MICROSACCADES IN PARKINSON'S DISEASE

by

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Abstract

Individuals with Parkinson's disease (PD) display deficits in voluntary saccade generation but improved automatic, visually-triggered saccade performance. This can be tested using prosaccades, saccades to visual stimuli, and antisaccades, saccades in the opposite direction from the visual stimuli. Voluntary saccade impairments resulting in antisaccade direction errors and longer saccadic reaction times (SRTs) are thought to be due to insufficient presetting of neural circuitry during saccade preparation in complex tasks involving suppression and selection. The basal ganglia, a major site of PD pathology, might be the cause of abnormalities in preparing for action selection in PD patients. Recently, microsaccade rates have been hypothesized to reflect the dual preparatory signals of saccade facilitation and suppression. In this thesis, we investigated the microsaccade behaviour of PD patients as they performed prosaccades and antisaccades. We hypothesized that deficits in voluntary movements in PD would result in impaired suppression of involuntary movements as reflected by increased microsaccade rates. Our findings demonstrate consistently elevated microsaccade rates in PD subjects compared to age-matched controls. Furthermore, positive correlations were found between antisaccade direction error rate and microsaccade rate as well as microsaccade rate and Hoehn-Yahr score, an indicator of disease severity in PD patients. We conclude that microsaccades reflect the impaired suppression of involuntary movements caused by voluntary movement deficits in PD pathology. Our findings indicate that microsaccades provide insight into action preparatory mechanisms and BG dysfunction. Therefore, measuring microsaccades in PD may provide a useful biomarker to follow disease progression and effectiveness of treatment therapies.

Co-Authorship

This research was conducted by Hailey McInnis under the supervision of Dr. Douglas P. Munoz and Dr. Giovanna Pari. The experimental paradigm was conceptualized by Dr. Douglas Munoz and implemented into eye-tracking capability by Don Brien. Dr. Giovanna Pari and Hailey McInnis recruited subjects with Parkinson's disease from the Movement Disorders Clinic at Hotel Dieu Hospital. Hailey McInnis conducted the behavioural experiments, performed data analysis, and reported the findings in this thesis.

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List of Abbreviations

A	anticholinergic
AN	automatic saccade neuron
ANOVA	analysis of variance
BG	basal ganglia
CN	caudate nucleus
CNS	central nervous system
COMT	catechol-O-methyl transferase
cVN	contralateral saccade-preferred volitional neuron
D1	D1- like dopamine receptors
D2	D2-like dopamine receptors
DA	dopamine
DBS	deep brain stimulation
DLPFC	dorsolateral prefrontal cortex
E	entacapone
FEF	frontal eye field
fMRI	functional magnetic resonance imaging
GABA	gamma-aminobutyric acid
GPe	globus pallidus, external segment
iVN	ipsilateral saccade-preferred volitional neuron
L	levodopa
L-CR	levodopa controlled-release
LATER	linear approach to threshold with ergodic rate
LCD	liquid-crystal display
LGN	lateral geniculate nucleus

LIP	lateral intraparietal area
LSD	least significant difference
M	amantadine
MAO-B	monoamine oxidase B
MoCA	Montreal Cognitive Assessment
MPTP	1-methyl-4 phenyl-1,2,3,6-tetrahydropyridine
P	pramipexole
PD	Parkinson's disease
R	ropinirole
S	rasagiline
SC	superior colliculus
SD	standard deviation
SE	standard error
SEF	supplementary eye field
SNc	substantia nigra pars compacta
SNr	substantia nigra pars reticulata
SRT	saccadic reaction time
STN	subthalamic nucleus
UPDRS	United Parkinson's Disease Rating Scale

Chapter 1

Introduction

Parkinson's Disease (PD) is a neurodegenerative disorder of the central nervous system (CNS). The core motor symptoms stem from the death of dopaminergic neurons in the substantia nigra pars compacta (SNc) of the basal ganglia (BG) (Greenfield & Bosanquet, 1953). As in other neurodegenerative diseases, however, lesions begin developing a considerable time earlier than the appearance of motor dysfunctions. Initially, lesions occur in the dorsal motor nucleus of the glossopharyngeal and vagal nerves and the anterior olfactory nucleus (Braak *et al.*, 2003). PD can also impact sensation and perception, cognition, sleep, and emotional functioning (Davidsdottir *et al.*, 2005; Jankovic, 2008; Wolters, 2009). Pertinent to this thesis, dopamine depletion in peripheral visual structures can affect basic visual processes (Harnois & Di Paulo, 1990).

PD affects approximately 50000 people in Canada and more men are affected than women, at a ratio of 1.8:1. The majority (79%) of individuals living with PD were diagnosed after the age of 65 (Statistics Canada, 2013). Once a diagnosis is made, medications are prescribed to alleviate symptoms by increasing dopamine (DA) levels in the brain or directly stimulating DA receptors to compensate for neurodegeneration. Common medication includes levodopa, which converts into DA in the CNS, dopamine receptor agonists, and monoamine oxidase B (MAO-B) inhibitors that prevent the breakdown of DA in the synapse (Rascol *et al.*, 2003). Once drug therapy is no longer sufficient, surgical treatments such as deep brain stimulation are considered. Current treatments can reduce symptom severity and increase quality of life but there is at present no cure for PD.

A diagnosis of PD relies on the clinical presentation of at least 3 of the motor symptoms of tremor, rigidity, akinesia/bradykinesia and postural instability (Bergman & Deuschl, 2002). Diagnostic accuracy is complicated by the variable presentation of the disorder along with the

similarity of other motor disorders such as essential tremor and progressive supranuclear palsy. Tremor is common between PD and essential tremor patients although PD patients tend to present with a resting tremor while essential tremor manifests as an action tremor (Chen & Swope, 2003). Progressive supranuclear palsy shares common motor symptoms with PD but can be differentiated due to the presence of postural instability and ophthalmoplegia in the former disorder (Rehman, 1999). These disorders have a poor response to dopaminergic treatment so neurologists often wait for a positive treatment response to confirm a PD diagnosis (Calne *et al.*, 1992). Therefore, the implementation of new screening tools could increase diagnostic accuracy at initial presentation and expedite appropriate treatment of disorders resembling PD.

Additional screening tools would lessen the reliance on treatment responses because neurologists and even general practitioners could properly discern PD from other related disorders upon presentation. A quantitative test could also be used to evaluate the efