-5 show the peak voxel locations of the active regions. Visually, controls showed activity in the right thalamus, Brodmann areas 22 and 13 (located in the superior temporal gyrus) and Brodmann area 40 (in the inferior parietal lobule), which were not seen in the PD group. These apparent differences did not survive a direct voxel-based comparison with the PD group.
Control Reflexive Task

(A)

Figure 5-4 – Voxels showing reflexive saccade activity greater than fixation for the control group only. (A) shows BOLD activation (black) for reflexive vs fixation overlaid on a transparent brain in coronal, sagittal and transverse planes. (B) shows the same areas of activation (orange) overlaid on an average elderly brain template in the transverse plane through the 3D scan. All areas of activation are corrected for multiple comparisons FWE, p < 0.05). BOLD activation can be seen in the thalamus, precuneus, posterior parietal cortex, the occipital lobe, FEF and SEF. The FEF region appears as a wide cluster with what appears visually to be two sub-clusters, one medial and one lateral. The parietal activation can also be seen with two clusters, one in the superior parietal lobule and one in the inferior.
**PD Reflexive Task**

(A)

(B)

Figure 5-5 – Voxels showing reflexive saccade activity greater than fixation for the PD group only. (A) shows BOLD activation (black) for reflexive vs fixation overlaid on a transparent brain in coronal, sagittal and transverse planes. (B) shows the same areas of activation (orange) overlaid on an average elderly brain template in a transverse plane through the 3D scan. All areas of activation are corrected for multiple comparisons FWE p < 0.05). BOLD activation can be seen in the precuneus, posterior parietal cortex, the occipital lobe, FEF and SEF. As with controls, the FEF region appears as a wide cluster with medial and lateral sub-clusters. The parietal activation also appears as sub-clusters in the superior and inferior parietal lobule.
Table 5-4 – Locations of peak BOLD activation for the Control group: reflexive greater than fixation contrast.

<table>
<thead>
<tr>
<th>Lobe</th>
<th>T value</th>
<th>Z value</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
</tr>
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<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Lateral FEF Brodmann Area 6 L</td>
<td>11.95</td>
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<td>-6</td>
<td>48</td>
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<tr>
<td>lateral FEF Brodmann Area 6 R</td>
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<td>5.85</td>
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<td>-10</td>
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<td>Precuneus L</td>
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<td>52</td>
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<tr>
<td>Precuneus R</td>
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<td>8</td>
<td>-52</td>
<td>58</td>
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Table 5-5 – Locations of peak BOLD activation for the PD group: reflexive greater than fixation contrast.

<table>
<thead>
<tr>
<th>Location</th>
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<th>Z value</th>
<th>X</th>
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<th>Z</th>
</tr>
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<tr>
<td><strong>Frontal Lobe</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Lateral FEF Brodmann area 6 L</td>
<td>10.05</td>
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<td>-40</td>
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<td>Lateral FEF Brodmann area 6 R</td>
<td>10.44</td>
<td>6.56</td>
<td>46</td>
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<td>50</td>
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<tr>
<td>Medial FEF Middle Frontal Gyrus L</td>
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<td>5.72</td>
<td>-25</td>
<td>0</td>
<td>56</td>
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<tr>
<td>Medial FEF Middle frontal gyrus R</td>
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<td>5.39</td>
<td>22</td>
<td>-5</td>
<td>50</td>
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<td><strong>Parietal lobe</strong></td>
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<tr>
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<td>52</td>
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<tr>
<td><strong>Occipital lobe</strong></td>
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<tr>
<td>Lingual Gyrus L</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Middle Temporal Gyrus R</td>
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<td>5.34</td>
<td>46</td>
<td>-48</td>
<td>6</td>
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<td>-15</td>
<td>-75</td>
<td>10</td>
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</table>
5.3.2.2 Predictive vs Fixation

Increased bilateral FEF and SEF activity was seen in the control group while performing the predictive task. In addition, activity was seen in the superior parietal lobule bilaterally, the right inferior parietal lobule, the superior temporal gyrus, the middle temporal gyrus and the lingual gyrus. These findings can be seen in Figure 5-6. A table of the peak voxel locations for each cluster is shown in Table 5-6. Areas seen to be active for the PD group were the FEF, the SEF and the left superior temporal gyrus. Interestingly, no significant parietal activation was seen in the PD-only analysis. These findings are displayed in Figure 5-7 with Table 5-7 showing the locations of peak voxel activation for each cluster. A direct voxel-based comparison of these apparent differences between PD and control groups yielded no significant difference.
Figure 5-6 – Voxels showing predictive saccade activity greater than fixation for the control group only. (A) shows BOLD activation (black) overlaid on a transparent brain in coronal, sagittal and transverse planes. (B) shows the same areas of activation (orange) overlaid on an average elderly brain template in a transverse plane through the 3D scan. All areas of activation are corrected for multiple comparisons FWE \( p < 0.05 \). Active areas (in orange) can be seen in the posterior parietal cortex, occipital lobe, SEF and FEF.
Figure 5-7 – Voxels showing predictive saccade activity greater than fixation for the PD group only. (A) shows BOLD activation (black) overlaid on a transparent brain in coronal, sagittal and transverse planes. (B) shows the same areas of activation (orange) overlaid on an average elderly brain template in the transverse plane through the 3D scan. All areas of activation are corrected for multiple comparisons FWE p < 0.05). Activity is seen in the FEF, SEF and in the left superior temporal gyrus. Of note, no significant parietal lobe activation can be seen.
Table 5-6 – Locations of peak BOLD activation for the control group: predictive greater than fixation contrast.

<table>
<thead>
<tr>
<th>Frontal Lobe</th>
<th>T value</th>
<th>Z value</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
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</thead>
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<td>6.27</td>
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<td>6.1</td>
<td>4</td>
<td>6</td>
<td></td>
</tr>
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</table>

| Parietal Lobe                 |         |         |      |      |      |
| Brodmann Area 7 Superior Parietal Lobule L | 6.38 | 4.94 | -28 | -58 |      |
| Superior Parietal Lobule R    | 7.46    | 5.45    | 22   | -66  |      |
| Inferior Parietal Lobule R    | 5.56    | 4.5     | 30   | -54  |      |

| Temporal Lobe                 |         |         |      |      |      |
| Middle Temporal Gyrus L       | 6.88    | 5.19    | -46  | -62  |      |
| Middle Temporal Gyrus R       | 7.15    | 5.31    | 44   | -66  |      |
| Superior Temporal Gyrus R     | 6.93    | 5.21    | 56   | -42  |      |

| Occipital Lobe                |         |         |      |      |      |
| Lingual Gyrus L               | 6.61    | 5.05    | -10  | -74  |      |
| Brodmann Area 18 - Lingual Gyrus L | 6.19 | 4.84 | -22 | -75 |      |
| Brodmann Area 19 - Lingual Gyrus R | 5.72 | 4.59 | 18  | -64  |      |

Table 5-7 – Locations of peak BOLD activation for the PD group: predictive greater than fixation contrast.

<table>
<thead>
<tr>
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<th>T value</th>
<th>Z value</th>
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<th>Y</th>
<th>Z</th>
<th>MNI coordinates</th>
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<td>4.47</td>
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<td>Medial FEF Middle Frontal Gyrus R</td>
<td>5.88</td>
<td>5.19</td>
<td>30</td>
<td>-5</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>SEF Superior Frontal Gyrus</td>
<td>9.43</td>
<td>6.22</td>
<td>6</td>
<td>6</td>
<td>62</td>
<td></td>
</tr>
</tbody>
</table>

| Temporal lobe                 |         |         |      |      |      |                 |
| Superior Temporal Gyrus L     | 9.12    | 6.11    | -50  | 6    | 4    |                 |
5.3.2.3 Reflexive vs Predictive contrast

A contrast of the reflexive over the predictive task shows which areas were more active specifically in the reflexive task compared to the predictive. Notably, greater bilateral medial FEF activity is seen in the control group (Figure 5-8) and PD group (Figure 5-9). No difference in lateral FEF activity was seen for either group for this comparison. Within the PD group, significantly greater activity was also seen in the PPC and PEF for the reflexive task vs the predictive task. These parietal activations were located in the superior and inferior parietal lobules and Brodmann area 7. Unlike the PD group, no parietal activation reached significant levels within the control group in this comparison. When a direct voxel-based comparison was made between the PD and controls, however, there were no significant group differences (FWE-corrected p < 0.05). Peak voxel locations for each group is shown in Table 5-8 and Table 5-9.

Figure 5-10 and Figure 5-11 give a side-by-side view of all the comparisons which have been made in this study, allowing for easy visual comparison of activation differences.
Controls Reflexive greater than Predictive

Figure 5-8 – Voxels in control group-showing activation greater for the reflexive task compared to the predictive task. (A) shows BOLD activation (black) overlaid on a transparent brain in coronal, sagittal and transverse planes. (B) shows the same areas of activation (orange) overlaid on an average elderly brain template in the transverse plane through the 3D scan. All areas of activation are corrected for multiple comparisons FWE $p < 0.05$). Greater activity was seen in the medial frontal eye field bilaterally. No differential parietal cortex activity was seen.
Figure 5-9 – Voxels in PD group showing activation greater for the reflexive task compared to the predictive task. (A) shows BOLD activation overlaid on a transparent brain in coronal, sagittal and transverse planes. (B) shows the same areas of activation overlaid on an average elderly brain template in a transverse plane through the 3D scan. All areas of activation are corrected for multiple comparisons (FWE p < 0.05). Activity is seen in the medial frontal eye field bilaterally as well as the superior parietal lobule bilaterally.
Table 5-8 – Locations of peak BOLD activation for the Control group - reflexive greater than predictive comparison.

<table>
<thead>
<tr>
<th>Frontal lobe</th>
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<th>Z value</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medial FEF L</td>
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</tr>
<tr>
<td>Medial FEF R</td>
<td>7.4</td>
<td>5.42</td>
<td>22</td>
<td>-2</td>
<td>54</td>
</tr>
</tbody>
</table>

Table 5-9 – Locations of peak BOLD activation for the PD group – reflexive greater than predictive comparison.

<table>
<thead>
<tr>
<th>Frontal Lobe</th>
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<th>Z value</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
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<tr>
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<td>5.32</td>
<td>-22</td>
<td>-4</td>
<td>56</td>
</tr>
<tr>
<td>Medial FEF R</td>
<td>8</td>
<td>5.68</td>
<td>22</td>
<td>-4</td>
<td>56</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Parietal lobe</th>
<th>T value</th>
<th>Z value</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brodmann Area 7 L</td>
<td>7.95</td>
<td>5.66</td>
<td>-16</td>
<td>-66</td>
<td>62</td>
</tr>
<tr>
<td>Brodmann Area 7 R</td>
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<td>5.71</td>
<td>22</td>
<td>-62</td>
<td>44</td>
</tr>
<tr>
<td>Precuneus L</td>
<td>6.08</td>
<td>4.78</td>
<td>20</td>
<td>-60</td>
<td>48</td>
</tr>
<tr>
<td>Precuneus R</td>
<td>7.58</td>
<td>5.5</td>
<td>18</td>
<td>-66</td>
<td>52</td>
</tr>
<tr>
<td>Superior Parietal Lobule L</td>
<td>6.82</td>
<td>5.16</td>
<td>-24</td>
<td>-66</td>
<td>60</td>
</tr>
<tr>
<td>Inferior Parietal Lobule R</td>
<td>5.5</td>
<td>4.46</td>
<td>42</td>
<td>-44</td>
<td>38</td>
</tr>
<tr>
<td>Inferior Parietal Lobule L</td>
<td>6.62</td>
<td>5.06</td>
<td>-36</td>
<td>-44</td>
<td>46</td>
</tr>
</tbody>
</table>
Control activation comparison for all tasks

Figure 5-10 – Summary of the activation differences for controls in the reflexive task greater than fixation, the predictive task greater than fixation and the reflexive task greater than predictive task. BOLD activity is seen in orange. In the reflexive greater than predictive comparison, the medial FEF subregion was significantly more active in the reflexive task than the predictive.

PD activation comparison for all tasks

Figure 5-11 – Summary of the activation differences for PDs in the reflexive task greater than fixation, the predictive task greater than fixation and the reflexive task greater than predictive task. BOLD activity is seen in orange. In the reflexive greater than predictive comparison, the medial FEF subregion was significantly more active in the reflexive task than the predictive. Parietal regions in the superior parietal lobule were also significantly more active for the PD group in the reflexive greater than predictive comparison.

5.3.2.1 Predictive greater than Reflexive contrast

No significant differential activity was observed either within PD or control groups or in a comparison between-groups for predictive activity greater than reflexive activity.
5.4 Discussion

5.4.1 Discussion of study aims

From our results, we are able to comment on general eye movement control from our findings. Overall, there were no significant functional group differences able to be detected between PD and controls. This argues against my initial hypothesis, with the results of this study suggesting saccade performance differences between groups are not reflected in a significant functional difference detectable using fMRI. The possible reasons behind these findings are covered further in the discussion section below. To list briefly, it is likely that PD-related functional changes are small and are unable to be detected using the limitations of our current fMRI setup. The lack of significant change may be due to inadequate patient numbers, however it should be noted that our study is a comparatively large one in the fMRI literature (Herz et al., 2014). The results may also be due to the relatively mild severity within the patient group, with the study confined to mainly PD-N and PD-MCI patients.

The finding of a differentiation between medial and lateral FEF activation satisfies the goal posed initially regarding broadening our knowledge of general eye movement control. This finding allowed new interpretations of FEF subregion function. The details of this finding are discussed in sections 5.4.3 titled “FEF subregions” below.

5.4.2 Discussion of study findings

Several differences, mainly seen in the parietal lobe, were noted between PDs and controls from their separate group analysis. The PD group visually showed less parietal lobe activity for the predictive task compared to controls. In the reflexive task, both groups showed large areas of parietal and frontal activation. When comparing reflexive activity to predictive activity, the PD group showed bilateral parietal activity in the superior and inferior parietal lobule specific to the reflexive task over the predictive task whereas controls did not show this. Taking the findings from each group separately, it would appear that parietal lobe activity in PD patients was more prominent in the reflexive task and less in the predictive task compared with controls. All these differences were significant after correcting for multiple comparisons within each single group, however, when PD and controls were directly compared, there were no significant differences. Hence we must bear in mind the “imager’s fallacy” noted earlier – although there appear to be compelling visual differences in group activations, these did not survive when directly compared in the model.

fMRI studies of eye movement performance in PD are limited but a number have reported parietal lobe activity differences in PD. A recent study by Lemos et al. (2016) comparing
antisaccade activation to prosaccades in a block fMRI paradigm suggests compensatory parietal lobe activity in prosaccades may be present in PD. Rieger et al. (2008) reported increased inferior parietal lobule activity in PD for a volitional (self-paced) saccade task over fixation compared to unaffected controls. Reiger et al. reported these differences using an indirect, visual-based comparison, which might not have remained in a direct voxel-wise comparison as described by the imager's fallacy. When a voxel-wise whole-brain comparison was made between PD and controls in our study, no significant difference remained. This suggests no significant group differences in cortical function for the reflexive and predictive task. Thus, either any possible differences between PD and controls in the reflexive and predictive task are too subtle to survive our correction criteria, or were not suitably elicited using our specific experimental paradigm.

FEF activity was observed as medial and lateral sub-clusters, in both the reflexive task and the predictive task for both groups. The medial FEF was more active in the reflexive task than the predictive task in both groups. This would suggest that while both the medial and lateral FEF are active in the reflexive and predictive tasks, the medial FEF has activity specific to the reflexive saccade task. In this next section, I discuss the present knowledge on FEF subregion function before presenting further evidence to support this claim.

5.4.3 FEF subregions

Both medial and lateral FEF activity was observed in our reflexive saccade task. When reflexive activity was compared to the predictive task, in each group we saw distinct medial FEF activation in the reflexive task over the predictive task, with no difference in the lateral FEF. The results of this study support the notion that the FEF comprises of functional subregions, with one region involved more in the process underlying saccade generation to unpredictable targets, and another region more involved in saccades to known predictable locations at known times. This idea of the FEF operating as separate subregions has been discussed in the introduction and there is uncertainty on the specific function of each subregion, or even how the FEF is functionally organised: A medial/lateral division has been described (Gagnon et al., 2002; Simo, 2005) as well as a superior/inferior division (Luna et al., 1998).

One unlikely but potential cause of task activation differences is the difference in cumulative total saccade amplitude between the reflexive and predictive tasks blocks in this study. This cumulative total saccade amplitude across all trials in a reflexive task block was not equal to
the cumulative total saccade amplitude in the predictive task. In the reflexive task, the mean saccade amplitude was 15 degrees of visual angle while the predictive task has an average saccade amplitude of 20 degrees. The number of trials per block was the same between reflexive and predictive task (36 trials) - thus the total saccade amplitude per predictive task block is greater than in each reflexive task block.

A possible mechanism by which this could cause activation differences is from the increase in time spent in saccade travel due to the greater visual angle “distance” covered. This may cause increased cortical activity, should the time spent in saccade travel relate to an increased duration of cortical activity needed to maintain the saccade over a longer distance. This increase in time, however, would be in the region of milliseconds per movement, but accumulated over multiple trials in a block-design task, could potentially cause activation differences. I am not aware of any functional imaging studies investigating the effect of cumulative saccade amplitude on BOLD signal changes and this is an unlikely cause for task activation differences. It has been shown before that saccade amplitude itself (from single saccades, not cumulative) does not correlate with BOLD signal changes (Kimmig et al., 2001). Rather it is the number of saccades generated which correlates with BOLD activity, a factor which was kept constant between tasks in this study. It is pertinent to note that the reflexive task showed increased FEF activity compared to the predictive, despite having a lower cumulative saccade amplitude. No cortical regions were significantly more active in the predictive task when contrasted with the reflexive task. Thus, from our results, no increase in cortical activity was observed in the task with the higher cumulative saccade amplitude, making it impossible to argue that cumulative amplitude causes any increase in BOLD activity. The alternative, opposite possibility of an increased cumulative saccade amplitude leading to decreased activity does not fit into any currently proposed mechanism of eye movement control I am aware of. The FEF is known to have a neural distribution reflecting a topographic map of saccade direction and size. Micro-stimulation of the FEF at any site produces a saccade with a specific amplitude (Bruce et al., 1985). At a cortical level, at least, a large saccade is generated not by an increase in signal, but by activation of the FEF area corresponding to the specific size of the saccade. This makes it less likely cumulative total saccade amplitude corresponds to greater activation. Rather it is more likely individual saccade size variation contributes to FEF sub-region activation. By design, the reflexive task elicits variable saccade amplitudes, while the predictive task elicits one single amplitude, which may contribute to differences in FEF activity. This will be discussed further but
beforehand, I will briefly summarise other potential functions of FEF subregions described in the literature.

5.4.3.1 **FEF subregion function in saccade tasks**

Petit et al. (1997;1999) and Berman et al. (1999) identified an FEF region related to smooth pursuit, located more inferior and lateral, than the saccade-related FEF. In attention studies, separate superior and inferior activations in the FEF for overt attention shifts that occur with eye movements and covert attention shifts with no eye movement have been reported (Beauchamp et al., 2001). In saccade-only trials, divisions of the FEF into a superior and inferior region have been described (Heide et al., 2001). In their self-paced task, a more inferior (and posterior) activation within the FEF was seen when compared to memory or visually-guided saccades.

A medial and lateral FEF division as described in our study has also been reported previously. Ettinger et al. (2008) found the (right) lateral FEF had a more selective involvement in antisaccade generation, with the medial FEF more associated with saccade inhibition than generation. Lobel et al., (2001) contrasted an fMRI study with a human intracerebral electrical stimulation study performed prior to surgery. They observed that the lateral FEF region was more active in the repetition of newly memorised saccade sequences than the medial portion. Their findings show similarity to our findings whereby the lateral FEF region was active in the predictive saccade task in which participants execute a saccade to a newly memorised location.

Grosbras et al. (2001; 2005) described dorso-medial and lateral FEF subregions. They reported that the dorso-medial FEF had more activity during novel saccades sequences while the lateral FEF showed no significant difference. They speculated, based on the study by Koyama et al. (2004) that the dorso-medial cluster is likely to represent the “proper” frontal eye field, equivalent to that in the macaque, whilst the ventral lateral cluster could be equivalent to part of the macaque pre-motor area. Luna et al., (1998) described a superior and inferior FEF in their reflexive saccade fMRI study, the Talairach (Talairach & Tournoux, 1988) coordinates of their superior FEF located roughly at the location of our medial FEF (See Table 5-10).
Luna et al. (1998) found a higher degree of activation in the superior than inferior FEF for the reflexive task. The reported results are broadly consistent with our observation that the medial FEF demonstrated greater activity in the reflexive task than the lateral FEF.

Other studies using different paradigms have described alternative functions for each FEF region. A combined electroencephalography/magnetoencephalography (EEG/MEG) study by McDowell et al. (2005) into pre-saccadic activity for anti and prosaccades found lateral FEF activity appeared first but levelled off 70 ms before antisaccade generation, whereas for prosaccades, the level of lateral FEF activity continued to rise until saccade generation. This pattern was said to reflect an inhibitory signal reaching the lateral FEF in the antisaccade task, preventing a programmed reflexive saccade made towards the cue. In contradistinction, the medial FEF showed a consistent rise in activity immediately prior to both pro and antisaccades, with no levelling off, resulting in higher pre-saccade activity in the medial FEF for antisaccades compared to prosaccades.

On initial reading, this report of higher medial FEF activity in an antisaccade (volitional) task, compared to a prosaccade (reflexive) task appear to contradict our own findings of more activity in the medial FEF for reflexive saccades compared to predictive (volitional) saccades. However, differences in test paradigm used by McDowell et al. mean the observations from their study cannot provide meaningful comparison with our results. The prosaccade task used by McDowell et al. has similar elements to both the reflexive and predictive task used in our study - the time interval between each trial was kept the same in each task. This introduced a predictable timing element to their prosaccade task, with only direction remaining as an unpredictable factor, as in our reflexive task. The antisaccade task is a more complex type of

<table>
<thead>
<tr>
<th>Study</th>
<th>Region</th>
<th>Left</th>
<th>Right</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>x</td>
<td>y</td>
</tr>
<tr>
<td>Luna -1998</td>
<td>FEF - superior</td>
<td>-30.2</td>
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</tr>
<tr>
<td>Heide -2001</td>
<td>FEF - superior</td>
<td>-32</td>
<td>-8</td>
</tr>
<tr>
<td>Feng -2017</td>
<td>FEF - medial</td>
<td>-29</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>FEF - lateral</td>
<td>-39</td>
<td>-2</td>
</tr>
</tbody>
</table>
volitional saccade task than the predictive task. It requires reflexive saccade suppression, as well as a self-generated saccade to a mirror-opposite location (to the target). The study by McDowell and colleagues examined pre-saccade activity at 150 ms before the saccade was made. Our block design fMRI activation also encompasses pre-saccade activity, in addition to the activity during and after the saccade. In spite of these experimental differences, it remains pertinent to note the antisaccade task is also associated with medial and lateral FEF specific activations, supporting the idea that the FEF may operate as functionally separate medial and lateral subregions.

5.4.3.2 **FEF subregions and saccade size contribution**

As previously mentioned, one hypothesis to account for the functional differences between the medial and lateral FEF includes the influence of saccade size (Gagnon et al., 2002; Heide et al., 2001). Heide et al., suggest the FEF comprises superior and inferior regions, corresponding to the medial and lateral FEF in our experiment respectively (Table 5-10). In their experiment, internally-triggered self-paced saccades showed a more inferior (lateral) FEF activation than a visually-guided (reflexive) or memorised saccades. These differences were attributed to saccade size; the self-paced task elicited a larger amplitude saccade and was said to activate the inferior (lateral) FEF, compared to the smaller amplitude saccades elicited in the reflexive and memorised saccade task, said to involve the superior (medial) FEF region. Gagnon et al. provided a similar explanation for medial and lateral FEF activations in their study. Gagnon et al. studied reflexive and predictive saccades and maintained a consistent 14-degree amplitude for all saccade tasks in their study. Participants were described as needing to generate a large initial saccade with smaller corrective saccades to reach the final eye position. These large and small saccades were thought to correspond with saccade size specific regions in the medial and lateral FEF, although it is not directly stated which FEF sub-region is responsible for larger or smaller saccades.

The explanations for medial and lateral FEF activation discussed above conflict with observations in other studies. In primate electrostimulation studies, large saccades (20-60 degrees) were generated by the dorso-medial FEF and smaller saccades (1-5 degrees) by the ventro-lateral FEF (Bruce et al., 1985; Robinson & Fuchs, 1969) in contradiction to the observations by Gagnon, Heide and colleagues. Paus (1996), who was referenced by both these studies, did address this issue in his review. Paus noted a variability of FEF location on the X-axis (left to right). The left FEF was seen to vary from X= -20 to -40 mm and the right from X = 21 to 40 mm using the Talairach co-ordinate system (Talairach & Tournoux, 1988) and Paus proposed that the apparent variation in FEF location could reflect regions of the FEF.
being active for different saccade sizes related to the different types of task. Paus did concede that the study by Robinson & Fuchs (1969) did not support the notion that larger saccades involved lateral FEF. Paus concluded, however, that the medial FEF region was involved in the generation of comparatively small saccades and lateral FEF region in large gaze shifts involving simultaneous eye and neck muscle activation, rather than large saccades alone. The studies reviewed by Paus used saccade amplitudes from 5 degrees to 90 degrees, with the larger stimulus amplitudes likely to involve some inadvertent gaze-related neck muscle activation. In eye movement laboratory testing, our study employed a maximum of 20 degrees of gaze shift for both reflexive and predictive tasks. Gaze shifts of under 20 degrees do not require a head movement contribution (Freedman & Sparks, 1997). It is important to note the visual angle of the stimuli presented using the goggle system within the MRI machine has not yet been determined by the manufacturer. The visual angles, however, are unlikely to differ from laboratory measures by significant amounts. While inadvertent neck muscle activation was not able to be directly controlled, patients were asked to remain as still as possible during testing and used head braces and chin rests as appropriate. Our comparatively small saccade amplitudes compared to studies described by Paus makes it less likely that recruitment of regions involved in large gaze shifts occurred in our tasks. Hence, we do not attribute lateral FEF activation seen in our study to larger saccades or gaze shifts and believe other processes may be involved.

Having reflexive saccades jump in 10, 15 and 20 degree increments and the predictive task all jumping in 20 degree increments allows an interesting comparison between saccade amplitude and cortical activation. The medial FEF has been previously noted to be responsible to generate saccades 20-60 degrees. Every cued saccade in the predictive task is 20 degrees of visual angle. Only a portion of the reflexive task saccades are 20 degrees, with 15 and 10 degree saccades mixed within the trial. Should saccade amplitude be the cause behind FEF activation differences, we would expect to see greater medial FEF activity in the predictive task, due to the higher number of saccades falling within the amplitude which is controlled by the medial FEF compared to the reflexive task. This was not seen, and instead, higher medial FEF activity was seen in the reflexive task, despite fewer saccades made in the amplitude range thought to be controlled by the medial FEF. Hence it is unlikely the cause of activation differences in the medial FEF is due to the generation of certain saccade amplitudes.

Another possible explanation for the differences observed is that in the predictive task - more corrective – and thus smaller saccades were required to reach target position, particularly when anticipatory saccades were generated prior to target illumination. Indeed, average initial
saccade gain was lower in the predictive task compared to the reflexive task - significantly so for the PD group. This should manifest as increased activity in the lateral FEF region responsible for smaller saccades. However, no FEF region, or indeed any region, was more active for the predictive task in comparison to the reflexive task. In contradistinction, the medial FEF demonstrated higher activity in the reflexive task compared with the predictive task. Thus the functional difference between medial and lateral FEF activation is likely more complex than can be explained by saccade size difference alone.

5.4.3.3 **FEF subregions – predictive and reflexive task differences**

Gagnon et al. (2002) found greater fMRI activity in the FEF in both medial and lateral regions in predictive tasks - where participants had advanced knowledge of target direction and timing - compared to a non-predictive, visually-guided reflexive saccade task. The authors argued that eye movements are controlled by systems within the FEF processing two parameters: saccade amplitude and timing of execution. They proposed that FEF activity is modulated by prior knowledge of both the timing and amplitude of a saccade. For the reflexive task, timing and amplitude are unknown in advance, while in the predictive task both these parameters are known. Gagnon and colleagues reported that in predictive tasks, both medial and lateral FEF activity increased over time as participants became aware of the predictive pattern and state functions were shared between FEF regions. They concluded that FEF neural processes involved in saccade timing and amplitude are highly overlapping.

Simo et al. (2005), in a similar saccade study, found more prominent FEF activity in visually-guided saccades than predictive saccades, contrary to the findings of Gagnon et al. (2002). Simo and colleagues used direction as the only unpredictable element in their visually-guided saccade trial, with inter-trial interval and saccade amplitude kept constant. This differs to the study by Gagnon et al. which used a variable trial interval, which was said by Simo et al. to contribute to the difference in findings between the two studies. Simo and colleagues reported the prefrontal and inferior parietal cortical regions, striatum, dorsomedial thalamus, cerebellum and hippocampus were more active in the predictive task compared to the visually-guided task. The authors observed that medial FEF, along with the superior parietal lobule and lingual gyrus (forming a fronto-parieto-occipital system) was more active in their visually-guided task over the predictive task. They proposed that these areas of activation reflected different processes, with the predictive task involving a learning and memory network and the visually-guided task involving sensory processing areas. Simo et al. suggested that the visually-guided saccade task was associated with a higher attention load, giving rise to greater activation in cortical regions related to attention processes.
Our study supports the idea proposed by Simo et al. (2005) with greater medial FEF activity in a reflexive than predictive saccade task. Our reflexive task differed in some key aspects to the visually-guided tasks employed by Simo et al., and Gagnon et al. The inter-stimulus interval was pseudo-randomised, so that target illumination occurred with unpredictable timing in our study, but were kept constant by Simo and colleagues. The amplitude and direction of the stimulus jumps were also randomised in our study, whereas the amplitude was constant in both the Simo et al. and Gagnon et al. studies. The presence of one predictable element, be it timing or saccade size could give rise to preparatory activity even in so-called “visually-guided” tasks. The variation in saccade size and timing ensured unpredictability in our reflexive task and minimised ability of participants to formulate any pre-emptive saccade plans. The limitation of keeping inter-trial intervals constant in a reflexive-type trial is that it introduces the potential for a participant to make anticipatory saccades, once the participant is familiar with the timing of the trials, even if they do not know the direction. With the study of Simo et al., therefore, it is not possible to determine whether the medial FEF activation was truly related to generating a saccade in reaction to the appearance of a stimulus or rather, whether it was related to error/corrective anticipatory saccades at the predicted trial intervals. By varying the trial timing and saccade distance, our study avoided these potential shortcomings. Significant activation of the medial FEF in the reflexive task over the predictive task in our study lends support to the notion that the medial FEF has activity specific to processing reflexive saccades and is functionally different to the lateral FEF.

Connectivity studies have demonstrated that medial and lateral FEF have different cortical connections, supporting the notion that the FEF operates as functionally distinct subregions. In macaque monkeys, there are separate connections between medial and lateral FEF to the SEF and temporal sulcus (Schall et al., 1993; 1995). Further, medial FEF receives peripheral visual field representations and the lateral FEF foveal representations (Schall et al., 1995), consistent with their putative respective size-specific saccade roles, with the more medial FEF region being concerned with large saccades and lateral FEF region with smaller saccades (Bruce & Goldberg, 1985; Robinson & Fuchs, 1969) as discussed above. Babapoor-Farrokhran et al. (2013) investigated the coupling of large saccades with reaching movements and small saccades with manual manipulation and object processing. Medial and lateral FEF demonstrated separate involvement in the two distinct visuo-spatial networks. Tomassini et al. (2007) using DTI-MRI, found that the medial FEF region has connections with superior parietal lobe, dorsal prefrontal cortex and cingulate gyrus with the lateral FEF region having connections with the anterior inferior parietal lobule and ventral prefrontal cortex. De Weijer
et al. (2010) also found the two FEF subregions to have differing connections. The authors used DTI to map white matter connections between the FEF and the SC or CN, with the right and left FEF containing two sub-areas, each with connections to the ipsilateral SC or CN, or both. While the delineation between the FEF-SC and FEF-CN areas were not a clean divide, FEF regions connecting to the CN tended to be located more inferior and lateral than FEF regions connecting to the SC. All FEF zones exhibited higher activity during antisaccades compared to prosaccades, with greater activation in FEF regions connected to the SC than the CN in the antisaccade task. The authors proposed that the FEF-SC connected region was responsible for antisaccade generation and the CN connected region active in withholding a prosaccade.

5.4.4 FEF - PD and control differences

No significant differences in FEF activity were observed in our study between PD and control groups in either reflexive or predictive tasks. Eye lab analysis did reveal significant differences in saccade performance, consistent with the wider literature of PD saccade abnormalities. The initial saccade made by PD patients was smaller (lower gain) than in controls. We might have expected expect to see this reflected by increased FEF activity ventro-laterally, the FEF region responsible for smaller saccades, but this was not observed. PD patients were also seen to initiate predictive saccades sooner than controls in the eye movement laboratory. Significant eye movement performance differences in the absence of significant differences in fMRI activity suggest that either the PD-control differences are too subtle to be detected by our current block MRI design, or the oculomotor control region was either too small an area to be detected at the resolution. The lack of a difference between PD and controls may also be a result of overall FEF hypo-activity, as reported by Rieger et al. (2008) and Lemos et al. (2016). Should there be a relative increase in lateral FEF activity, potentially due to the PD group making more small corrective saccades, but within an overall underactive FEF, the increased activity that would be expected to show may not reach detection threshold. However, this is speculation given the failure to show activation differences between the PD group and controls.

5.4.5 Parietal cortex

Parietal activation was seen as two adjoining clusters for both groups in the reflexive task. The two adjoining parietal areas are located in the superior and inferior parietal lobules, continuous with the intraparietal sulcus in between – these regions form the PPC. Superior parietal lobule activity was also seen to be greater in the reflexive task than predictive task within the PD group only comparison. However no significant difference was evident in 182
between-group comparisons. The PPC is known as a complex area, having a number of tightly positioned regions with a number of functions such as memory/attention/saccade generation. As no significant group difference was seen, we are unable to make further inferences on group differences in PPC function at this stage. However, further discussion will take place within the next chapter regarding PPC function, which was investigated using a different saccade paradigm and fMRI design (memory-guided saccades using an event-related fMRI design).

In general, parietal cortex activity has been reported in nearly all saccadic eye movement studies for visually guided saccades, predictive saccades, memory-guided saccades and antisaccades (Anderson et al., 1994; Colby et al., 1996; Dyckman et al., 2007; Gaymard et al., 1998; Heide & Kömpf, 1998; Heide et al., 2001; Pierrot-Deseilligny, et al, 1991; Simo et al., 2005). It is thought that reflexive saccades are triggered by the parietal eye field (Pierrot-Deseilligny et al., 2004). Our findings are consistent with this proposal, with substantial areas of the parietal cortex active in the reflexive task but smaller areas of parietal activation in the predictive task, for both groups. In the single group analyses, and in contrast to controls, no parietal activity was seen for the PD group in the predictive task. This difference within groups was not evident in the direct comparison between PD and control groups. When the reflexive was compared with the predictive task, no difference in parietal activity was seen in controls, but bilateral posterior parietal activations were apparent in the PD group. Again, this within-group difference did not remain significant in the between-group comparison. A raised level of parietal cortex activity in PD for reflexive saccades would fit with the suggestion by Lemos et al. (2016) that the parietal cortex shows compensatory hyperactivity during prosaccades, but though the within-group analyses might be supportive of this hypothesis, the lack of significance in the between-group comparison does not allow such a conclusion.

Lesion and TMS studies have found that PPC dysfunction increases reflexive and predictive saccade latency (Braun et al., 1992; Gaymard et al., 2003; Heide & Kömpf, 1998; Machado & Rafal, 2004). We did not observe any such increase in reflexive latency in the eye movement laboratory recordings, perhaps due to the relatively small sample size, but larger studies have reported that reflexive saccade latencies are increased in PD, particularly in later disease states with cognitive decline and dementia (MacAskill et al., 2012; Mosimann et al., 2005). It might be expected then that PD patients would exhibit altered activation – either reduction or compensatory increase - in cortical regions controlling reflexive saccades underpinning performance deficits, particularly at later stages of the disease. Failure of the present study to reveal such differences may be because there is no such altered cortical function in PD, or
because the disease status of the PD participants was not sufficiently severe enough to be associated with a detectable change in activation as major cognitive impairment was an exclusion criteria in this study.

5.4.6 Other activations

5.4.6.1 Visual cortex

Significant lingual gyrus activity was observed in both PD and control groups for the reflexive task and to a lesser extent in the predictive task. The lingual gyrus corresponds to the visual areas V1, V2 and the lower V3 (McKeefry et al., 1997). Visual information is first captured by the retina and relayed via optic nerve and thalamus to primary visual cortex (V1). This visual information then passes to the V2/V3 regions in the extrastriate cortex for visual information processing (Orban, 2008) and thence to the parietal cortex via a hypothetical pathway termed “the dorsal stream”. Here, these signals contribute to an integration of visual environment information with viewer/self factors. Such integration is considered essential for eye movement control (Greenlee, 2000).

No occipital lobe activation differences between control and PD groups were observed in the reflexive task. In the predictive task, the control group, when analysed separately (i.e. within-group comparison), exhibited lingual gyrus activity but the PD group did not. This observation might reflect more reliance by the control group on visual information than the PD participants who exhibited greater anticipatory and, therefore, less stimulus-driven behaviour, evidenced by more pre-emptive, negative latency saccades in the predictive task, which, in turn, may be compensation for their hypometria. This difference in activation is, however, rather only slight, and did not survive the between-group comparison.

5.4.6.2 Supplementary Eye Field

The SEF was active in both the reflexive task and predictive task, for both PD and control groups. These results are consistent with the general literature, with a number of studies reporting SEF activity in functional imaging saccade studies for both reflexive and predictive saccade paradigms (Gagnon et al., 2002; Lukasova et al., 2014; Simo, 2005). There were no differences in SEF activation for reflexive or predictive saccades in both groups. This indicates likely no significant functional SEF differences between PD and controls for both tasks.

The SEF has neural connections to every cortical eye control region (Huerta et al., 1986; Shook et al., 1990). The SEF is believed to be important in the temporal control of memorised
saccade sequences (Gaymard et al., 1998; 1993) and antisaccades (Dyckman et al., 2007). There is some debate over SEF function, with Parton et al. (2007) arguing against a proposed SEF role in memorised saccade sequences, reporting a patient with a focal SEF lesion who would still perform memorised saccade sequences correctly but with hypometria. A hallmark of PD saccadic deficit is hypometria, particularly during voluntary saccades (Anderson & MacAskill, 2013; Crawford et al., 1989b; Ventre et al., 1992). This was observed in the laboratory-based predictive saccade recordings in the PD group in the present study. There was, however, no difference in SEF activity between the PD and control groups, hence suggesting the source of hypometria of voluntary saccades PD is not likely to arise from SEF dysfunction.

5.4.6.3 Temporal lobe

When analysed as individual groups, temporal lobe activations in the middle and superior temporal gyrus were seen in the reflexive task for the control group, while the PD group only showed middle temporal gyrus activation. In predictive saccades, both middle and superior temporal gyrus activation was seen in controls while the PD group showed superior temporal gyrus activation only. These differences were not significant in the group comparison, indicating no significant temporal lobe activity differences between PD and controls for reflexive and predictive saccade tasks.

The middle and superior temporal gyrus is not well known as a saccade control region. Instead, this region contains the middle temporal area (area MT) and medial superior temporal area (MST), which are well known for visual motion and smooth-pursuit-related activity (Bremmer et al., 1997; Komatsu & Wurtz, 1988). However, saccade-related activity of these pursuit-related regions has been demonstrated in the primate (Bakola et al., 2007). A number of human imaging studies have reported superior temporal lobes activity in reflexive (Law et al., 1997; Simo, 2005), predictive (Simo, 2005) and memory-guided tasks (Anderson et al., 1994; Ozyurt et al., 2006). Studies are limited regarding the nature of superior temporal cortex involvement for saccadic eye movements but this region is thought to encode for oculomotor information on top of its usual smooth-pursuit-related functions (Bakola et al., 2007).

Another region located in the superior temporal lobe is the superior temporal polysensory region (STP) (Bruce et al., 1981). This region is thought to be involved in visuospatial processes and lesions of this area have produced an increase in reflexive saccade latency,
similar to lesions of the posterior parietal cortex (Scalaidhe et al., 1995). Superior temporal lobe activity may reflect STP visuospatial processes during saccade generation.

Limited information is available regarding middle temporal gyrus involvement in saccade control. The middle temporal gyrus is involved in cognitive processes such as episodic memory and object naming (Cabeza & Nyberg, 2000; Chao et al., 1999). Middle temporal lobe activity could reflect these processes occurring, unrelated to the main saccade task, for example, should the participant be recalling past episodic memories during saccade task performance.

5.5 Conclusion and future directions

In summary, PD participants in our study had a reduced primary gain in the predictive task and to a lesser extent, the reflexive task, in the laboratory-based eye movement recordings. These differences have not been explained by any corresponding observable imaging differences in the comparison between PD and control groups. Differences seen in individual group analyses (i.e. “within-group” analysis) did not remain after the “between-group” comparison analysis. While differences in cortical activity between the two experimental groups were not definitively demonstrated in this study, the differences seen in separate within PD and control analyses could indicate potential eye field differences, possibly in the parietal cortex, which may not be identifiable with a block fMRI design. Thus there is a need for more detailed fMRI study designs. An event-related fMRI design would add a temporal resolution to the cortical activity for each task. The next chapter concerns event-related fMRI to investigate memory-guided saccades in PD and controls. Future studies should use the event-related design for reflexive and predictive task studies. The predictive task involves a preparatory element before the saccade is generated. A block design study is unable to separate pre-task preparatory activity but future event-related study design would, potentially giving further insight into the functional nature of the FEF division.

This study supports the proposition that rather than being a single functional area, the FEF operates as separate subregions for saccade control. Comparisons between reflexive and predictive activity revealed more medial than lateral FEF involvement in unpredictable, reflexive saccade tasks. Both medial and lateral FEF were seen to be active in the reflexive task over the fixation baseline, showing that lateral FEF regions do have a role in the reflexive task too. Further research is needed to show how the increased medial FEF activity relates to
saccade control and to what extent the two regions are separate in their control of the different saccade types.
6 Memory-guided saccades in PD and controls

6.1 Introduction

The memory-guided saccade task, also known as the oculomotor delayed response task, is a type of volitional or voluntary saccade task used to investigate saccadic eye movement performance. It requires the subject to initially remain fixated on the fixation stimulus while a target is briefly flashed to either side. This target position must be kept in working memory while the patient continues to remain fixated on the original stimulus. After a certain interval, the fixation stimulus disappears, cueing the patient to make a saccade to the memorised location of the flash (see Figure 1-5 in the Introduction on page 28). The first part of this task involves deploying visual attention to the flashed target while also suppressing a reflexive saccade to the target. The next part of the task involves maintaining memory of the target location and then a production of a saccade to the target when the fixation stimulus disappears.

Studies of memory-guided saccades have shown task-related activations in the FEF, regions of the posterior parietal cortex (PPC), as well as the SEF and DLPFC (Anderson et al., 1994; Chafee & Goldman-Rakic, 2000; Geier et al., 2007; Pierrot-Deseilligny, Ploner et al., 2002; Sweeney et al., 1996) (See introduction chapter for an overview of these areas. Section 1.2.3.8 to section 1.2.3.11 covers cortical control of saccadic eye movements).

6.1.1 Aims of study

The aim of this study follows from the previous chapter, which is to test the hypothesis that abnormal memory-guided saccade performance in PD will be reflected in functional changes within the brain able to be detected by fMRI. I hypothesise that the eye fields and also the DLPFC, which is a region known to be specifically involved in memory-guided saccades, would show a decrease in functional activity as measured by the BOLD signal compared to controls. In addition, this study will use an event-related MRI sequence in addition to the block design, allowing the construction of a BOLD timecourse. My hypothesis was that the BOLD timecourse will show diminished BOLD activity during memory guided saccade execution within regions known to be involved in memory-guided saccade control in PD.

As previously stated, given this was a reasonably large study in this field, we had a secondary aim of improving our understanding of eye movement control in the general population. In this study, I aimed to use the technique previously described by Sugiura et al., (2004), using a principal component analysis (PCA) of BOLD data to extract components of the functional
time course. PCA allows us to find patterns from complex data sets that may not be immediately obvious visually. This analysis can potentially give us more insight into what constitutes the BOLD timecourse pattern for the memory-guided task for each of the analysed regions of interest. My hypothesis was that these principal components will be able to reflect specific elements of saccade control (e.g. saccade generation), with regions known to be involved in these elements expressing more of that principal component, and regions not involved, expressing less. Furthermore, I am to use a cluster analysis to group functionally-related regions based on PC expression. Should the amount of expression of a principal component reflect function, my hypothesis is that a cluster analysis, with no prior information, should be able to group functionally similar regions together, using only PC expression values.

Using PCA, I also aim to test if PC expression is able to detect PD related changes during saccade task performance. Should regions in the brain be adversely affected in PD, for example, having less involvement in saccade generation, this should be reflected either as a separate PD distinct PC, or regions that are affected in PD being clustered separately. Chapter 6 is a cross-sectional study using a block and event-related fMRI design and principal component analysis.

6.1.2 Memory-guided saccades in PD

PD patients make abnormal memory-guided saccades, of smaller amplitude (hypometric) and longer latency compared to controls (Le Heron et al., 2005; Lueck, Crawford, et al., 1992; Lueck, Tanyeri, Crawford, Henderson, & Kennard, 1990; MacAskill, Anderson, & Jones, 2002). PD patients also have impairments in the suppression of erroneous reflexive saccades (van Stockum et al, 2008) during the delay period. The mechanism of reflexive saccade disinhibition in PD and the source of hypometric saccades remain speculative.

It has been proposed that the voluntary saccade system is responsible for the inhibition of reflexive saccades (Amador et al, 2006). A study by Chan et al. (2005) found PD patients made more erroneous anticipatory saccades, being less able to maintain their gaze on central fixation. This is thought to be a result of a general deficit in reflexive response inhibition, by which a defective voluntary saccade system in PD is less able to control and inhibit the reflexive saccades. van Stockum et al. (2008) have suggested there may be other mechanisms leading to saccade disinhibition rather than just an impaired voluntary saccade system, such as an increased excitability of saccade triggering SC neurons, due to a dysfunctional basal ganglia and cortical inputs, or a general deficiency in maintaining the focus of attention in PD.
The programming of volitional saccades for the memory-guided task also involves the manipulation of spatial information. Spatial working memory may be impaired in PD (Chan et al., 2005) with disruption of the DLPFC (a region known to be involved in working memory processes) suggested as a possible cause of memory-guided saccade deficits in PD (Le Heron et al., 2005; MacAskill et al., 2002).

Previous functional imaging studies have yet to reveal a definitive source for PD-related impairment in saccade disinhibition and working memory. Some have reported dissimilarities between PD and control cortical activity during certain saccade tasks but these findings still do not fully explain the differences in parameters. Cameron et al. (2012) probed the antisaccade task using fMRI and suggested that the observed diminished FEF activity in the PD group could represent decreased neural signals from other cortical areas; signals needed to establish correct saccade task programming, source unknown. Rieger et al. (2008) also reported reduced FEF activation in PD, but with a self-paced task and variations between individuals in the number of saccades completed may have contributed to differences in activation. These two studies did not involve tasks with a working memory component and so their results do not allow any conclusions to be drawn about the memory-guided task in PD.

Our study aims to investigate underlying cortical mechanisms that might explain the impairment in PD task performance in memory-guided saccades. The memory-guided task is unique in that it allows separation of the attention and motor components of saccade production. It has not been previously studied with functional imaging in PD. Thus the present study aimed at investigating differences between PD and controls in cortical activation during the memory-guided task so as to provide further insights into potential sources of deficiency in PD eye movement control.

### 6.1.3 Parietal subregions

The PPC is a key region for memory-guided saccade control. It contains subregions responsible for attention (Corbetta & Shulman, 2002; Rushworth et al., 2001), working memory (Brown et al., 2004; Gaymard et al., 1998) and saccade execution (Pierrot-Deseilligny et al., 2002; Rushworth et al., 2001; Sweeney et al., 1996). The PPC is an anatomical area comprising the superior parietal lobule (SPL), inferior parietal lobule (IPL) and intraparietal sulcus (IPS). Like the FEF, the PPC has been extensively examined in monkeys. A region within the monkey PPC called 7a is thought to be involved in attention processes, whereas the lateral intraparietal area (LIP) is involved in generating visually-guided saccades (see the Introduction page 45 for further background). It is believed that there
are human homologues of these monkey parietal regions. The human parietal eye field (PEF) corresponds to the monkey LIP (Culham et al., 2006) and may lie in the horizontal portion of the IPS (Müri et al. 1996) and regions of the superior parietal lobule (Konen & Kastner, 2008). The Area 7a equivalent has not been determined but is thought to correspond to portions of Brodmann areas 39 and 40 in the inferior parietal lobule in humans (Leigh & Zee, 2000) and may also comprise parts of the SPL and IPS (Konen & Kastner, 2008). The anatomical mapping of this area has proven difficult in humans and uncertainties remain over the exact location of each parietal region. Often, the term “PPC” is used as a broad area description to refer to these parietal regions. As a result of the usage of broad anatomical areas, some questions arise when discussing the PPC involvement in memory-guided saccades (Chen & Crawford, 2017; Christopoulos et al., 2015; Kapoula et al., 2011; Sweeney et al., 1996). For example, does the PPC refer to one or both of areas 7a and LIP?

The PEF surprisingly is seldom referred to in human eye movement studies. The LIP tends to be the preferred term despite being strictly speaking a region defined in monkey rather than man. The terms PEF and LIP are used often used interchangeably and the areas are considered homologous (Culham et al, 2006). For these regions to be so used requires valid human studies that demonstrate that the two are indeed functionally equivalent. There is a need for better consistency in the naming of the parietal cortex subregions Here I aim to clarify what has been reported about these areas by incorporating my results with the available to literature, in order to assist understanding our own results and those from studies in the future.

6.1.4 Principal component analysis

In complex datasets, it can be difficult to determine important overall patterns which may not be immediately apparent within the abundance of information. Principal component analysis (PCA) is a technique which can be used to capture the underpinning patterns which make up complex data sets. This analysis can be used to express data using functions or “principal components” (PCs), which account for most of the variance in the data. This allows the dimensions (size) of the data to be reduced and allows the PCs that capture most of the variance in the data to be visualised.

PCA can be used to extract key features from complex data in many settings. For example, PCA has been widely employed to develop computer facial recognition systems by detecting characteristic feature patterns from facial images (Turk & Pentland, 1991). Likewise, it has been employed in neuroimaging studies to identify spatial patterns in samples of diseased and control study participants (Eidelberg et al., 1994; Huang et al., 2007; Melzer et al., 2011).
PCA has also been used to characterise patterns in event-related time courses of BOLD activity (Sugiura et al., 2004), a technique which was employed in the present study. The PCs in our study represent the independent component patterns of the BOLD activity time courses. These components may represent aspects of the BOLD time course related to separate events within the memory-guided task.

The “expression” or the closeness of the shape of a BOLD time course to a certain PC may be used to infer additional information on the function of each region for the studied task. During the PCA, each region is given a loading value for each PC, which represents how close the shape of the actual BOLD time course of a region is to the shape of a PC. Should a PC be seen, for example, to relate to an aspect of the memory-guided task e.g. saccade execution, then it is possible to suggest that regions showing a BOLD time course shape close to this PC (higher loading value) are involved more in saccade execution, and regions showing a shape more dissimilar (lower loading value) to this PC are less related to saccade execution.

Loading value expression can, therefore, be used to differentiate regions on the basis of function. Regions showing similar PC expression can also be identified using a cluster analysis. This technique, which was employed by Sugiura and colleagues (Sugiura et al., 2004), was able to separate frontal and parietal clusters using BOLD activity data alone, demonstrating that it is possible to group regions into functionally similar groups without explicit information provided for the location of each region. A PCA, in summary, allows additional information on regional function to be derived from the underlying BOLD time course data, and which may not be apparent with simple visual assessment. From the loading values determined in the PCA, functionally similar regions can then be grouped together using a cluster analysis.

This study builds from the technique used by Sugiura et al. to investigate functional BOLD activity in the memory-guided task in both a PD and a control group. In addition, this study utilised a localiser task (using a block design fMRI method – see below) to detect active regions of interest – a step which was not employed by Sugiura and colleagues. I aimed to use the PCA method to differentiate the roles of active regions. Differences between PD and control groups were then examined with PCA and cluster analysis to identify disease-related components and group functionally related active regions.

6.1.5 Functional MRI

As previously covered in the introduction (see page 58), fMRI is a neuroimaging technique which allows researchers to image physiological activity within the brain. Oxygenated blood
and deoxygenated blood have different magnetic resonance signalling properties (different magnetic susceptibilities). fMRI works by detecting these signal changes in the blood oxygen level during task activity. The difference between an active and inactive region is shown by contrast – the difference between intensity in the signal of certain regions during task activity and baseline. The experimental design of an fMRI study needs to be properly devised to satisfactorily evaluate these differences. Two major classes of fMRI experimental design exist – the block design and the event-related design (See sections 1.4.2.1 and 1.4.2.2 pages 62 to 63 in the introduction chapter for an in-depth explanation of block and event-related fMRI design). Both these designs were used in this study. Event-related fMRI was only used in this chapter. Because of this, additional detail on how the BOLD activation is modelled in event related fMRI designs are discussed below in sections 6.1.5.1 to 6.1.5.3.

### 6.1.5.1 Modelling Event-related design

Standard general linear modelling is used to test how well the observed data fits the model of expected haemodynamic response. The expected BOLD activity can be modelled by convolving the onset of each task or “event” with a haemodynamic response function (HRF) (Friston et al., 1994). A canonical HRF has been developed which represents the expected BOLD signal change dynamics to an activated neural cell (Boytont et al., 1996) and is used in this study. Other types of modelling exist using Fourier sets and finite impulse response, which assume no prior haemodynamic response shape. These can easily mis-model the haemodynamic response, and also can fit noise into the model (Kay et al., 2008). This may lead to an “overfit” of the data and misattribute unrelated activity to the task. Thus these other forms of modelling are not used in this study to estimate the BOLD response.

A general linear model may be used to determine which areas of activity most closely correspond to the expected task activity (Friston et al., 1995). This can be visualised using parametric mapping, with identifies the voxels in which activation most closely matches to the expected task activity and can be considered to be “active” for the studied task. These “active” voxels can be highlighted on a map of the brain providing a visual image of regions which show greater BOLD activity in a task compared to a baseline condition. This analysis is undertaken on an individual basis, or first level, and then at a group, or second, level.

### 6.1.5.2 Informed basis set

The canonical HRF is the summation of two gamma functions (Boytont et al., 1996) and is a widely used estimate of the biological haemodynamic response (Calhoun et al., 2004; 194
Lindquist et al., 2009). Actual haemodynamic response can vary between persons and cortical regions (Aguirre et al., 1998; Duann et al., 2002; Handwerker et al., 2004). Using a strict HRF function may inaccurately fit the actual response, causing true significant activations to be missed (Aguirre et al., 1998). To account for this, derivative functions can be added during the first level analysis to account for small changes in time to peak (temporal derivative) and width (dispersion derivative) of the haemodynamic response, this being known as the informed basis set (Friston et al., 1998).

The beta weights of temporal and dispersion derivatives are typically treated as “regressors of no interest”, much like motion correction parameters at the first level and are commonly excluded from second level group analysis (Calhoun et al., 2004; Steffener et al., 2010) as the addition of beta weights from each derivative has been found to add excessive variation between subjects at the second level, leading to a decrease in significance of group differences (Cignetti et al., 2016). Techniques exist that add derivative functions into the second level group analysis; e.g. combining the canonical beta weights with derivative functions at a specific weighted ratio, known as a “derivative boost” (Calhoun et al., 2004; Lindquist et al., 2009). These approaches are however not yet fully established and more studies are needed to validate them (Cignetti et al., 2016).

6.1.5.3 Event-related Time course

One limitation of using the canonical response is that BOLD activity with a more broad shape than described by the canonical shape are unable to be modelled e.g. sustained working memory processes (Aguirre et al., 1998; Courtney et al., 1997). There is no established “canonical” model of sustained activity and there is debate whether sustained activity can even be detected using a univariate GLM analysis as used in this study (Riggall & Postle, 2012) (see Introduction page 66 for details on the GLM analysis). Another technique can be used to directly visualise the BOLD signal change in selected regions of interest and was used in our experiment. This is known as the BOLD time course and allows the average BOLD percent signal change of every trial to be plotted without any need for prior estimation of response shape. This is achieved using a finite impulse response model (FIR) (Glascher, 2009; Goutte et al., 2000), which is able to determine the average BOLD signal change through time. This process is carried out by functional neuroimaging software (my own study used a toolbox for SPM called RFXplot - see Glascher (2009) for an overview of the software). In brief, the time period to be plotted is parcellated into time segments known as "bins". The duration of each bin is effectively the time resolution of the plot – e.g. a one
second bin gives a mean BOLD signal change value for every one second segment for the
duration of the time course. The mean BOLD signal change for each time bin is determined
using an FIR model and these values are used to plot the time course. Time courses are
extracted for every participant at the first level and then combined as group averages at the
second level. Should the specified time period of the plot cover closely spaced overlapping
trials, the overlapping bins are selectively removed by the software, taking care to preserve
the most important part of the haemodynamic response, which is considered the peak, usually
4-5 seconds after onset. If bins for two trials overlap, the first 2 seconds after stimulus onset
are first removed. If this does not resolve the ongoing overlap, further bins are removed from
the end of the time course period. This ensures the average BOLD signal of the trials is
correctly determined despite overlap from closely spaced individual trials.

6.2 Methods

6.2.1 Study participants
The participants for this study was the same group as described in the reflexive and predictive
task chapter (Chapter 5 Table 5-2 page 154), with the exclusion of one control participant
who did not perform the memory-guided saccade task correctly in the scanner. In total then,
16 PD patients and 15 controls were included in this study.

6.2.2 Memory-guided saccadic eye movement task
The eye movement tasks were initially carried out in the eye movement laboratory at the New
Zealand Brain Research Institute. A memory-guided saccade task was used for this
experiment (see Introduction chapter page 28 for an overview of the task). Figure 6-1
additionally illustrates correctly executed and error trials using an eye-trace example. The eye
movement tasks were performed in the same sequence that were subsequently presented to the
participants during MRI scanning. This ensured that they understood how to perform the task
and also allowed for the precise recording of eye movements using the using the
SensoriMotoric iView X Hi-speed 1250 Hz video oculography system (SMI Berlin), as
opposed to the less capable and reliable eye tracker used in the MRI scanner. As previously
noted, the Avotec eye tracking system proved unreliable in maintaining gaze tracking within
the scanner. Ideally, trials with error anticipatory saccades should be excluded as these are
likely to arise from a different mechanism from the main memory-guided task. One effect
from this is the potential for error saccades to influence activity measured during what is
named the “memorisation” event, or the event when the flash appears but the participant is
instructed to maintain fixation on the original stimuli. Should an error reflexive saccade be
made at this stage, this would activate a separate reflexive saccade mechanism, rather than the expected fixation/spatial memory mechanism for this event. This point is further acknowledged during the discussion of the BOLD timecourse results (sections 6.4.3.7 for PPC activity and section 6.4.6.2 for FEF activity).

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**Figure 6-1** – Schematic of the difference between a correctly executed memory-guided saccade trial and an error trial. The red bars represent the stimulus used in this task through time (x-axis). The y-axis represents the amplitude or the visual angle of the presented stimulus. The thin black line represents a tracing of the location of a participant's gaze. An error trial (B) occurs when an unintended reflexive saccade is made to the target flash despite instruction to maintain fixation during this period. In a correctly performed trial (A), there should be no saccade executed until the fixation stimulus is extinguished. Note, the eye trace (thin black line) is an illustrated example and not from actual results.

### 6.2.3 MRI task design

#### 6.2.3.1 Memory-guided saccade task - block design

The block-design task involved a run of the memory-guided task followed by a run of fixation, which would form a block design for the fMRI analysis (i.e. 36 seconds spent
performing the memory-guided task followed by 36 seconds of fixation). This was repeated 6 times giving a total time of 7 minutes 12 seconds (36 seconds memory + 36 seconds fixation = 72 seconds. 72 seconds × 6 = 432 seconds or 7 minutes 12 seconds) for the block-design task. A memory-guided task trial began with an initial fixation phase on a red square target. After 0.9, 1.2 or 1.5 seconds (average 1.2 seconds), a peripheral target “flash” of 400 ms occurred, ranging from 5, 10 or 15 degrees of visual angle left or right from the fixation target. Participants were instructed to keep their gaze on the fixation point when the target stimulus flashed but to remember the spatial location of that flash. After an additional 1.4 seconds, the initial fixation point disappeared and participants were required to make a saccade to the remembered location of the “flash”. After 1 second, the location of the peripheral flash was re-illuminated and became the fixation point for the next trial. Each trial lasted an average of 4 seconds and 9 trials were held in each 36 second block of memory-guided saccades. The target stimulus amplitudes of each trial were pseudo-randomised with the average target amplitude kept the same between leftward and rightward eye movements. For fixation, a green square stimulus was projected to the centre of the screen for 36 seconds with participants instructed to keep their gaze on the green stimulus.

6.2.3.2 Memory-guided saccade task - Event-related design

The second part of the memory-guided saccade study was an event-related design. This was made up of two runs of 18 memory-guided saccade trials in 378 seconds (6 min 18 seconds). The sequences began with a fixation stimulus (red square). After 12, 13 or 14 seconds (average 13 seconds) of fixation, a target “flash” stimulus (red square) appeared for 400 ms in positions 5, 10 or 15 degrees of visual angle left or right of the fixation stimulus. Participants were instructed to keep their eye on the fixation point but to remember the flash location. After an additional 5.6 seconds, the fixation stimulus was extinguished and participants were required to re-fixate on the remembered location of the flash. After 2 seconds, the flash stimulus reappeared and became the fixation point for the next trial. The target stimulus amplitudes between each trial were pseudo-randomised with the average target amplitude kept the same between leftward and rightward eye movements. Figure 6-2 illustrates the task timings for both block and event-related memory-guided tasks in this study.
Figure 6-2 – Timings used for block design and event-related design memory-guided saccade experiments. Red bars represent the visual stimulus at certain visual angles (y-axis) through time (x-axis). Dotted grey vertical lines represent the beginning and end of each phase of the memory-guided task. The duration of each phase is labelled in the diagram (figure durations not to scale).

6.2.3.3 Memory-guided saccade task events

Each component or “event” of the memory-guided task occurs at a certain time within the trial known as an “onset”. The memory-guided task involves the “perceive”, “memory” and “saccade” events (Figure 6-3). For the plotting of the BOLD time course, time zero was set at the moment when the target “flash” stimulus was presented. This is known as the “perceive” event. 400 ms after the flash was extinguished marks the beginning the “memory” period, also known as the delay period. After a further 5.6 s, the fixation stimulus extinguished, signalling the generation of a saccade to the remembered location. This is known as the “saccade” period.
Figure 6-3 – The event-related memory-guided saccade task event names (grey arrows). Each trial begins with fixation. This is considered the baseline activity and not designated an “event”. The time point of the appearance of the target (peripheral) flash is labelled as the “perceive” event. Following this event, on the extinguishing of the flash, the “memory” event occurs with a “delay” period lasting from the disappearance of the flash to the disappearance of the fixation stimulus, cueing the participants to make a saccade to the remembered location. The “saccade” event occurs at this cue to perform a memory saccade.

6.2.3.4 Trial jittering
An interleaved stimulus presentation was used for this study. This technique, sometimes called “jittering”, describes the technique of presenting each event so every task cycle aligns with a slightly different point to one TR. The fixation duration was randomised to vary by one second between trials (12, 13 or 14 seconds; average 13 seconds). This range offset each task and avoided the MRI scanner capturing the same points of the haemodynamic response in every trial. This allowed a better effective temporal resolution of the haemodynamic response as more points of the response are sampled.

6.2.3.5 MRI parameters
MRI parameters used in this study were identical to those in the previous chapter (Chapter 5) and was detailed in Chapter 2 (see page 85).
6.2.3.6 Peristimulus time histogram (PSTH)

The block-design task was used as a localiser for the event-related task. That is, areas which were seen as significantly active in the block-design task were selected as the regions of interest (ROI) for the event-related time course analysis. Coordinates for the ROIs were visually determined from the centre of the active regions from the memory-guided block-design task corresponding to the FEF, SEF, SPL and IPL, DLPFC and insula. From these coordinates, active voxels within a 2 mm radius sphere were selected as the region of interest for the event-related analysis. The BOLD signal change at each time point of the task was calculated and plotted as a time course using RFxplot. The time course plot was aligned with the “perceive period” (time of the presentation of the peripheral “flash”) being at zero seconds. Two FEF subregions were selected – a medial and lateral. The SEF was selected as two subregions with an anterior pre-SEF region and a more posterior SEF region. The insula was also selected. While noted in the past as active in memory-guided tasks (Baumann et al., 2007; Geier et al., 2009), further investigation using event-related fMRI has not been undertaken for the insula region.

6.2.4 Principal component analysis methods

The individual BOLD time courses for every participant in every selected ROI were entered into a PCA. The time course data for each individual comprised of the 20 second period, with one observation or value at every second for every individual. Fourteen regions were used in the PCA analysis (see Table 6-1), with the ROI coordinates obtained from the block design experiment. From this, a $14 \times 14$ correlation matrix was calculated. From this, a principal component analysis was performed (Hastie et al., 2009). The principal components explaining 80% of the variance were considered meaningful and used in subsequent analyses (these were PC1, PC2 and PC3 – see results). When PC loading values are plotted against another for every ROI, regions showing close distances between loading value expression show similar PC component expression and hence a similar time course activation pattern (Sugiura et al., 2004).

6.2.5 Clustering analysis

Loading values from the PCA can be analysed using a clustering-based analysis. Using the data points with no additional prior information, a clustering analysis will find a specified number of clusters by minimising variance between data points within a cluster, while maximising variance between each cluster. This will find data clusters of the most closely related data points with the most difference from other clusters. A K-means clustering analysis was performed with the loading values from the PCA. This determines clusters of
regions with similar loading values and hence similar BOLD activation pattern. We selected K = 4 (4 clusters) for this study. To select this, we ran K = 3, 4 and 5 cluster analyses. At K = 3, prefrontal, frontal and parietal cluster were formed. However, the insula regions were unable to be differentiated - one insula formed part of the prefrontal cluster and another parietal cluster. We considered K = 3 too coarse of a clustering level to allow ROI differentiation. At K= 5, clustering differentiation of prefrontal, frontal, parietal and insula regions was achieved. However, one frontal and one parietal region formed a separate cluster outside of the main parietal and frontal clusters. We felt 5 clusters didn’t offer additional meaningful information regarding ROI separation over 4 (for completeness 3 and 5 cluster figures are in Appendix Figures 9-14 and 9-15). We decided 4 clusters offered a good compromise of ROI differentiation/functional specificity and not over-segmenting and results from this cluster analysis are presented in the following results section (Figure 6-22).

6.3 Results

6.3.1 Eyelab results

Saccade performance measures of memory-guided saccade latency and primary gain are reported in Table 6-1 for the block-design task. No significant group difference in latency and gain measures were detected between PD and controls for this task. However, PD patients made a significantly higher proportion of erroneous anticipatory saccades during the “flash” period, despite instruction to hold gaze on the fixation stimulus. Prolonged trials during the event-related tasks caused calibration issues with the eye tracker, mainly due to patient head-drift during the long periods of fixation. Eye movement performance values were thus unable to be accurately measured and hence were not able to be reported for the eyelab trials of the event-related task.

Table 6-1 – Group mean and standard deviation (SD) values for memory-guided saccade performance measures from the eyelab analysis for the memory-guided block-design task. No significant difference in latency or gain was detected between the groups. PD patients, however, generated a significantly higher proportion of erroneous anticipatory saccades compared to controls during the “cue” or flash period.

<table>
<thead>
<tr>
<th>Task parameter</th>
<th>Control (SD)</th>
<th>PD (SD)</th>
<th>p - value</th>
</tr>
</thead>
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<tr>
<td>Memory-guided saccade latency (ms)</td>
<td>313 (44.2)</td>
<td>293 (65.1)</td>
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<tr>
<td>Memory-guided saccade gain</td>
<td>0.81 (0.07)</td>
<td>0.76 (0.13)</td>
<td>0.22</td>
</tr>
<tr>
<td>Proportion anticipatory saccades</td>
<td>0.11 (0.07)</td>
<td>0.30 (0.19)</td>
<td>0.003</td>
</tr>
</tbody>
</table>
6.3.2 Memory-guided block-design results

The areas of activity are visualised in two forms below - as overlayed on a transparent brain and as slices through the brain. Activation for the control group is shown in Figure 6-4 and Table 6-2. PD activity is shown in Figure 6-5 and Table 6-3. The observed pattern of activation fits with locations described in previous studies on human eye movement control. Namely, the regions corresponding to the FEF, SEF, PEF and PPC and DLPFC are seen active for the memory-guided task in the block design in both groups, all which have been described to be involved in the memory-guided task (Anderson et al., 1994; Sweeney et al., 1996). Of note is bilateral activation within the insula, which had not been observed during our reflexive and predictive tasks (see Chapter 5 results page 159).
**6.3.2.1 Control Group – Memory-guided vs fixation**

(A)

(B)

Figure 6-4 – Significant BOLD activity for the memory-guided task activity greater than fixation for the control group. The top panel (A) shows cortical activation (dark areas) over a transparent brain. The lower panel (B) shows the same cortical activation (orange to yellow areas) over an axial view of the brain. The colour scale corresponds to the T value of the activation. Activity is broadly similar to regions seen for the PD group (Figure 6-5) and activation can be seen bilaterally in the parietal cortex and frontal cortex corresponding to known locations of the FEF, SEF, PEF and PPC. Anterior to this are regions of activity consistent with the locations of the DLPFC. Activity in the anterior insula is also seen bilaterally. Activity is corrected for multiple comparisons using FWE (p < 0.05).
6.3.2.2 PD group – Memory-guided vs fixation

Figure 6-5 – Significant BOLD activity in the PD group in the memory-guided task greater than fixation. The top panel (A) shows cortical activation (dark areas) over a transparent brain. The lower panel (B) shows the same cortical activation (orange to yellow areas) over an axial slice view of the brain. The colour scale corresponds to the T value of the activation. Activity is seen bilaterally in the parietal cortex and frontal cortex. Activity is seen throughout the PPC with SPL, IPL with continuous activity joining both lobules covering the IPS. Activity is observed in the frontal areas which include the FEF and SEF. Anterior to this are regions of activity consistent with the locations of the DLPFC. Activation in the anterior insula cortex is seen bilaterally. Corrected for multiple comparisons FWE p < 0.05.
### 6.3.2.3 Control Group – Memory-guided vs fixation

Table 6-2 – Locations of BOLD activations greater in the memory-guided task compared to fixation for the control group.

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<tr>
<th>MNI coordinates</th>
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<th>Y</th>
<th>Z</th>
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<tr>
<td>FEF Middle Frontal Gyrus L</td>
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<td>Medial FEF - Superior Frontal Gyrus R</td>
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<tr>
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<tr>
<td>DLPFC Superior Frontal Gyrus R</td>
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### 6.3.2.4 PD Group – Memory-guided vs fixation

Table 6-3 – Locations of BOLD activations greater in the memory-guided task compared to fixation for the PD group.

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<tr>
<th>Lobe</th>
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<td>FEF Middle Frontal Gyrus L</td>
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<td><strong>Other</strong></td>
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<td>Putamen L</td>
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<td>5.55</td>
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6.3.3 Memory-guided event-related tasks

6.3.3.1 Perceive event activity
Figure 6-6 and Figure 6-7 show regions showing memory-guided saccade BOLD activity during the perceive period for control and PD groups respectively. Regions in the SEF are seen active in both groups. Within-group analysis showed additional BA 7 and middle temporal gyrus activity in the controls, which was not seen in the PD group. Table 6-4 and Table 6-5 show the locations of the significant clusters for control and PD within-group analysis. When a between-group analysis was performed, however, no significant group differences between PD and control groups were found.

6.3.3.2 Saccade event activity
Figure 6-8 shows the active regions during the “saccade” event for controls. Activity is seen in the right middle and inferior frontal gyrus. In addition, Figure 6-9 shows uncorrected data. While uncorrected results cannot be considered sufficient to report as significant (see multiple comparisons problem, page 68 in the Introduction chapter), the uncorrected images can often show useful information about the activity which is overlooked by a stringent corrections criteria. In this case, activity in the uncorrected image can be seen in the middle frontal gyrus bilaterally, as well as in posterior parietal areas, all areas known to be involved in memory-guided saccade control. Table 6-6 provides a list of the active regions seen in the corrected comparison for the control group.

Figure 6-10 shows activity during the saccade period for the PD group. Activity in the frontal eye fields, supplementary eye fields, DLPFC and posterior parietal cortices are seen. In addition, right insula activity is seen for this saccade period. Additionally, Table 6-7 provides a list of the active regions seen in this task for the PD group.
6.3.3.3 Control group - Perceive event activity

Figure 6-6 – Significant BOLD activity for the control group during the memory-guided “perceive” event greater than fixation. Top panel (A) shows activity (in grey/black) over a transparent brain. The lower panel (B) shows activity (in yellow) over an axial view of the brain. Once corrected for multiple comparisons (FWE p < 0.05), activity is sparse and seen in the SEF region and in the middle temporal gyrus. There was a small cluster seen active in the right Brodmann area 7.
6.3.3.4 PD group – Perceive event activity

(A)

(B)

Figure 6-7 – Significant BOLD activity for the PD during the “perceive” event greater than fixation. As with the previous diagrams, the top panel (A) shows cortical activation (dark areas) over a transparent brain. The lower panel (B) shows the same cortical activation (orange to yellow areas) over axial view of the brain. The colour scale corresponds to the T value of the activation. Activity for this event involves small areas of activity in the SEF and IPL. Activity is corrected for multiple comparisons using FWE (p < 0.05).
6.3.3.5 Control group - Perceive event activity

Table 6-4 – Locations of BOLD activations greater in the memory-guided task compared to fixation for the ‘perceive’ event for the control group.

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<th>X</th>
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<th>Z</th>
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</tbody>
</table>

6.3.3.6 PD group – Perceive event activity

Table 6-5 – Locations of BOLD activations greater in the memory-guided task compared to fixation for the ‘perceive’ event for the PD group.

<table>
<thead>
<tr>
<th>Frontal Lobe</th>
<th>T value</th>
<th>Z value</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supp motor area R</td>
<td>6.19</td>
<td>4.81</td>
<td>6</td>
<td>18</td>
<td>52</td>
</tr>
<tr>
<td>SEF Brodmann area 6 L</td>
<td>5.86</td>
<td>4.64</td>
<td>-10</td>
<td>10</td>
<td>54</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parietal lobe</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Brodmann area 40 R</td>
<td>6.19</td>
<td>4.81</td>
<td>46</td>
<td>-46</td>
<td>44</td>
</tr>
<tr>
<td>Inferior Parietal Lobule L</td>
<td>5.72</td>
<td>4.56</td>
<td>-48</td>
<td>-38</td>
<td>42</td>
</tr>
</tbody>
</table>
6.3.3.7 Control group - Saccade event activity- corrected for multiple comparisons

(A)

(B)

Figure 6-8 – Significant BOLD activity for the control group during the memory-guided “saccade” event greater than fixation. Top panel (A) shows activity (in grey/black) over a transparent brain. The lower panel (B) shows activity (in yellow) over an axial view of the brain. Once corrected for multiple comparisons (FWE p < 0.05), BOLD activity is seen with small clusters in the right FEF region.
6.3.3.8 Control group - Saccade event activity- uncorrected

(A)

(B)

Figure 6-9 – Uncorrected view of the same analysis from above (control group saccade event of the memory-guided task greater than fixation). Top panel (A) shows activity (in grey/black) over a transparent brain. The lower panel (B) shows activity (in yellow) over an axial view of the brain. In the uncorrected image, frontal and parietal activity is seen during the saccade event, much like the block design task. It is possible the FWE correction for multiple comparisons applies a too stringent correction threshold to allow activity in the event-related design to be detected as strongly as the block design task as the event-related task has fewer total trials.
6.3.3.9 *PD group – Saccade event activity*

Figure 6-10 – Significant BOLD activity for the PD group in the saccade event greater than fixation. The top panel (A) shows cortical activation (dark areas) over a transparent brain and the lower panel (B) shows the same cortical activation (orange to yellow areas) over an axial view of the brain. The colour scale corresponds to the T value of the activation. Activity for this event involved significant activity in the FEF, SEF and PPC. The FEF activity appeared to be more prominent in the right hemisphere of the brain with much smaller significant areas of activation seen on the left FEF. The analysis depicted is corrected for multiple comparisons (FWE p < 0.05).
### Control group - Saccade event activity

Table 6-6 – Locations of BOLD activity greater in the saccade event than fixation for the control group in the memory-guided task.

<table>
<thead>
<tr>
<th>Frontal Lobe</th>
<th>T value</th>
<th>Z value</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Middle Frontal Gyrus R FEF</td>
<td>6.45</td>
<td>4.94</td>
<td>26</td>
<td>-4</td>
<td>54</td>
</tr>
<tr>
<td>Inferior Frontal Gyrus R</td>
<td>6.44</td>
<td>4.94</td>
<td>50</td>
<td>6</td>
<td>28</td>
</tr>
<tr>
<td>Brodmann area 44 Precentral Gyrus R</td>
<td>5.61</td>
<td>4.5</td>
<td>52</td>
<td>14</td>
<td>10</td>
</tr>
</tbody>
</table>

### PD group - Saccade event activity

Table 6-7 – Locations of BOLD activity greater in the saccade event than fixation for the PD group in the memory-guided task.

<table>
<thead>
<tr>
<th>Frontal Lobe</th>
<th>T value</th>
<th>Z value</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medial FEF Middle Frontal Gyrus R</td>
<td>9.25</td>
<td>6.1</td>
<td>26</td>
<td>-2</td>
<td>54</td>
</tr>
<tr>
<td>Lateral FEF Middle Frontal Gyrus R</td>
<td>8.3</td>
<td>5.75</td>
<td>38</td>
<td>-4</td>
<td>56</td>
</tr>
<tr>
<td>Medial FEF Brodmann area 6 L</td>
<td>5.59</td>
<td>4.49</td>
<td>-24</td>
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</tr>
<tr>
<td>Medial FEF Middle Frontal Gyrus FEF L</td>
<td>5.54</td>
<td>4.46</td>
<td>-24</td>
<td>-6</td>
<td>54</td>
</tr>
<tr>
<td>SEF Brodmann area 6 R</td>
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<td>5.09</td>
<td>4</td>
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<td>64</td>
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<tr>
<td>Brodmann area 8 R</td>
<td>6.97</td>
<td>5.19</td>
<td>4</td>
<td>20</td>
<td>48</td>
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<tr>
<td>Supplementary motor area Superior Frontal Gyrus L</td>
<td>5.82</td>
<td>4.61</td>
<td>-10</td>
<td>0</td>
<td>72</td>
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<tr>
<td>DLPFC Middle Frontal Gyrus R</td>
<td>5.84</td>
<td>4.62</td>
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<td>40</td>
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</tr>
<tr>
<td>Brodmann area 44 R</td>
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<td>5.95</td>
<td>52</td>
<td>14</td>
<td>8</td>
</tr>
<tr>
<td>Inferior Frontal Gyrus R</td>
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<td>5.02</td>
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<td>26</td>
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<tr>
<td>Medial Frontal Gyrus L</td>
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<th>Z</th>
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<tbody>
<tr>
<td>Inferior Parietal Lobule R</td>
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<td>56</td>
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<tr>
<td>Brodmann area 40 Inferior Parietal Lobule R</td>
<td>8.29</td>
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<td>38</td>
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<tr>
<td>Brodmann area 40 L</td>
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<td>52</td>
</tr>
<tr>
<td>Brodmann area 40 L</td>
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<td>4.98</td>
<td>-44</td>
<td>-38</td>
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<tr>
<td>Superior Parietal Lobule L</td>
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<td>5.04</td>
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<tr>
<td>Inferior Parietal Lobule L</td>
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<td>34</td>
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<tr>
<td>Brodmann area 7 R</td>
<td>5.97</td>
<td>4.69</td>
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<td>52</td>
</tr>
<tr>
<td>Brodmann area 7 R</td>
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<td>4.55</td>
<td>22</td>
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<table>
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<th>Z</th>
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<tr>
<td>Insula Brodmann area 13 R</td>
<td>6.18</td>
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<table>
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<th>Z</th>
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<tr>
<td>Brodmann area 19 Fusiform L</td>
<td>6.18</td>
<td>4.8</td>
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<td>-70</td>
<td>-14</td>
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<tr>
<td>Fusiform Gyrus R</td>
<td>6.16</td>
<td>4.79</td>
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<table>
<thead>
<tr>
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<th>Y</th>
<th>Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clastrum putamen R</td>
<td>5.55</td>
<td>4.47</td>
<td>34</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>
6.3.4 Manually-identified ROI

Automatic clustering can often merge adjacent clusters together, missing important, closely spaced clusters of activation. ROI were visually identified from the block experiment and the results are shown in Table 6-8.

Table 6-8 – Coordinates of visually identified active ROI from the block design experiment. These regions were subsequently used in the event-related time course and PCA analyses. DLPFC – dorsolateral prefrontal cortex, FEF – frontal eye field, IPL – inferior parietal lobule, SPL – superior parietal lobule, SEF – supplementary eye field.

<table>
<thead>
<tr>
<th>ROI</th>
<th>Region</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>L DLPFC</td>
<td>-34</td>
<td>46</td>
<td>24</td>
</tr>
<tr>
<td>2</td>
<td>R DLPFC</td>
<td>34</td>
<td>46</td>
<td>24</td>
</tr>
<tr>
<td>3</td>
<td>L Lateral FEF</td>
<td>-40</td>
<td>-4</td>
<td>48</td>
</tr>
<tr>
<td>4</td>
<td>R Lateral FEF</td>
<td>42</td>
<td>-2</td>
<td>50</td>
</tr>
<tr>
<td>5</td>
<td>L Medial FEF</td>
<td>-29</td>
<td>-2</td>
<td>53</td>
</tr>
<tr>
<td>6</td>
<td>R Medial FEF</td>
<td>24</td>
<td>-6</td>
<td>54</td>
</tr>
<tr>
<td>7</td>
<td>L Insula</td>
<td>-36</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>R Insula</td>
<td>36</td>
<td>23</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>L Parietal (IPL)</td>
<td>-39</td>
<td>-46</td>
<td>46</td>
</tr>
<tr>
<td>10</td>
<td>R Parietal (IPL)</td>
<td>45</td>
<td>-46</td>
<td>47</td>
</tr>
<tr>
<td>11</td>
<td>L Parietal (SPL)</td>
<td>-26</td>
<td>-60</td>
<td>55</td>
</tr>
<tr>
<td>12</td>
<td>R Parietal (SPL)</td>
<td>28</td>
<td>-62</td>
<td>48</td>
</tr>
<tr>
<td>13</td>
<td>SEF</td>
<td>-1</td>
<td>3</td>
<td>57</td>
</tr>
<tr>
<td>14</td>
<td>Pre SEF</td>
<td>-2</td>
<td>20</td>
<td>47</td>
</tr>
</tbody>
</table>
6.3.5 Memory-guided event-related time course

Figure 6-11 to Figure 6-17 show event-related time courses for the selected ROI from Table 6-8. The time courses showed a common bimodal pattern. An initial peak in signal change is seen at 3 to 4 seconds after trial onset and a second peak at 10-12 seconds. The peak sizes also differed, with the second peak amplitude being larger than the first peak for most regions. The pattern of activation is compatible with the nature of the memory-guided task, with the first peak representing activity from the visualisation and initial memory of the flashed peripheral target during the “perceive” event. The second, more pronounced peak, likely represents the activity related to the generation of the saccade to the remembered location for the “saccade” event.

The IPS, bilaterally, on visual inspection, showed a group difference between the PD and control group for the second peak period: the PD group showed greater activity during the second peak. This was evident bilaterally. In contradistinction, the medial FEF visually showed a greater BOLD signal for the control group compared to the PD group in the first peak, more prominent on the left side. No bilateral group differences in the lateral FEFs were seen. No other regions showed bilateral group differences in BOLD activity for the memory-guided task.
The event-related time course of the right and left medial FEF. Time at 0 s represents the “perceive” event when the target stimulus was flashed. Error bars show standard error. After this flash, we can see an increase in the bold response of both PD and control groups, peaking at 3-4 seconds before reducing. After 5.6 seconds in, the fixation point was extinguished, cueing the saccade to the memorised position. We can see a larger rise in activation, thought due to saccade generation, from the 6 second mark peaking 4 seconds later, before declining. The activity continues to decline to a post-stimulus undershoot before returning to baseline as the participant fixates on the new position. Interestingly, there was no separate increase in BOLD activity relating to stimulus reappearance after the saccade – possibly due to only a small corrective saccade being needed. Both PD and control groups showed very similar levels of activation. In the first peak at 3-4 seconds, controls show, on visual inspection, slightly greater BOLD signal change than PD in the right medial FEF. In the left medial FEF, during the first peak, we see a slight difference in timing between the two groups with PD peaking at 3 seconds and 4 seconds for the controls. The peak signal change appears greater in the control group compared to the PD group for the first peak, with a greater difference than for the right FEF. The second peak at 10 seconds showed no visible difference between the two groups.
Figure 6-12 – Time courses for the right and left lateral FEF. Error bars show standard error. The activation pattern shown here is similar to the medial FEF region, with two-peaks, the first peak at 3 seconds and the second peak at 10 seconds, believed to correlate with visual attention/initial memorisation and saccade generation events respectively. For the right lateral FEF, the PD group, on visual inspection, showed a greater BOLD signal compared to controls at the second peak of the task – corresponding to saccade generation. This, however, was not evident on the left lateral FEF. No bilateral group differences in BOLD activation was seen for this region.
Figure 6-13 – Time courses for pre-SEF and SEF activity during the memory-guided task. The shape of the response curve showed a two-peak shape with the second peak being of higher amplitude than the first. The approximate timing of the peak is similar to that observed in the FEF (Figure 6-11 and Figure 6-12), with the first peak at 4 seconds and the second peak at 10 seconds. No bilateral group difference was seen found between PD and controls.
Figure 6-14 – Time courses for both PD and controls in the IPL (right – upper image, left – lower image). The time course shape is similar other eye control regions, being characterised by two peaks, with a higher second peak amplitude compared to the first. A difference in activity can be observed on visual inspection between PD and control groups for the second peak. For both left and right IPL regions, the PD group showed a larger amplitude for the second peak compared to the controls (within thin grey lines).
Figure 6-15 – Time courses for both left and right SPLs for the memory-guided task. Both sides show the two-peak shape typical of other eye movement regions, with the second peak being of higher amplitude than the first. This pattern is seen in both PD and controls. Both PD and control time courses show a similar shape with the signal change close between the two groups at each time point. No bilateral group difference was seen found between PD and controls.
Figure 6-16 – Time courses for both left and right sided DLPFC activity. The right DLPFC followed the two-peak shape seen in other areas of eye movement control. The PD group for the right DLPFC showed a higher second peak amplitude than the first whereas both peaks of the control group are roughly equal in amplitude. The left DLPFC showed a much different pattern compared to the right. Overall, the maximum percent signal change is lower on the left DLPFC compared to the right DLPFC with the left DLPFC peaking at roughly 0.16%, compared to the right DLPFC, which showed a 0.4% maximum signal change. A three peak pattern can be seen for the PD group, with a third peak seen at 14 seconds. The control has the first peak at 3 seconds with no second peak. The decrease in activity after the first peak is not linear, with a levelling off close to where the second peak would be expected. With a much lower overall percent signal change, it is unclear if the shape of the haemodynamic response relates to meaningful task-related activity or if contributions from underlying sustained processes are occurring, which do not follow a peaked BOLD response shape.
Figure 6-17 – Time courses for the right and left insula region. The activity showed a very similar pattern when comparing both left and right insula regions, with the noted two-peak pattern. The pattern is also very similar between the two groups, indicating no pronounced group difference in activity for the insula region for memory-guided saccades. The similarity of the BOLD response shape to the response seen in known saccade control regions would suggest the insula could potentially operate in a similar way as an eye field during memory-guided saccades.
6.3.6 PCA – results

Figure 6-18 shows the percentage variance explained by each principal component. The loading values of each ROI for PC 1, 2, and 3 are shown in Figure 6-19, Figure 6-20 and Figure 6-21 respectively. Plots of the loading values for PC 2 against PC 3 are shown in Figure 6-22. The K-means clustering analysis was used to separate the 14 ROIs into 4 clusters. One cluster comprises all the frontal and supplementary eye fields. A second cluster separated both DLPFCs. The posterior parietal regions were mostly grouped in a third cluster, with the left SPL and both anterior insula ROI forming the fourth cluster. Finally, an illustration of the shape of each principal component is shown in Figure 6-23.

![Figure 6-18](image_url) – Plot of the variance of the BOLD time courses of every ROI explained by each PC. The first three components explained 80% of the variance between the BOLD time courses of every ROI and were retained for subsequent analysis.
Figure 6-19 (above) – Loading values for each ROI shown for PC 1. It can be seen that all 14 ROIs were loaded strongly positive for PC 1. This indicates a shared common component of BOLD activity shared for every ROI for the memory-guided saccade task.

Figure 6-20 – Loading values for each ROI shown for PC 2. In contrast to PC1, PC 2 is differentially expressed by each ROI. This indicates this component captures some aspect of their functioning that differentiates their contributions to the task, with FEF and SEF regions showing higher PC 2 expression, DLPFC and insula regions showing intermediate PC2 expression and posterior parietal areas, in general, showing the lowest PC 2 expression. FEF – Frontal eye field, SEF – Supplementary eye field, DLPFC – dorsolateral prefontal cortex, IPL – Inferior parietal lobule, SPL – Superior parietal lobule.
Figure 6-21 – Loading values for each ROI shown for PC 3. The DLPFCs bilaterally were particularly negatively loaded on this component. Other regions such as the FEF and posterior parietal cortex regions showed weakly positive PC 3 loadings. FEF – Frontal eye field, SEF – Supplementary eye field, DLPFC – dorsolateral prefrontal cortex, IPL – Inferior parietal lobule, SPL – Superior parietal lobule.

Figure 6-22 – The loading values of PC3 vs PC2. Also shown are results from the K-means clustering analysis with 4 clusters. Ovals and colours indicate separate clusters. Cluster 1 is formed by both DLPFC. Cluster 2 contains all posterior parietal ROI except the L SPL, which is located in cluster 3, along with both insula regions. Cluster 4 shows the cluster formed by all frontal and supplementary eye field ROIs.
Figure 6-23 – Shape of each principal component. PC 1 (black) follows a two-peak activation pattern. This is similar to the general two-peak BOLD activity shape seen in the individual time courses for the majority of the ROI and it follows that this PC was strongly expressed by every ROI. PC 2 (red) shows peaks before the peaks of PC 1. PC 2, therefore, may represent preparatory or anticipatory activity preceding the general task activity that is represented by PC 1. PC 2 is strongly expressed in the FEF and SEF regions and negatively expressed for the posterior parietal regions. PC 3 (blue) shows a relatively flat shape before a more pronounced peak during the second peak of PC 1. This pronounced peak occurs at the time of saccade generation. Therefore, PC3 may represent activity related specifically to saccade generation. This PC is negatively expressed by the DLPFC, with all other eye field ROI, along with the insula cortices, showing a higher PC 3 expression.
6.4 Discussion

In this study, we observed BOLD activity in the FEF, SEF, posterior parietal cortex (PPC), DLPFC and insula for the memory-guided task. We also found activity within the PPC as two continuous clusters - one in the SPL (parietal area A) and one in the IPL (parietal area B), with additional activation present in the IPS between these two areas. Parietal areas A and B are illustrated in Figure 6-24. Figure 6-25 and Figure 6-26 illustrate the anatomy of the PPC, showing regions within each lobule, such as the supramarginal gyrus and angular gyrus, both located within the IPL.

A subtle difference was found on visual inspection between PD and controls in the IPL bilaterally - a greater change of BOLD activity was seen during activity related to memory-guided saccade generation in PD than the control group. These observations would suggest firstly, that the PPC has two regions involved in the memory-guided saccade task, and secondly that IPL activity may be altered PD. In the frontal cortex, the medial FEF showed a subtly reduced BOLD activity in the memory phase in the PD group, with controls showing a slightly higher activity during the first time course peak thought to correspond to initial stimulus perception and memory events. This may indicate altered FEF activity in PD for the memory-guided task. No other pronounced bilateral BOLD signal changes were observed between PD and control groups.

6.4.1 Discussion - Aims of study

This experiment shows that significant functional changes are not detected for PD in memory-guided saccades compared to controls, using event related fMRI. This does not support the first aim, in testing the hypothesis that eye control regions would show detectable functional changes in PD while performing the memory-guided saccade task.

This study satisfies the second general question of improving our understanding of eye movement control by using PCA and cluster analysis. The expression of principal components of each region of interest are consistent our present knowledge of eye movement control. Notably, this consistency with prior understanding arose despite these analyses arising from a completely data driven approach, free of any prior assumptions. The cluster analysis was also able to group anatomically and functionally related regions together without prior information, supporting the ability of this technique to determine functionally related regions from PC expression.
Figure 6-24 – Active (orange) PPC regions for the memory-guided task. Red squares indicate PPC regions selected for the time course analysis. “A” represents the superior parietal lobule (SPL) and “B” the inferior (IPL).

6.4.2 Posterior parietal cortex anatomical areas

The PPC consists of the superior parietal lobule (SPL), inferior parietal lobule (IPL) and intraparietal sulcus (IPS; Figure 6-25 and Figure 6-26). The inferior parietal lobule consists of the supramarginal gyrus (SMG) anteriorly and the angular gyrus (AG) posteriorly. The lower, inferior region of the inferior parietal lobule contains the temporal parietal junction (TPJ).

Figure 6-25 – Diagram of the PPC region in the human and primate brain (Bisley & Goldberg, 2010). Diagram a (left) represents the human brain and b (right) represents the macaque monkey brain. From the human brain: SPL, superior parietal lobule; Ang, angular gyrus; Smg, supramarginal gyrus; IPS, intraparietal sulcus; IPL, inferior parietal lobule; TPJ, temporoparietal junction. From the primate brain: V1-V4, visual cortices; PO, parieto-occipital area; PIP, posterior intraparietal area; MIP, medial intraparietal area; LIP, lateral intraparietal area; VIP, ventral intraparietal area, AIP, anterior intraparietal area. Figure permissions obtained (Bisley & Goldberg, 2010).
6.4.3 Parietal lobe activity for the memory task

During the memory-guided task, subjects shift attention to the target flash while maintaining visual fixation on the fixation stimulus, in a process known as covert attention (Corbetta & Shulman, 2002). Pierrot-Deseilligny et al. (2002) have described three more processes in the memory-guided task that occur after stimulus presentation – spatial integration, memorisation and saccade triggering. The PPC has been implicated in all these functions. Here, we will attempt to summarise the literature available for this area to help our understanding of these parietal areas and what the BOLD activation differences in PD observed in the present study might indicate. The following sections on PPC function is complex, given the multitude of functions ascribed to posterior parietal subregions and the resulting lack of consensus (See section 1.2.3.11 in the introduction). Three subsections cover PPC involvement in each process of the memory-guided task. PPC attention-related processes are covered first (Sections 6.4.3.1 to 6.4.3.4). The next sections cover PPC spatial-memory processes (section 6.4.3.6 and 6.4.3.6). The PPC’s role in saccade triggering has already been discussed in the introduction section (1.2.3.11) and won’t be repeated here. Finally, section 6.4.3.7 ties these functions to my result findings seen in Figure 6-14 (This figure visually depicts increased IPL BOLD activity during the memory-guided saccade event for the PD group compared to controls.)
6.4.3.1  **Attention and the PPC**

The PPC plays an essential role in visual attention. Most prominently, unilateral brain lesions in the PPC cause a condition called visual neglect whereby the patient is unable to attend to stimuli and make saccades to the contralateral side of the lesion (Morrow & Sharpe, 1993; Moscovitch & Behrmann, 1994). All regions within the PPC – SPL, IPS and IPL have been noted to be involved in spatial attention (Friedrich et al., 1998; Posner et al., 1984; Vandenberghe et al., 2012). Spatial attention is closely related to saccade programming, with attention always being directed to the end-location of a planned saccade – saccades cannot be generated to a location different to the attended location (Deubel & Schneider, 1996; Hoffman & Subramaniam, 1995; Kowler et al., 1995).

6.4.3.2  **Attention and the LIP**

Within the PPC, the LIP is thought to have both attention and saccade functions (Bisley & Goldberg, 2010). Biesley and Golberg argued that the LIP forms a priority map of the world, in which attention is given and a saccade generated based on the importance of the object or location. The monkey LIP corresponds to the human PEF (Culham et al, 2006) and is located in the human IPS (Rushworth et al., 2001) and parts of the SPL (Konen & Kastner, 2008; Sereno, 2001). We believe parietal area A seen in our study is the PEF, or the human equivalent to the LIP. Sereno (2001) proposed the human homologue of the LIP (PEF) to be at coordinates x = 32, y = -68, z = 46 very close to our parietal area A. (Our parietal area A has the coordinates of x = 29, y = -58, z = 43). Coordinates from a more recent human study (Konen & Kastner, 2008) places regions equivalent to the human LIP within the IPS and SPL, also very close to our parietal area A (see Table 6-9 for a comparison of human equivalent LIP coordinates between studies).

**Table 6-9 – Talaraich coordinates of potential human LIP locations described by Sereno (2001) and Konen & Kastner (2008). Coordinates from parietal area A in our study were transformed from MNI space to Talaraich by the methods described by Lacadie, Fulbright, Constable, & Papademetris (2008) to allow comparison.**

<table>
<thead>
<tr>
<th>Study</th>
<th>Region</th>
<th>Right</th>
<th>Left</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sereno 2001</td>
<td>Possible human LIP</td>
<td>32</td>
<td>-68</td>
</tr>
<tr>
<td>Konen &amp; Kastner 2008</td>
<td>IPS1</td>
<td>25</td>
<td>-72</td>
</tr>
<tr>
<td></td>
<td>IPS2</td>
<td>24</td>
<td>-71</td>
</tr>
<tr>
<td></td>
<td>SPL1</td>
<td>18</td>
<td>-68</td>
</tr>
<tr>
<td>Feng 2017</td>
<td>Parietal area A</td>
<td>29</td>
<td>-58</td>
</tr>
</tbody>
</table>
A review by Corbetta & Shulman (2002) describes the IPS as being part of an attention network linked with the FEF, forming what is known as the dorsal attention system. This dorsal attention system is thought to mediate "top-down" attention - that is attention controlled by cognitive factors such as previous knowledge about the locations to pay attention to – as in the memory-guided saccade task. By contrast, the ventral attention system, which is described as more to do with unattended attention shifts (e.g. an alarm going off unexpectedly) – is not likely to be so relevant to our remembered task. Silver et al. (2005) conducted an fMRI study of covert attention whereby stimuli were presented around a central fixation point. Participants were asked to identify locations of the peripheral stimuli while maintaining fixation. Two regions within the IPS were involved in this covert attention task, both of which were reported candidates of the human LIP/PEF. It is likely that parietal area A, which we believe to be the PEF/LIP, showed activation that represents, at least in part, the attention function of this area during memory-guided saccades.
6.4.3.3 **Attention and area 7a**

A second saccade control area known as area 7a is present in the PPC. Contrary to the LIP, which is thought to have both attention and saccade roles, area 7a is thought to be involved mostly in directing attention (Steinmetz et al. 1995). Stimulation studies have been unable to elicit saccades in area 7a (but can in the LIP) (Thier & Andersen, 1998). The discharge of 7a neurons have also been found to be related to eye (Andersen et al., 1990) and head position (Brotchie et al., 1995) and this link is thought to represent the involvement of area 7a in the synthesis of information in directing visual attention (Brotchie et al., 2003). Steinmetz et al. (1995) and Mountcastle et al. (1981) noted macaque 7a neurons fired when a peripheral stimulus was presented during an active fixation task. A peripheral stimulus presentation occurs during fixation in our memory-guided task during the peripheral flash period. We can, therefore, expect the human 7a to activate during the memory-guided task relating to attention.

There does not appear to be a consensus on the human region that corresponds to area 7a. Hutchinson et al. (2009) suggested the ventral PPC contains area 7a and Konen & Kastner (2008) report 7a could also be within certain IPS regions and the SPL. Monkey studies show 7a to be in the IPL (Andersen et al., 1990) with Leigh & Zee (2000) suggesting it is possible the human inferior parietal lobule contains area 7a. However, this would run contrary to the more recent study done by Konen & Kastner (2008) suggesting the 7a may comprise regions of the IPS and SPL. Our task showed activation in both the superior and inferior parietal lobule. Without a firm consensus on the human 7a location, we can only speculate that either one of parietal area A or B corresponds to 7a activation during the memory task. Should parietal area B represent the human 7a, then it would suggest that 7a might be affected in PD, with a different BOLD response curve during the memory-guided task compared to unaffected controls. However, this is speculation given the dearth of studies confirming parietal area B within the IPL corresponds to the human area 7a.

6.4.3.4 **Attention and the inferior parietal lobule**

The IPL is thought to contribute to spatial attention function (Friedrich et al., 1998; Posner et al., 1984). However, there is a degree of uncertainty regarding the nature of attention-related inferior parietal lobule function. A review by Husain & Nachev (2007) highlights the issues in our understanding of the IPL for attention processes. For example, this region does not completely fit into the conventionally defined dorsal or ventral attention systems as described by Corbetta & Shulman (2002). Husain and Nachev suggested that the IPL contains further
subregions which are part of attention networks that are non-spatial in nature e.g. auditory attention (Linden et al., 1999) or related to the temporal aspects of attention e.g. sustained attention (Husain, 2005). Factors contributing to the uncertainty of IPL function include inter-individual anatomical variability and the large number of networks involving the IPL (Igelström & Graziano, 2017). The IPL has been described as a network “hub”, or an area with a particularly high number of network connections, and is involved in a broad range of cognitive functions not limited to attention (Igelström & Graziano, 2017). Examples of these functions include self-body perception (Blanke et al., 2002), mind-wandering (Buckner et al., 2008) and a perception-to-action system used to envisage the process of manipulating objects in peri-personal space, and which may have allowed humans to first conceptualise the use of tools (Kastner et al., 2017). A number of these IPL networks have only recently been found so there are few, if any, follow up confirmatory studies. Using resting-state functional connectivity imaging, 5 subregions were found in the left IPL and 7 in the right (Wang, Xie, et al., 2016; Wang, Zhang, et al., 2016). These subregions were considered to be involved in attention processes as well as sensory/motor processing, movement imagination, spatial cognition and higher order functions such as social cognition (Wang, Zhang, et al., 2016).

Non resting-state fMRI studies have implicated the IPL in attention functions. An investigation by Perry & Zeki (2000) found the supramarginal gyrus (SMG), particularly the right side, to be involved in covert attention (and interestingly, the anterior insula which will be discussed later). The angular gyrus, particularly the right side, has been seen to be more active in reflexive saccades rather than voluntary (Mort et al., 2003), which the authors suggest supports a role for the IPL in exogenous (directed by external stimuli, rather than internal volition) attention shifts. With evidence from fMRI and lesion studies for a role of the IPL in attention processes, it is highly likely that the activity within parietal area B in the present study, located in the IPL, does relate, in part, to attention processes, despite our limited understanding of this region. It follows that the BOLD activation differences seen in the IPL between groups in our study may well relate to attention process impairments in the PD disease state. However, it is equally likely that the differences between the two groups reflects a number of other processes or networks in which the IPL is involved. Cognitive processes such as movement imagination and spatial cognition are likely to occur during the memory-guided task so we cannot necessarily attribute IPL activity group differences to altered attention function alone. The activity seen in the IPL in our study appears only as one broad region of activation. Higher resolution fMRI scans in the future may be able to visualise
differential functional activity during task performance in various IPL subareas defined in the recent network studies and provide more insight into the functions of IPL subregions.

6.4.3.5 \textit{Spatial memory processes in the PPC}
TMS methodology has indicated that the PPC is critically involved in the initial several hundred millisecond period of spatial integration and memorisation of a target (Muri et al, 1996). Interruption of PPC function via TMS during the early memory period results in defective memory-guided saccades (Muri et al., 1996; Oyachi & Ohtsuka, 1995). Furthermore, TMS of the PPC immediately following stimulus presentation impairs accuracy of the saccade, but not when applied some 500ms after the stimulus presentation, suggesting that after the initial several hundred millisecond period, spatial memory is retained elsewhere (Brandt et al., 1998; Muri et al., 1996). The PPC has a number of connections to the frontal lobe (Cavada & Goldman-Rakic, 1989) and SC (Lynch et al., 1985). Damage to the PPC affects memory-guided saccade performance, with patients making saccades with decreased gain. However, those with damage to the parietal-SC pathway, but not PPC itself, do not have these memory-guided saccade deficits. As a consequence it is widely believed that PPC signals for the memory-task are transmitted to the frontal lobe, specifically to the DLPFC (Chafee et al., 2011; Chafee & Goldman-Rakic, 2000; Gaymard et al., 1998; O'Sullivan et al., 1995; Pierrot-Deseilligny et al., 1991; Salazar, 2012; Sweeney et al., 1996) rather than directly to the SC. This is felt to occur some 300 ms following initial stimulus presentation (Le Heron et al., 2005; Muri et al., 1996).

More recent event-related functional imaging studies of memory-guided saccades have given a differing picture of the role of the PPC in memory-guided saccades. Rather than confirming the idea of the PPC being only involved in very early spatial memory as reported in previous neurostimulation studies, a number of event-related fMRI studies have described sustained activation in the IPS, well after the 300 ms mark, and attributed to memory processes: Brown et al. (2004) described a fronto-parietal system that is active for the memory-guided saccade task. They report greater activity in the right ventral IPS, right medial FEF and the SEF during the delay period of a memory-guided saccade task compared to a comparator visually-guided saccade task, and they attributed this increase in activity to be part of working memory processes during the delay phase of the memory-guided task. Brignani et al. (2010) observed sustained IPL activity during a memory-guided saccade task which they concluded supported the notion that the IPL is involved in short-term maintenance of spatial information.
Other reports have been more cautious in their conclusions. McDowell et al. (2008) in a review, describe “robust” delay period-related activity in the PPC in multiple studies including Brown et al. (2004) and Brignani et al. (2010), but do not discuss further whether this delay period activity is related to memory retention or other processes. Srimal & Curtis (2008) reported delay-related BOLD activity in the parietal cortex for the memory-guided task. The authors could not determine if the activation was from sustained retaining of the memory cue location or if it was a sustained planned motor response for the future. Geier et al. (2007), using a memory-guided task comparing short (2 s) and long (10 s) delay periods, found a sustained response pattern in the right supramarginal gyrus in the IPL during the delay period. The authors attributed this supramarginal gyrus activity to a state of preparedness to generate a saccade rather than to the maintenance of spatial coordinates. Schluppeck (2006) also used a variable length memory-guided paradigm and found sustained activity in IPS1 and IPS 2 regions during the delay period. Schluppeck reported these IPS areas to represent potential human homologues of the monkey LIP, and associated with attention and memory functions. A memory-guided study by Curtis & D’Esposito (2006) utilised a paradigm that included four locations that were briefly illuminated and then participants were cued to make a saccade to one of the remembered four locations following a delay period. Area IPS did not show sustained activity during the delay in their task but the FEF did. On the other hand, the IPS was activated after the participant was cued to make a saccade to a memorised location (before the saccade was made). This was taken to imply that the FEF was involved in maintaining spatial memory of saccade goals while the IPS was especially concerned with conversion of the instructional cue into spatial coordinates for the saccade.

Those reports that suggest that the sustained PPC activation is related to the retention of spatial memory of the cue location, particularly for long delays of 10 seconds or over, contradict conclusions from earlier TMS studies. Those earlier studies reported that TMS to the PPC (where the IPS is located) during a period of several hundred milliseconds after stimulus presentation did not disrupt memory-guided saccades. A potential weakness of these studies, such as by Geier et al. (2007) arises from using the shape or sustainment of the haemodynamic response to make inferences on working memory function. It may not be possible to determine if sustained activity represents memory maintenance activity or other processes such as response preparation or attention processes simply from the shape of the hemodynamic response (Lebedev et al., 2012). Furthermore, sustained delay period activity is not consistently seen in regions believed to be involved in task memory processes (Zaksas &
Pasternak, 2006). Riggall and Postle (2012) suggest memory storage processes may involve a number of distributed cortical regions with subthreshold activity not able to be detected using current univariate MRI methods.

The shape of what is considered delay activity in the haemodynamic response curve varies between studies. As previously mentioned, Geier and colleagues (2007) compared short (2.5 s) to long (10 s) delay period memory-guided saccades. They described delay or maintenance related activity as a single broad peak. A response curve with a biphasic shape with two peaks was described as showing response preparation (see Figure 6-27).

![Figure 6-27](image_url)

**Figure 6-27** – Maintenance BOLD activity compared with response preparation-related BOLD activity according to Geier et al. (2007). Red traces indicate short delay period trials. Blue traces indicate longer trials. Geier and colleagues concluded that maintenance-related activity should not track downward during prolonged delay phases. Figure permission obtained (Geier et al., 2007).

By contrast, Brown et al. (2004) compared visually guided saccade activity to memory-guided activity with a delay period of 9.8 s. The response curve from the right IPS in their study showed a biphasic shape. From the description of the expected time courses provided by Geier et al., the shape of the haemodynamic response in Brown’s study would be more associated with response rather than maintenance. However, Brown et al. compared the BOLD time course of the memory task to a visually guided task and reported that activity in the period between the two peaks in the memory-guided task was greater than in the visually-
guided task. This activation was interpreted as representing maintenance activity, despite the time course having a biphasic shape. (See Figure 6-28).

![Figure 6-28](image)

Figure 6-28 – BOLD time courses as found by Brown et al. (2004) during a memory-guided saccade task for the ventral intraparietal sulcus (vIPS) and rostral intraparietal sulcus (rIPS) (solid black time course) compared to a visually-guided saccade task (dotted black time course). Vertical dotted lines indicate events, in order from the left; trial start, stimulus “flash” and reappearance of the fixation stimulus. Both graphs show a bimodal shape with two peaks. Delay period activity is greater in both IPS regions in the memory-guided task vs visually-guided task. Adapted from Brown et al. (2004) - figure permission obtained.

Both parietal regions A and B in the SPL and IPL in our study showed a biphasic response similar to the observations of Brown and colleagues. The aim of the present investigation was to compare PD and control BOLD activity. The procedure was not set up to optimise detection of delay related BOLD activity. We did not vary the delay period and we used a relatively short 5 second memory period and did not employ a comparator visually guided task. Therefore, whilst on visual inspection, the BOLD response in our experiment appears similar to the plots in the study of Brown et al., we cannot confirm that the activity between the peaks reflects delay-related activity.

6.4.3.6 Memory processes and the inferior parietal lobule

Aside from Brignani et al, (2010), few other studies have described IPL as having visuospatial memory-related function. Several have, instead, suggested that the IPL is involved in episodic memory (Cabeza et al., 2008), which may also involve regions of the IPS (Wagner et al., 2005). Baldassano et al. (2016) described a cortical network comprising the caudal IPL as well as the retrosplenial complex and anterior parahippocampal region. This network was shown to exhibit high resting-state hippocampal coupling, suggesting involvement in episodic memory. The relationship of episodic memory and visuospatial memory is unclear but one
study found that visuospatial (and verbal) memory performance is related to episodic memory retention (Janssen et al., 2015). Of note, parietal lesions do not typically cause episodic memory loss. Wagner et al., (2005) have theorised that parietal contribution to episodic memory may be such that it is not important for memory expression, or that impairments may only be shown under specific conditions which have not been tested.

Spatial memory and attention processes in PD. In memory-guided saccades, hypometria, or the undershooting of saccades is a consistent finding in PD compared to controls (Lueck et al., 1992; Lueck et al., 1990). Le Heron et al., (2005) found impairments in the percentage error in final eye position for PD patients with memory delay periods of 3 seconds. Chan et al. (2005) also found PD patients to make undershooting memory-guided saccades and were less able to make memorised sequences of eye movements correctly. They suggested that there is a deficit in spatial working memory in PD that leads to the difficulty in generating the correct saccade sequence. A number of other investigations have also revealed working memory impairments in PD. Helmuth et al. (2000) found deficits in PD in learning a finger button pressing sequence. They, however, found no impairment in PD patients when learning a spatial location sequence. Ketcham et al. (2003) reported that PD patients had impairment transforming spatial information into motor action. They suggested that this problem is related to dopamine depletion in the caudate nucleus. Rottschy et al. (2013) used a typing-sequence recall task to investigate working memory in PD. PD subjects had mostly decreased activation during the memorisation and execution of their memory task in regions that included the superior parietal lobule, intraparietal sulcus and right inferior parietal lobule. There was also increased activation in the posterior parahippocampal gyrus and the authors suggested that detrimental aberrant activity in this region was responsible for impaired performance in PD.

Our fMRI study employed a very similar paradigm to that in the study by Le Heron and colleagues (2005), differing only in the delay period (ours being 5.7 seconds versus 3.0 seconds). We found altered activity in the IPL in the PD group during the memory-guided saccade task. The difference in IPL activation was seen during the latter of the two activation peaks, and corresponding to the time we would expect to see neural activity during the saccade. No difference was seen between PD and controls during the memory delay period before the saccade. It seems less likely then that the difference in activation between PD and controls is related to working memory during the delay. Previous studies suggest the PPC is only involved in very early memorisation, only in the first 300 ms and not the entire 5.7 seconds of memory needed for our study. More likely, this IPL activity difference may relate
to memory retrieval at the time of the saccade, or the use of visuospatial information to direct a voluntary saccade to the remembered location.

It is possible that the increase in IPL activity seen in PD could represent aberrant activity similar to that described by Rottschy et al. (2013). The IPL is thought to be involved in the visuospatial integration process which calculates saccade amplitude (Pierrot-Deseilligny et al., 1995). Drawing from the proposals by Helmhuth et al. (2000) and Ketcham et al. (2003) regarding an impairment of the visuospatial to motor movement conversion in PD (in finger-pointing tasks), it is possible that aberrant activity in the IPL seen in the present study could also reflect impairment of visuospatial memory to oculomotor movement conversion.

Another possibility is that altered BOLD activity in PD could potentially be related to the memory retrieval processes in the memory-guided task. More activity in PD at the point of saccade generation may represent a higher percentage change necessary to recall/utilise visuospatial memory information, leading to the impairments in final eye position seen in the Le Heron et al. study.

Geier et al. (2007) concluded that sustained activity in the IPL represents preparedness to make a saccade to an area. IPL is also active in voluntary saccade planning (Ptak et al., 2011). TMS over bilateral IPL impairs sustained spatial attention (Lee et al., 2013). If preparedness to make a saccade to a certain location is linked with sustained attention process to that location, then the altered activation we observed in the IPL could reflect impaired visuospatial sustained attention processes in PD. If attention to the planned saccade location is impaired, this could lead to the inaccurate memory saccades consistently encountered in PD.

6.4.3.7 Memory guided saccade generation in PD

The PPC plays a critical role in saccades and in particular, the LIP is strongly active during remembered saccades (Sweeney et al., 1996). Pierrot-Deseilligny et al., (2002) describes the LIP as being involved in triggering reflexive saccades to targets in the visual environment. The LIP is also active in monkeys when withholding a saccade and is thought to be involved in planning anticipated saccadic movement (Barash et al., 1991; Pare & Wurtz, 1997).

The IPL has a particular role in saccades directed by a reflexive shift of attention. Mort et al. (2003) reported activation in the angular gyrus (in particular the right side) during a reflexive saccade task greater than a predictive task. Perry & Zeki (2000) observed that the supramarginal gyrus (located within the IPL) was activated with covert attention shifts and reflexive saccades, especially the right supramarginal gyrus. Sheliga et al. (1995) describe a
premotor theory of attention, whereby attention shifts - which activate saccade generating areas - can activate these same regions even when saccades are not executed.

With these understandings, we can generate an explanation for our observations of increased BOLD activity in the IPL in the PD group which we propose reflects increased IPL activation during attention shifts in PD. Drawing from the premotor theory of Sheliga et al. (1995) and the theory of attention by Bisley & Goldberg (2010) where attention-related activity is thought to relate to priority, with saccades automatically generated to the highest attention points, increased IPL activity during attention shifting processes could reflect the assignation of a higher priority to each attention shift, and as a result predispose PD to generate more erroneous anticipatory saccades. However, that the timing of the BOLD signal changes between PD and controls, with greater IPL activity only seen during the later “saccade” event rather than the “perceive” event when erroneous reflexive saccades are made, is counter to this proposal. Additionally, it is possible that the number of trials was not sufficient to permit the development of a true group difference during the “perceive” event. Alternatively, it might be that any true effect is masked or confounded by PD participants’ making more erroneous anticipatory saccades. Future studies using more reliable eye-tracking equipment in the MRI scanner may be able to identify and exclude, or specifically study error trials to ascertain the effects of erroneous anticipatory saccades on BOLD activity.

Chan et al. (2005) discussed the notion of adaptive behaviour in PD. Since PD patients are slower to initiate voluntary goal-directed movement, the saccade inhibition threshold may be set lower to help facilitate movement. Saccade initiation may need to be bolstered by a more readily activated attention switching system in PD. This, in turn, could give rise to a compensatory increase in activity in the IPL and reflected in our observations of increased IPL activity during the saccade period in PD compared to controls.

As mentioned previously, a limitation of our study was that we were unable to determine the proportion of anticipatory saccades made within the MRI scanner. Our equipment could not reliably track and capture erroneous saccades during MRI scanning, which means we could not specifically exclude error trials during analysis. A possible reason for the difference between PD and controls in IPL activity may relate to the PD group simply making more saccades. This possibility though is made less likely by the timing of the IPL activation difference. The difference in the BOLD response between the groups was around the second peak relating to the “saccade” event rather than the first peak relating to the “perceive” event. The second peak is more related to the time of saccade generation to the memorised location,
and at the expected timing for a correct remembered saccade. The first peak, which occurs during the period when erroneous anticipatory saccades are made, showed no difference between PD and control groups in the IPL. Should erroneous anticipatory saccades be causing an activation difference, a higher number of saccades made by the PD group should equate to greater BOLD activity in saccadic eye fields during the “perceive” event. As no group difference was seen during this event period, it becomes less likely that the differences seen in IPL activity are explained purely by an increased number of erroneous anticipatory saccades by the PD group.

6.4.4 DLPFC

No significant group activation differences in the DLPFC were present in either block or event-related experiments. Clear DLPFC activity was detected in the memory-guided task for both PD and control groups in our block design experiment. No significant DLPFC activation, however, was detected in either “perceive” (initial appearance of target stimulus “flash”) or saccade (generating saccade to memorised location) events in PD and control groups. This observation (of the absence of DLPFC activity in the event-related paradigm) may be explained by the block paradigm simply having a higher number of trials (54 memory trials in the block experiment vs 18 trials in the memory experiment). However, this may also relate to the nature of sustained delay period activity during the memory period, being sufficiently different from the canonical haemodynamic response to be able to be detected in a whole-brain analysis using GLM (see pages 194 to 195 in the introduction section of this chapter for further explanation). In the following sections, I discuss DLPFC function and location and how this relates to DLPFC activity differences detected in our memory-guided tasks.

The DLPFC is believed to be involved in the process of spatial memory (Curtis, 2006; Curtis & D’Esposito, 2003). There are several theories regarding the nature of DLPFC involvement in working memory; whether it participates in all components of short-term memory storage, manipulation and utilisation, or only one component (Levy & Goldman-Rakic, 2000). The DLPFC may also contain functional divisions, each with separate contributions in handling different information types and for different cognitive tasks (Cieslik et al., 2013). The DLPFC, not being a specific eye field, has not been reported to play a role in saccade generation. However, through its role in working memory, DLPFC dysfunction in PD is thought to cause performance deficits in memory-guided saccades (Le Heron et al., 2005; MacAskill et al., 2002).
6.4.4.1 DLPFC subregions

The DLPFC is located within the frontal cortex in Brodmann areas 46 and 9 and is thought to comprise several subregions. Levy & Goldman-Rakic (2000) suggested that the DLPFC is segregated according to the type of sensory information being processed, with some regions responsible for visuospatial processing and others for nonspatial processing such as faces and object recognition. In a review, Cieslik et al. (2013) posited that the DLPFC can be separated into two subregions: anterior-ventral and posterior-dorsal. The anterior-ventral region appears to be more involved in attention and action inhibition processes and tasks requiring conflict resolution - examples being the go/no-go task (in which stimuli are presented in a continuous stream and participants perform a binary decision to respond or not to each stimulus), and the Stroop task (a task that assesses executive functions of selective attention and cognitive flexibility). The posterior-dorsal DLPFC appears to be more related to action execution and working memory; for example, the n-back task – in which, from a continuous stream of stimuli, subjects are asked to recall whether the current stimulus is the same as one presented n steps ago.

6.4.4.2 DLPFC and memory timing

DLPFC activity is related to the memory delay period time (Muri et al., 1996; Nyffeler et al., 2002; Pierrot-Deseilligny et al., 2003). Muri et al. (1996) described the PPC as being involved in very early memorisation – in the first 300 ms with the DLPFC having a role after this initial memorisation period. A review by Pierrot-Deseilligny et al. (2002) concluded that the DLPFC was involved in the memorisation phase about 1 second after the target presentation and for up to some 20 seconds subsequently. For delays longer than this time, Pierrot-Deseilligny and colleagues suggested that the critical structure was the parahippocampal cortex in the medial temporal lobe.

TMS over the DLPFC induces inaccuracy of memory-guided saccades (Muri et al., 1996). Likewise, prefrontal cortex (PFC) lesions also cause scattered and inaccurate memory-guided saccades (Funahashi et al., 1993; Ploner et al., 1999). TMS over medial frontal gyrus/DLPFC during the delay period of a memory-guided saccade impaired its accuracy, whereas TMS over the medial frontal gyrus/DLPFC around the time of target presentation or saccade execution had no such effect (Brandt et al., 1998; Muri et al., 1996). These observations are consistent with the notion that the PPC is involved in very early working memory around the time of target presentation and DLPFC is more involved during the delay period. Tanaka et al. (2014) found that single pulse TMS over the left DLPFC during a saccade and over the right DLPFC 100 ms after a saccade induced improved performance on a visual working memory
task that required the memorisation and reporting of changes in the orientation of a visual stimulus. Though their paradigm did encompass a memory-guided saccade task, they suggested that TMS over the DLPFC improves recall after making a saccade by the TMS wiping the DLPFC free of irrelevant spatial information stored during the saccade. This in turn implies that the DLPFC might form a live dynamic system of updating and storage of visual information during saccades.

6.4.4.3 DLPFC delay-related activity

A number of electrophysiology studies in the monkey have shown delay-phase memory activity in the DLPFC - for a review see Curtis (2006). This sustained activity was thought to represent either the maintenance of sensory or motor information or the neural code for the planned movement. Takeda & Funahashi (2004) separated memorised spatial location from saccade location. They observed that activity in the DLPFC initially coded for the memorised spatial location but then, midway through the memory delay period, coded for the planned movement. Thus DLPFC activity may represent both spatial memory and planned movement at different time points of the memory-guided task.

fMRI studies have provided a mixed picture of DLPFC activation during the memory period. Baumann et al. (2007) found that the medial frontal gyrus (MFG; containing the DLPFC) was active in their block memory task (1 second delay) but not in their event-related memory task (9 second delay). Heide et al. (2001) did not observe DLPFC activation in their memory saccade study and postulated that the DLPFC is only active during retention times above 500 ms, a delay period that their experiment did not exceed. In contrast, our block design experiment, using a 2 seconds delay, showed robust DLPFC activation. Our event-related experiment though, with a memory period of 5.6 seconds, failed to detect significant DLPFC activation during the “perceive” and “saccade” events, with the “memory” event not assessed in our paradigm. As described earlier, the HRF for sustained memory processes isn’t modelled by the canonical HRF used in the present study. In addition, sustained cortical activity during working memory processes are thought to be subtle and often subthreshold using current fMRI detection methods (Riggall & Postle, 2012). As discussed previously, one explanation for observing DLPFC activity in the block experiment only might be that the event-related experiment contained fewer total trials, due to longer trial duration, compared to the block experiment, thereby providing less overall statistical power. Our experiment was also not designed to compare different memory durations. Activations in the block and event-related experiments are unable to be directly compared using a voxel-based analysis as these were separate scan procedures. Future studies may be able to address this by using rapid
event-related design methods and thus providing more power, combined block and event-related fMRI designs, or varying the memory period within a single scan run to allow a direct comparison between different memory periods.

6.4.4.4 DLPFC and reflexive saccade disinhibition

DLPFC (and FEF) lesions cause disruption in inhibition of reflexive saccades (Guitton et al., 1985; Ploner et al., 2005). In the eye movement laboratory, we found PD patients made significantly more erroneous anticipatory saccades than controls during the flash period (30% vs. 11%), indicating impaired reflexive saccade inhibition. One explanation for this abnormality could be that DLPFC function was compromised in the PD patients. This is speculative however as no significant difference was seen in DLPFC activation in fMRI data. This speculation could be specifically investigated in future studies once more reliable MRI compatible eye tracking systems are available.

6.4.5 Insula

Bilateral anterior insula activation was noted in our experiment for the memory-guided task. Insula activation in memory-guided tasks has been noted before in previous studies. A PET study by Anderson et al. (1994) noted insula activation in their memory-guided task with the authors surmising that the insula had a role in spatial attention, memory, or motivational aspects of oculomotor control. Similarly, Baumann et al. (2007) noted left insula activation (in a combined region with the superior temporal gyrus and caudate nucleus) for the memory-guided task but did not explore further.

The shape of the insula BOLD activation time course in the present study was very similar to that observed in the classic eye fields (e.g. PPC, FEF, SEF). However, we observed insula activation only in the memory-guided saccade task and not in more simple reflexive and predictive tasks (see results in Chapter 5 page 159). These findings suggest that the insula plays role in memory and other higher-order cognitive processes harnessed in the memory-guided task and are not integral to the less cognitively demanding reflexive and predictive tasks.

The insula is involved in aspects of attention and decision making (Weller et al, 2009) and proposed to be part of an overlapping attention and working memory cortical network (LaBar et al., 1999). In fMRI studies, the insula has shown activity during tasks comprising object recognition and detection of salient or relevant stimuli (Downar et al., 2002) as well as letter identification, motor timing, object naming and visual search tasks (Dosenbach et al., 2006). Perry & Zeki (2000) found the anterior insula (along with the supramarginal gyrus in the IPL.
as discussed in the parietal cortex section previously) to be active during both a matching task - where participants made saccades to a peripheral object when the object shape matched a central fixation stimulus - and covert attention shifts whereby participants, having identified that no peripheral object matched the shape of a central fixation stimulus, did not make a saccade. Interestingly, the anterior insula was more responsive to ipsilateral rather than contralateral stimuli as seen in most cortical regions. The memory-guided task involves a number of task elements with participants needing to actively withhold a saccade, attend to the surroundings of the fixation stimuli, memorise a spatial location and to generate a saccade during specific points in the task. It is therefore possible that the insula activation in the memory-guided task reflected covert attention and cognitive processing of the individual components within the memory task, whereas the less complex predictive and reflexive saccade tasks did not require the same degree of task processing and so insula activation was not seen.

The insula plays a role in vestibular function. The vestibular system is important to the control of eye movements by integrating information about relative body and eye positioning in external space and, for example, memory-guided saccades become inaccurate if patients are rotated during the delay period (Israël et al., 1995; Israël et al., 1999). Specific cortical regions that receive vestibular input have been identified in primate studies with Eickhoff et al. (2006) describing the primary vestibular region in monkey to be the parieto-insula vestibular cortex (PIVC), an area with dense vestibular inputs. Eickhoff et al. suggested the human equivalent is located in the posterior parieto-insular cortex. One could speculate then that the insula activation in our study might relate to the integration of body positioning and vestibular function with spatial perception and memory. However, not only were the participants motionless in the scanner, but the location of insula activation in our study was the anterior insula, whereas the PIVC is believed to be located in the posterior insula (Eickhoff et al., 2006).

**6.4.6 Frontal Eye Field**

The FEF is well known to be involved in eye movement control and was active in all our saccade tasks. Early studies using the macaque monkey precisely located the monkey FEF equivalent by direct microstimulation. The localisation of the FEF has been more variable when identified with other techniques such as fMRI and TMS in humans. In their review, Vernet et al. (2014) described the FEF as being located in the lateral aspect of the precentral sulcus and the most dorsal aspect of the superior frontal sulcus.
The FEF is reported to be involved in fixation (Petit et al. 1995), reflexive and memory saccades, with or without visual cues (Fox et al. 1985), and even suppressed or imagined saccades (Law et al. 1997). Studies by Bruce & Goldberg (1985) and Robinson & Fuchs (1969) described saccade size-specific-neurons within the FEF, with larger saccades being evoked from regions in the dorsomedial portion and smaller saccades from the ventrolateral region. It has been suggested the FEF has two foci of activation – medial and lateral - and there exist several ideas, some conflicting, regarding the functional split between these two regions (Gagnon et al., 2002; McDowell et al., 2005; Simo, 2005). There is a more extensive discussion on the FEF medial and lateral split in the previous chapter (Chapter 5).

### 6.4.6.1 FEF function during memory delay period

Functional imaging studies of the memory-guided saccade task have shown the FEF to be active during working memory delay (Brown et al., 2004; Geier et al., 2007). This delay period activity may either represent the maintenance of a spatial image of the location or planned motor outputs responsible for moving the eyes to the new location (Curtis, 2006). Curtis & D’Esposito (2006) reported that the FEF might be more involved in holding the motor plan for making the saccade rather than retaining the spatial memory of the location. However, Srimal & Curtis (2008) found sustained FEF activity in the delay period for both a memory-guided task, in which a saccade was generated, and a spatial item recognition task in which no saccade executed. The authors suggested that FEF activity may not be related to saccade planning but rather, attention to the spatial location of the stimulus cue. Others have supported this notion of the FEF being involved in maintaining spatial information, irrespective of the need to generate a saccade (Armstrong et al., 2009; Clark, Noudoost, & Moore, 2012). Brignani et al. (2010) reported that the activity of the FEF during the delay phase was not consistent during the whole of the memory period as expected if the region was maintaining spatial information. Instead, the FEF was predominantly active only during the initial memory phase. The authors concluded that the FEF is involved in converting the initial spatial representation of the target to a motor plan.

### 6.4.6.2 FEF and anticipatory saccades

On visual inspection, the present study showed the medial FEF was less active in PD compared to controls during the early peak in the BOLD time course. This effect was present bilaterally but was more prominent on the left medial FEF. There were no other bilateral frontal eye field differences seen between groups.
The activation impairment in the medial FEF in the PD group was observed in the first peak period. This is the period where the flash is first seen (“perceive” event), whereby the subject is instructed to hold gaze while remembering the location of the flash. This is also the time when an error reflexive saccade can be made to the flash. In the eye movement laboratory, PD patients were seen to make significantly more error reflexive saccades during the memory delay period than controls. As discussed earlier, we were unable to measure the frequency of error saccades in scanner trials due to limitations in the eye tracking equipment. With PD patients making significantly more anticipatory saccades in the eye movement laboratory, however, it is reasonable to assume that the same behaviour persisted within the scanner.

Error reflexive saccades are more commonly studied as part of the antisaccade task where, through a similar mechanism, a peripheral stimulus triggers a reflexive saccade to the target despite instruction otherwise. Ptak et al. (2011) showed that error saccades in an antisaccade task were associated with stronger EEG activity in the right FEF. Further, Pa et al. (2015) reported that right lateral FEF activation inversely correlated with antisaccade performance (percentage correct saccades) in healthy elderly and suggested that an elevated signal represented aberrant neural activity that was detrimental to performance. In contradistinction, in the present study PD patients exhibited decreased BOLD signal compared to controls. Pertinently, Guitton et al. (1985) reported that FEF lesions resulted in saccade disinhibition in some patients to targets both ipsilateral and contralateral to the lesion. Thus, the diminished FEF activation in the PD group might be the basis for their failure to inhibit error anticipatory saccades.

FEF activation differences between the two groups could relate to differences in other functions such as spatial memory, rather than direct saccade execution. This notion is supported by the observation that the activation differences were seen at the time of flash presentation and the initial memorisation period, rather than the saccade period. PD subjects make more inaccurate memory-guided saccades (Chan et al., 2005; Le Heron et al., 2005; Lueck, Crawford, et al., 1992; Lueck et al., 1990). If impairment in working memory processes or the ability to convert spatial information to motor commands is the cause of memory-guided saccade inaccuracy, then it would be expected that associated impaired BOLD activity would be in the first peak, as indeed we observed. As previously discussed, working memory processes have been described as diffusely distributed throughout multiple cortical regions and sub threshold when measured using the GLM analysis technique employed in the present study (Riggall & Postle, 2012). Hence, there is the possibility that any true group differences related to working memory in the FEF, that may have been present,
may not have been detectable with our methodology. A distinction between medial and lateral FEF involvement for spatial memory processes has not been described in the literature (Curtis & D’Esposito, 2006). In conclusion then, the medial FEF activation reduction seen in the PD group might potentially have been due to spatial memory process impairments, but this speculation is not supported by the prevailing literature.

6.4.7 SEF

The SEF is located on the dorsomedial surface of each hemisphere in the postero-medial portion of the superior frontal gyrus within the medial portion of BA6 (Petit et al., 1996). The SEF activates in relation to memory-guided saccades, antisaccades, predictive saccades and reflexive saccades (Alvarez et al., 2010; Kimmig et al., 2001; Lu et al., 2002; O’Driscoll et al., 1995; Petit et al., 1996; Schall, 1991).

The SEF seems to be important for encoding the order of remembered saccade sequences (Isoda, 2002). However, Parton et al. (2007) studied a patient with a highly focal lesion in the SEF. The patient showed no impairment in generating the correct sequence for multi-step memory saccades but showed hypometria (reduced saccade amplitude) in both single and multi-step memory saccades tasks. In that study they noted how similar the deficits were to those in PD. Nachev et al. (2008), in a review of the supplementary motor complex (SMC; containing the SEF), highlighted a number of studies showing reduced SMC activity in PD (Buhmann et al., 2003; Grafton, 2004; Playford et al., 1992). It is surprising then that we were not able to discern any impairment in SEF activation in our PD group compared to controls.

6.4.8 PCA findings and discussion

PC1 alone explains over 70% of the variance of all the analysed time courses (Figure 6-18 page 225). The shape of PC1 was very close to the shape of the average BOLD time course, with two peaks corresponding to the initial perceive/memorisation event and the saccade event respectively (results - Figure 6-23 page 228). Figure 6-19 (results, page 226) showed that all ROIs expressed this component, indicating that they shared a common component of their temporal activation. PC2 and 3, however, revealed more functional specialization across the various ROIs.

As PC1 is expressed in every region, this PC reveals that BOLD activity in all regions during the memory-guided task conforms to a general pattern, as visualised by the PC 1 time course. This finding is in accordance with the results from the block design voxel-based analysis, in which active regions showed activity related to a model made by convolving an HRF with stimulus onset and durations. The active regions found in the block design study, all
exhibiting activity significantly similar to the HRF convolved model, were used as the ROI for the time course analysis. As PC1 explains a significant majority of the variance of the BOLD time course of every region, this component offers little differentiation in regional contribution to the task. PC1 may therefore represent general memory-guided related activity of each region.

![Plot of the PC loading value clusters](image)

**Figure 6-29** – Plot of the PC loading value clusters as previously shown in the results section. Illustrative vertical dotted lines show approximately the cluster differentiation based on PC2 loading values – The FEF and SEF cluster show a higher PC2 expression. DLPFC and insula cluster regions show intermediate expression and parietal cluster regions show the least expression.

PC2 encompasses roughly 7% of the total variance between all the time course plots in every region. The shape of the principal component appears to show a first peak or “hump” at 2.5 seconds, before the time of the first peak of PC1. PC2 shows a second “hump” at 10 seconds, which is about 2 seconds before the second peak of PC1. PC2 may, therefore, represent preparatory-related BOLD activity, before saccade events occur, e.g. these may represent memory retrieval or attention shifting processes, which occur before a volitional saccade is generated. PC2 offers better differentiation of cortical regions compared to PC1. In the clustering graph, the parietal cluster shows a low (negative) “expression” of PC2, with frontal/supplementary eye field clusters showing more expression of this PC. The DLPFC, insula and left PPC (SPL) show an intermediate expression of PC2, in between the other two
clusters (Figure 6-29). These findings suggest that frontal saccade-related regions are involved in pre-saccade and memory-related processes that occur before memory-guided saccade generation. This is in agreement with a study by Connolly et al. (2002) who, using another volitional saccade task (the antisaccade), found the FEF and not parietal regions (IPS) to show preparatory activity, coding readiness and saccade intention, prior to saccade generation. This conclusion fits a general theory that the FEF are more concerned with volitional saccades, whereas the PPC is more concerned with reflexive-type saccades. Correspondingly there are differences in preparatory activity in these regions for reflexive and volitional saccade tasks and highlighted by the PC differences seen in the present study.

PC3 explains approximately 5% of the variance between all the BOLD time courses. The shape of PC3 appears to show a relatively flat period during the first perceive/memory event, with a more raised peak at the second saccade event, suggesting that this PC is related to activity during saccade generation. From the cluster analysis, the eye field region clusters - PPC, SEF and FEF - exhibit a higher expression of this PC whereas the DLPFC cluster shows a lower negative expression of this PC (Figure 6-30). This observation is consistent with eye

Figure 6-30 – Plot of the PC loading value clusters as previously shown in the results section. The dotted horizontal line illustrates approximately the cluster differentiation based on PC3 loading values – The eye fields - FEF and SEF along with the PPC regions show a higher PC3 expression. The DLPFC show lower expression of PC3.
field activity being unsurprisingly related to saccade events in keeping with current understanding. The DLPFC regions show a lower (negative) expression of this PC, indicating a relatively weaker relationship with saccade generation. Again, this finding is entirely consistent with our understanding of the DLPFC, which is known to be involved in general executive function and memory processes, rather than specifically dedicated to the generation of saccades.

The clustering analysis, with no prior information on the function of each region, showed the PC loading values of the FEF and SEF regions to form one cluster, both DLPFCs forming a second, and most of the PPC in a third, with only the left PPC (SPL) and both insula regions forming a separate fourth cluster (See results Figure 6-22 page 227). Overall there was a consistent clustering pattern, so that related bilateral regions of similar function clustered together.

The clustering of the left SPL with the insula cortices likely reflects asymmetry in parietal cortex function, which has been noted in previous studies, particularly relating to attention processes (Perry & Zeki, 2000). The PPC has been widely implicated in attention (Corbetta & Shulman, 2002; Vandenberghe et al., 2012). The right IPL (note inferior parietal lobule) exhibits activity relating to covert and externally guided attention shifts (Perry & Zeki, 2000), suggesting attention processes in the PPC may be divided unilaterally. In our study, there was no clustering distinction between the IPLs - the right IPL in our study was found to cluster with the left IPL. Instead, it was the SPL (superior parietal lobule), which showed a lateralised distinction, which doesn’t fit exactly with what was proposed by Perry and Zeki. However, at least one study has reported potential lateralisation of SPL function – in a human lesion study, participants having right SPL lesions performed a visuospatial manipulation task (Spatial Span - Backward task, which involving reordering a memorised spatial sequence backwards) significantly worse than participants with left-sided SPL lesions (Koenigs et al., 2009). The study by Koenigs and colleagues was limited by the relatively small sample of left-sided lesions (n = 4) compared to right (n = 9), however, this does raise the possibility that lateralised processes occurring in the SPL, related to a currently unstudied visuospatial function, may be responsible for the non-bilateral clustering of the SPL observed in our study.

The study of Sugiura et al. (2004) served as the catalyst for performing the PCA analysis of the memory-guided BOLD time courses in our study. Both our study and that of Sugiura et al. were able to use clustering analysis, without explicit training, to group ROI into frontal and parietal regions, or at least groups that were consistent with different functional roles, using
BOLD time course data in the memory-guided task. There are some differences in findings between the two studies. Sugiura et al. did not detect or investigate activation within the DLPFC regions, and which are known to be involved in the memory-guided task. DLPFC activity was identified in our study in the block task and these regions were then examined in our event-related analysis. Our study also identified bilateral anterior insula activation, again not detected by Sugiura and colleagues. It is possible that these differences arise from differing methods employed to identify ROI. Sugiura and colleagues determined their ROI from the same event-related task session used for the PCA analysis. Our study employed a separate block-design task session which served as the localiser for ROIs for the subsequent event-related PCI analysis. As previously discussed, in using a separate block-design localizer paradigm, we were able to get participants to perform many more trials within each run, compared to the event-related runs (54 trials in the block task vs 18 in the event-related task). It was only from the block design runs that we were able to detect clear, bilateral DLPFC activity. DLPFC activation was not evident in the whole-brain analysis of the event-related results, both in our study and that of Sugiura et al. A likely explanation for this lack of expected detection is the relatively small BOLD signal changes in the DLPFC during the memory task. Thus, this comparatively subtle BOLD activity might only be able to be detected through repeated summation of closely spaced trials in a block design. Another reason why DLPFC activity may not be detected in the whole-brain event-related analysis is differences in the time series shape of BOLD activation, also previously noted. If DLPFC activity relates to prolonged working-memory processes, the BOLD signal may not follow the canonical HRF shape. In the whole-brain analysis for the event-related experiment, only regions determined to show activity most closely correlated to the canonical HRF were detected. The methodology would be less likely to detect regions showing responses deviating from the canonical HRF shape in the task. This potential limitation is less of an issue in the block design, since activity summates across numerous trials, giving a broad activity curve, regardless of the shape of the BOLD response in any individual trial.

Another region of activation not detected by Sugiura et al. was the insula. Bilateral anterior insula activity was seen prominently in the whole-brain block design session in our study, yet only weakly in the event-related session (significant activity was seen only in the right insula for the PD group and not at all in controls). This illustrates the advantages of the additional step employed by this study by using a separate block-design localizer task to identify ROI, being able to detect activity which may not be seen if using only an event-related design.
Conversely, Sugiura et al. found active occipital regions in their experiment and included this in their PCA analysis. Our experiment did detect small clusters of memory-guided occipital activity in the control group in the block-design task, but this was not observed in the PD group. The difference between the two studies may be due to a difference in the memory-guided saccade paradigm used. Sugiura and colleagues employed a design where all possible “flash” stimulus locations were presented during the saccade execution period, with the participants needing to generate a saccade to the memorised location of the cue, selected from the various presented locations. The memory-guided task used in our study requires the participant to make a memory-guided saccade to an empty, cue-less space. It would, therefore, make sense that occipital regions would be activated much more in the Sugiura paradigm given the additional visual stimulation.

Differences in the memory-guided paradigm may explain another variation in findings between the two investigations. Sugiura et al. reported parietal areas to have sustained activity during the delay period, or the period before saccade execution, resulting in a single-humped BOLD time course shape. Parietal regions in our study did not show this pattern for sustained delay period activity, and instead exhibited a double-peaked shape. This difference may have arisen from different working-memory processes demanded by the different memory-guided task paradigms. Delay period working memory processes needed to maintain target location can be coded in the sensory domain (memory of a location within an internal sensory map), or in the motor domain (as a pre-programmed saccade to the target). In the Sugiura et al. study, all possible locations of the “cue” were presented before the participant executed a saccade; a strategy they argued that supported a predominantly sensory domain pathway that retained the memory-guided saccade location in order to select the specific memorised cue location from a selection of presented targets, as opposed to a programmed motor command to a memorised location. This sensory-domain maintenance of target location was argued to fit with a role of the PPC in visuospatial attention (Mesulam, 1981) and leading to sustained PPC activity during the delay phase. A sensory-domain PPC-related memory process did not likely pertain to our study as no spatial information was provided during the saccade-execution phase (participants executed a memorised saccade to a location on a blank screen), and consequently an absence of the delay period activity in the posterior parietal regions that was observed by Sugiura and colleagues. We suggest that a motor-domain working memory pathway was predominantly recruited by our memory-guided task. This pathway is proposed to involve the frontal region (Mesulam, 1981; Sugiura et al., 2004) and is likely the basis for the higher PC2
expression in the frontal eye fields and which we posit represents preparatory activity prior to memory-saccade generation in our study.

Bilateral IPL regions, and to a lesser extent, the medial FEFs, showed subtle group differences; with the PD group exhibiting increased IPL BOLD activity during the saccade event and reduced medial FEF during the perceive event compared to controls. These findings, however, were not reflected as a separate principal component and identifying the IPL and FEF as disease-affected regions. Clustering analysis also did not separate out these subregions from the general parietal and frontal clusters. That PCA did not detect any specific PD component suggests that group differences in BOLD time course activation are small and may not be able to be detected using the PCA technique, a conclusion that might be addressed in any future studies.

6.5 Conclusion

This study has highlighted a number of potential areas and mechanisms by which memory-guided saccade performance is affected in PD. PD subjects make more anticipatory errors and more inaccurate saccades than controls. This could be the result of impaired working memory processes, attention processes or both saccade generation and inhibition processes. From our results, we propose that the PPC, particularly the IPL, is implicated in these impairments.

Understanding of theories behind saccadic impairment in PD is not aided by the complexity of the IPL. This area is known to be extremely difficult to study due to the multiplicity of functions, many of which are overlapping. We are only starting to understand the number of networks and functions that are linked to the IPL. It would seem the only firm conclusion we can make is that more research is needed in the future to further establish the role of the IPL in the variety of processes involved in the memory-guided saccade.

PCA and clustering analysis was able to cluster groups of functionally similar regions together from BOLD time course data alone. This ability supports the use of this technique to determine functionally related regions using the PCs of time course data from event-related studies. The lack of clustering of PD regions overall suggests that the group differences in BOLD time course detected using event-related fMRI technique are subtle at least in the memory-guided task utilized in the present investigation.
6.5.1 Study limitations and further research

Another limitation was that the PD patients recruited to this study were mostly unaffected by cognitive impairment or had only mild cognitive impairment, with no dementia/major cognitive impairment at the time of the study. We were uncertain of the ability of more severely impaired patients to perform the eye movement tasks correctly in the MRI scanner. This proved to be a valid concern, as a number of patients with mild PD fell asleep in the scanner due to fatigue. This does, however, mean that our patients were limited to relatively early stage of PD, where differences in performance and disease status are not as apparent. Recruitment of a PD patient population with a greater spread of disease severity might yield more prominent results but some thought would need to go into designing the procedures so as to be understood correctly and at the same time minimizing fatigue.

Any differences contributing to the performance deficit may not be great enough to be detected using the current resolution of our MRI scanner. PD saccade performance deficits are subtle and the functional differences are likely to be small. It is possible some minor changes in neural activity may occur in regions too small or close together to cause a significant BOLD signal change. This is particularly so for the PPC which has tightly located subregions. In the future MRI scanners with greater spatial and/or temporal resolution could provide more detailed data, both spatially and temporally, of cortical activity during saccade tasks.

As discussed, the eye tracking system we used within the MRI scanner is crude and inaccurate compared to the system used in the eye movement laboratory. This MRI compatible eye tracking system was unable to reliably detect error anticipatory saccades and often would lose tracking and on occasions could not be properly calibrated. As a camera, it did allow us to determine if the overall sequence for the task was being correctly performed. As a consequence, the data of one participant had to be excluded due to overwhelming errors in task execution. The in-scanner camera system also allowed us to ensure patients were continuing to performing the task and had not drifted off to sleep. Beyond this, the eye tracking system used in the fMRI provided very limited information and a superior system would allow a more detailed analysis and matching of fMRI with saccade performance data.
7 Summary and conclusions

In this thesis, I have compared saccadic eye movement performance in PD with a range of MRI techniques - structural T1, ASL, DTI and fMRI. The objective was to gain further understanding of the causes of characteristic eye movement deficits in PD. Overall, the studies in this thesis show eye movement performance changes between groups are not reflected by a corresponding detectable change using brain imaging. In Chapter 3, I failed to detect PD-specific changes in grey matter volume, perfusion or white matter integrity related to eye movement performance. Chapter 5 and 6 did not demonstrate significant functional activity differences during reflexive, predictive and memory-guided saccade tasks in PD compared to controls. As differences on the whole were not significant even at the group level, this means these measures are even less likely to be useful at an individual level. Chapter 4 was able to show broad association of eye movement performance with the degree of expression of a PD-specific perfusion pattern. However the variability in this association was large and one cannot therefore usefully predict individual perfusion pattern expression through eye movement performance alone. These results therefore mean that we are unable to provide supporting evidence for the use of eye movements in determining PD severity, at least at the cross-sectional level.

These experiments however have resulted in novel findings in general eye movement control. In Chapter 5, I identified that FEF activity appeared to be divided into subregions, with medial FEF regions appearing to be more involved in predictive saccades rather than reflexive. These findings contribute to our general knowledge of eye movement control. Chapter 6 showed the PCA technique is able to detect information within a BOLD response during the memory-guided saccade task, which can be used to group functionally-related regions. This satisfies the broader aim of improving our understanding of eye movement control in general.

The studies in this thesis used patients representing a cross-section of mild-moderate PD progression and offer a point of comparison for future studies. On a longitudinal level, changes in eye movement performance may still be able to provide useful information on PD progression within each subject. However, with my studies being of a cross-sectional design, they are unable to answer this question, and this is left for future studies. The study participants are part of a longitudinal study, with ongoing follow-up scanning and eye
movement recordings. Future studies will be able to build upon the results of this current study, potentially using targeted techniques for the identified regions.

7.1.1 Chapter 3 - Structural, ASL, DTI and eye movement correlation

In this chapter, comparisons were made between a range of MRI scanning techniques and reflexive, predictive, memory-guided and antisaccades, representing both volitional and visually-guided saccades types. A voxel-based whole-brain comparison found group-level correlation differences in the left temporal pole, precuneus and caudate nucleus with eye movement performance. Overall, few regions of correlation arose amongst the extensive number of comparisons, indicating minor structural and perfusion differences in eye movement control regions between PD and controls when studied using a whole-brain voxel-based approach and there are suspicions that the results may have arisen from artefacts. As the current results stand however, it is interesting to note that several regions, namely, the left temporal pole and precuneus, which were seen to correlate with eye movements and show group differences, are known to have integrative functions, relating to a wide variety of cognitive tasks not limited to eye movements. It may be possible then that entire cortical networks, which involve the left temporal pole and precuneus, are disrupted in PD, rather than single brain regions. This potential network disruption in PD, involving a number of brain regions, may not be able to be detected using the current voxel-based method. Network analysis could, therefore, potentially be used to study eye movement deficits in PD, which was further explored in Chapter 4.

7.1.2 Chapter 4 –ASL PD-perfusion pattern and eye movement correlation

In this chapter, I compared a previously described ASL-derived PD-related perfusion pattern with eye movement performance in PD. This pattern, described by a network score indicating the extent of pattern expression within an individual, is thought to represent general PD status, as it correlates with both PD motor scores and cognitive impairment measures. This hypothesis was tested using a comparison of the network score with eye movement performance measures thought to be characteristically impaired in PD. In addition, previously in Chapter 3, I found group differences in voxel-level comparisons with eye movement performance in certain cortical regions known to have integrative functions. This may suggest PD-related changes may occur across a network of widespread cortical areas. Thus, a network comparison may provide insight into the sources of PD-related eye movement dysfunction. A consistent pattern of correlation was found between expression of the network score and all tested eye movement tasks - that is, higher network scores were associated with increasing eye movement performance deficits. Multiple comparisons showed a strong multicollinear
effect, indicating correlation between predictors. This supports the idea that the ASL PD-related perfusion pattern, as it correlates with measures of both PD motor status and cognitive impairment, along with eye movement performance, may represent a general marker of PD progression. Alongside the network score correlation findings, this chapter indicated that memory-guided saccade latency deteriorated steeply past a threshold network score. This effect was also seen past certain UPDRS and cognitive Z-score thresholds. This raises the possibility of using memory-guided saccade latency as a marker point of PD progression in the future.

7.1.3 Chapter 5 – fMRI of reflexive and predictive saccade tasks in PD
In this chapter, I investigated reflexive and predictive saccades in PD using a blocked fMRI design. A within-group analysis showed significant activity in the FEF, PPC and SEF for the reflexive task and predictive tasks. No significant group difference in cortical activity for either predictive or reflexive saccades was seen between PD and control groups. However, a difference in between-task activity was detected in the medial and lateral FEF. The medial FEF showed greater activity in the reflexive task compared to the predictive task. This indicates that regions of the FEF may operate as functional subunits, one of which may be more strongly involved in reflexive saccades. This finding is novel and has not been directly reported before. This may be a site for future investigations.

7.1.4 Chapter 6 - fMRI of memory-guided saccades in PD
In this chapter, I investigated memory-guided saccades using both a blocked and event-related fMRI design. In the block design task, significant activity in the FEF, PPC, DLPFC, SEF and insula was seen for both PD and control groups. However, a between-group analysis revealed no difference. An event-related time course analysis showed subtle between-group task activation differences, seen most clearly in the inferior parietal lobule bilaterally, with the PD group showing a greater BOLD signal change during the saccade event. PCA of all the BOLD time courses of regions found to be active in the block design was performed, with a K-means clustering analysis of the loading values of PC expression of each region of interest. This cluster analysis, from the time course data only, was able to identify groups of functionally-related regions without any prior information of region function. This helps to validate the ability of the PCA and clustering analysis of PC expression to group functionally-related regions.
7.1.5 Implications

Overall, the observed group differences between PD and controls were subtle, with the majority of comparisons revealing no significant group difference. This may implicate that VBA, or whole-brain analysis techniques, may not be sensitive enough to detect changes, particularly if those changes are widespread and involve distributed changes in a network of brain regions. However, the group differences that were observed in this study were novel findings that have not been previously reported before. This adds to our understanding of eye movement control and of potential differences in PD contributing to saccade performance impairment. In future studies, investigators might consider more targeted MRI techniques to investigate changes in the discussed regions, rather than a whole-brain approach. Subtly decreased frontal and increased parietal BOLD signal change seen in fMRI of memory-guided saccades (Chapter 6) generally support previous studies reporting frontal hypoactivity and parietal hyperactivity in PD for other volitional saccade tasks such as self-paced saccades (Rieger et al., 2008) and antisaccades (Lemos et al., 2016). However, this study made an additional comparison of a perfusion network score to eye movement performance. Increased network score expression, indicating a more severe network of cerebral hypoperfusion, was associated with impaired saccade performance in PD. This perfusion network describes a number of regions showing characteristic hypoperfusion in PD, including the posterior parietal cortex. The implication of this is that if posterior parietal hypoperfusion is present in PD, relative increases in BOLD signal may not necessarily mean hyperactivity, but rather the increase in the BOLD activity detected in the PD group is only proportionately increased from a lower baseline BOLD signal. The increased BOLD signal change may, therefore, simply reflect a higher percentage change from baseline, not actual parietal cortex hyperactivity as reported by past studies (Lemos et al., 2016; Rieger et al., 2008).

The results of this study strongly suggest that the FEF may operate as functional subunits for components of the predictive and reflexive task. While previous studies have described possible medial and lateral FEF subunits, no study has explicitly stated the unique involvement of the medial FEF in reflexive saccades compared to predictive saccades. This has implications for our overall knowledge of cortical eye movement control, with these findings contributing to this understanding.

The fMRI technique used, while not showing extensive between-group difference, demonstrated strong within-group task activity. The implication for future research is that the number of trials and durations are sufficient to detect significant task activity for the saccade paradigms used. The ability of the PCA/cluster analysis to functionally group-related regions, 262
independent of any prior information, shows that in regions that are all functionally-related to the task, there is temporal information in the BOLD response that allows us to purpose some degree of functional specialization and commonality between clusters of regions.

7.1.6 Study limitations and future work

DTI scans in the whole-brain VBA (Chapter 3) showed artefacts, potentially from inaccurate normalisation. This indicates a need for future studies to use more refined analysis techniques specifically to minimise these effects. A tract-based skeleton approach for DTI could be used to minimise the effect of distortion and mis-normalisation as described in Smith et al. (2006) and Melzer et al. (2013). This skeleton approach can be used at a whole-brain level, selected regions or selected tracts. Using diffusion MRI, future studies may also use tractography analysis seeded from the identified areas in this study, particularly from the FEF subregions and dense functional areas such as within the PPC. These will allow connections to other cortical/subcortical regions to be seen, which will provide more information on the functions of these regions and possible changes in PD. More advanced diffusion MRI techniques may be used such as High Angular Resolution Diffusion Imaging (HARDI) or diffusional kurtosis imaging. Single voxels often contain fibre tracts running in multiple directions, however, DTI can only provide one diffusion direction per voxel. HARDI measures a higher number of diffusion gradient directions than DTI, which allows better detection of multidirectional fibres within a single voxel (Tournier et al., 2008; Tuch et al., 2002). The randomness of water diffusion itself may also be used to provide information on tissue type. A simplistic model of the probability of water diffusion can be described by a Gaussian distribution. Different tissue types, however, may cause diffusion probability to differ from the Gaussian distribution. Diffusional kurtosis imaging is a diffusion technique which can determine tissue structure based on the extent of non-Gaussian water diffusion behaviour (Jensen et al., 2005). These more advanced diffusion techniques should provide more accurate and sensitive information about the integrity of the white matter, and may, therefore, provide additional information for the future development of PD biomarkers.

Future studies could explore the identified regions with an ROI approach. Such an approach would limit the multiple comparisons problem. In fMRI scans, limiting the scan area to defined regions would also allow closer scan slices, allowing increased spatial resolution, and also better temporal resolution, shortening TR and allowing more detailed time course plots. Future fMRI investigations using the memory-guided task may compare different memory period lengths. This current study used only a single delay period duration. Le Heron, MacAskill, & Anderson (2005) found memory-guided saccade performance to be less
impaired in longer delay (over 20 seconds) compared to shorter delay periods (several seconds) in PD. Their theory was that the long-delay memory pathway bypasses areas, such as the DLPFC, which are thought to be affected in PD, leading to less impaired performance during longer delays. Future fMRI studies could therefore contrast activity in the DLPFC, for example, between short and long delay working memory tasks to confirm this theory. This could also show other regions, not limited to the DLPFC, which may be affected in PD, related to working-memory processes for short and long durations. More advanced eye-tracking hardware, able to provide accurate measurements during scanning may be employed for future studies. Accurate eye-tracking can allow the exclusion of error trials. Activity from error trials may also be compared to correct trials and baseline activity, allowing comparisons which may detect different cortical processes responsible for the error trials in PD. These differences may help us understand the source of increased PD saccade disinhibition.

This current study uses only a cross-section of data from a point in time of a group of patients enrolled in a longitudinal study. The future development of the biomarkers based on eye movements and imaging will need to compare changes in saccade measures and MRI scans over the progression of the disease. In Chapter 4, memory-guided saccade latency was seen to steeply decline past a certain threshold in network score expression, as it did with UPDRS and cognitive Z-score. Longitudinal eye movement studies could investigate the possibility of a memory-guided saccade latency value cut-off point as a marker of disease progression. Using longitudinal data collected over time, we may assess whether individual participants show this drop off pattern of decline in memory-guided saccade latency at certain points of disease progression and cognitive impairment.

As described in Chapter 4, all eye movement tasks studied showed a correlation with the ASL network score. The network score itself describes hypoperfusion in areas including the middle frontal gyrus (containing the FEF) and posterior parietal-occipital cortex (containing the PPC) – regions which have been reported to be affected in PD (Lemos et al., 2016; Rieger et al., 2008) and also showing subtle between-group differences between PD and controls during memory-guided saccades (Chapter 6). Imaging findings of regions showing group differences associated with saccade performance may allow eye movement measures to be developed as a more cost-efficient marker of changes detected in imaging. Future developments of the ASL network score may be compared to eye-movement performance longitudinally to compare changes over time to validate this possibility. Imaging comparisons with eye movements are also not limited to MRI scans. Other potential future imaging markers of PD progression, such as values derived from PET or SPECT or transcranial sonography, may also be
compared to eye movement performance to cross-validate the potential of other imaging measures as future biomarkers.

7.1.7 Final conclusion

In this thesis, I found a number of imaging and eye movement association differences between PD and control groups. From these, I have been able to compare analysis techniques and suggest avenues for future research. In addition, our knowledge of the associations between eye-movements and disease-related cerebral changes detected by imaging has grown. Finally, I was able to find functional subregions within the FEF and also attempted to clarify the current literature regarding PPC function using our results, contributing one small step in our quest to decipher the complexities of the human oculomotor system.
8 References


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9 Appendix

9.1 Chapter 3 - Supplementary figures

Figure 9-1 to Figure 9-10 show findings from voxel-based comparisons not pictured in the results section.

*Voxels showing increases in grey matter volume relative to increasing memory-guided saccade latency in controls - within-group analysis*

Figure 9-1 – Glass brain images in three planes showing significant voxels of positive correlation (increasing grey matter volume correlated with increasing memory-guided saccade latency) in the control group. Images are corrected for multiple comparisons (FWE p < 0.05). Scattered subgyral clusters were found to be significant.

*Voxels showing a more positive association between grey matter volume and memory-guided saccade latency in controls than PD – between-group analysis*

Figure 9-2 – Significant voxels showing more positive correlation (increasing grey matter volume correlated with increasing memory-guided saccade latency) in the control group compared to the PD group. Images corrected for multiple comparisons (FWE p < 0.05). Scattered subgyral clusters were found to be significant.
**Voxels showing increases in FA relative to increasing antisaccade gain in controls - within-group analysis**

![Figure 9-3](image1)

Brodmann area 8

Figure 9-3 – Significant voxels showing positive correlation (increasing DTI FA value correlated with increasing antisaccade gain) in the control group. Images corrected for multiple comparisons (FWE p < 0.05). A single cluster in the right Brodmann area 8 was significant.

**Voxels showing increases in MD relative to increased reflexive latency in PDs - within-group analysis**

![Figure 9-4](image2)

Superior temporal gyrus

Figure 9-4 – Significant voxels showing positive correlation (increasing DTI MD value correlated with increasing reflexive latency) in the PD group. Images corrected for multiple comparisons (FWE p < 0.05). A single cluster in the left superior temporal gyrus was significant.
Voxels showing increases in MD relative to increased memory-guided latency in PDs - within-group analysis

![Image of brain scans with marked voxels]

Figure 9-5 – Significant voxels showing positive correlation (increasing DTI MD value correlated with increasing memory-guided latency) in the PD group. Images corrected for multiple comparisons (FWE p < 0.05). Small clusters in the left insula cortex were significant.

Voxels showing increases in MD relative to increased reflexive gain in PD within-group analysis

![Image of brain scans with marked voxels]

Figure 9-6 – Significant voxels showing positive correlation (increasing DTI MD value correlated with increasing reflexive gain) in the PD group. Images corrected for multiple comparisons (FWE p < 0.05). An undefined cluster in the base of the brain, between hemispheres, was found to be “significant”.
Voxels showing decreases in MD relative to increased reflexive gain in controls - within-group analysis

Figure 9-7 – Significant voxels showing negative correlation (decreasing DTI MD value correlated with increasing reflexive gain) in the control group. Images corrected for multiple comparisons (FWE p < 0.05). Subgyral clusters were found to be significant.

Voxels showing a more negative association between MD and reflexive gain in PD than controls – between-group analysis

Figure 9-8 – Voxels of significantly more negative correlation (decreasing DTI MD values correlated with increasing reflexive gain) in the PD group compared to the control group. Images corrected for multiple comparisons (FWE p < 0.05). A cluster in Brodmann area 17 was found to be significant.
Voxels showing decreases in grey matter volume relative to increased cognitive Z-score in PD - within-group analysis

Figure 9-9 – Significant voxels showing negative correlation (decreasing grey matter volume correlated with increasing cognitive Z-score) in the PD group. Images corrected for multiple comparisons (FWE p < 0.05). A subgyral cluster was found to be significant.

Voxels showing decreases in ASL perfusion relative to increased cognitive MDS-UPDRS Part III score in PDs - within-group analysis

Figure 9-10 – Significant voxels of negative correlation (decreasing ASL perfusion values correlated with increasing MDS-UPDRS part III score) in the PD group. Images corrected for multiple comparisons (FWE p < 0.05). A subgyral cluster was found to be significant.
Figure 9-11 – Linear regressions between UPDRS part III score and reflexive, predictive, memory-guided and antisaccade primary gain (left graph column) and latency (right graph column). The x-axis represents the network score of each participant. The y-axis represents primary gain/latency in each task. Straight lines indicate least squares regressions for each measure. The grey intervals around each regression line indicate the 95% confidence interval of the regression. The cognitive category of PD participants (normal (PDN), mild cognitive impairment (PD-MCI) or dementia (PDD)) is indicated by the colour of the plotted points.
### Table 9-1 – P and R squared values for each eye movement and UPDRS comparison. Eye movement performance measures and UPDRS-III score are significantly correlated for every task analysed.

<table>
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<th>Estimate (gain)</th>
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<th>t value</th>
<th>p-value</th>
<th>r squared</th>
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Figure 9-12 – Linear regressions between cognitive Z-score and reflexive, predictive, memory-guided and antisaccade primary gain. The x-axis represents the network score of each participant. The y-axis represents primary gain in each task. Straight lines indicate least squares regressions for each measure. The grey intervals around each regression line indicate the 95% confidence interval of the regression. The cognitive category of PD participants (normal (PDN), mild cognitive impairment (PD-MCI) or dementia (PDD)) is indicated by the colour of the plotted points.
Figure 9-13 – Linear regressions between cognitive Z-score and reflexive, predictive, memory-guided and antisaccade latency. The x-axis represents the network score of each participant. The y-axis represents latency (ms) in each task. Straight lines indicate least squares regressions for each measure. The grey intervals around each regression line indicate the 95% confidence interval of the regression. The cognitive category of PD participants (normal (PDN), mild cognitive impairment (PD-MCI) or dementia (PDD)) is indicated by the colour of the plotted points.
Table 9-2 – P and R squared values for each eye movement and cognitive Z-score comparison. Eye movement performance measures and cognitive Z-score are significantly correlated for every task analysed.

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9.3 Chapter 6 - Supplementary figures

9-14 – PCA Clustering K = 3 (3 clusters). Regions plotted against loading values for PC2 and PC3. Colour of points and dotted grey ovals indicates cluster.

UK PARKINSON’S DISEASE SOCIETY BRAIN BANK CLINICAL DIAGNOSTIC CRITERIA

Step 1. Diagnosis of Parkinsonian Syndrome

- Bradykinesia
- At least one of the following
  - Muscular rigidity
  - 4-6 Hz rest tremor
  - Postural instability not caused by primary visual, vestibular, cerebellar, or proprioceptive dysfunction

Step 2. Exclusion criteria for Parkinson’s disease

- History of repeated strokes with stepwise progression of parkinsonian features
- History of repeated head injury
- History of definite encephalitis
- Oculogyric crises
- Neuroleptic treatment at onset of symptoms
- More than one affected relative
- Sustained remission
- Strictly unilateral features after 3 years
- Supranuclear gaze palsy
- Cerebellar signs
- Early severe autonomic involvement
- Early severe dementia with disturbances of memory, language, and praxis
- Babinski sign
- Presence of cerebral tumor or communication hydrocephalus on imaging study
- Negative response to large doses of levodopa in absence of malabsorption
- MPTP exposure

Step 3. Supportive prospective positive criteria for Parkinson’s disease

Three or more required for diagnosis of definite Parkinson’s disease in combination with step one

- Unilateral onset
- Rest tremor present
- Progressive disorder
- Persistent asymmetry affecting side of onset most
- Excellent response (70-100%) to levodopa
- Severe levodopa-induced chorea
- Levodopa response for 5 years or more
- Clinical course of ten years or more
Dear Professor Anderson

Saccadic function in Parkinson’s Disease: an fMRI study
Investigators: Prof. Tim Anderson, Dr Michael MacAskill, Dr Richard Watts, Dr Elizabeth Franz, Dr. Marcus Hetiger, Dr Scott Wells, Saskia Van Stockum
Locality: Van der Veer Institute for Parkinson’s and Brain Research
Ethics Ref: URB/07/03/010

The above study has been given ethical approval by the Upper South B Regional Ethics Committee. A list of members of this committee is attached.

Approved Documents
Information sheet for control participants, dated 28 May 2007
Information Sheet for patients with Parkinson’s disease dated 28 May 2007
Consent Form dated 28 May 2007

Certification
The Committee is satisfied that this study is not being conducted principally for the benefit of the manufacturer or distributor of the medicine or item in respect of which the trial is being carried out.

Accreditation
The Committee involved in the approval of this study is accredited by the Health Research Council and is constituted and operates in accordance with the Operational Standard for Ethics Committees, April 2006.

Progress Reports
The study is approved until 31 August 2008. The Committee will review the approved application annually and notify the Principal Investigator if it withdraws approval. It is the Principal Investigator’s responsibility to forward a progress report covering all sites prior to ethical review of the project in June, 2008. The report form is available on http://www.newhealth.govt.nz/ethicscommittees. Please note that failure to provide a progress report may result in the withdrawal of ethical approval. A final report is also required at the conclusion of the study.

Requirements for SAE Reporting
The Principal Investigator will inform the Committee as soon as possible of the following:
• Any related study in another country that has stopped due to serious or unexpected adverse events
• withdrawal from the market for any reason
• all serious adverse events occurring during the study in New Zealand which result in the investigator or sponsor breaking the blinding code at the time of the SAE or which result in hospitalisation or death.
• all serious adverse events occurring during the study worldwide which are considered related to the study medicine. Where there is a data safety monitoring board in place, serious adverse events occurring outside New Zealand may be reported quarterly.

All SAE reports must be signed by the Principal Investigator and include a comment on whether he/she considers there are any ethical issues relating to this study continuing due to this adverse event. If the adverse event is local and does not have the sponsor’s report attached, an opinion on whether the event is thought to be related to the study should be given along with any other pertinent information. It is assumed
by signing the report, the Principal Investigator has undertaken to ensure that all New Zealand investigators are made aware of the event.

Amendments
All amendments to the study must be advised to the Committee prior to their implementation, except in the case where immediate implementation is required for reasons of safety. In such cases the Committee must be notified as soon as possible of the change.

Please quote the above ethics committee reference number in all correspondence.

The Principal Investigator is responsible for advising any other study sites of approvals and all other correspondence with the Ethics Committee.

It should be noted that Ethics Committee approval does not imply any resource commitment or administrative facilitation by any healthcare provider within whose facility the research is to be carried out. Where applicable, authority for this must be obtained separately from the appropriate manager within the organisation.

Yours sincerely

Di Rutledge
Upper South B Regional Ethics Committee Administrator
Email: di_rutledge@moh.govt.nz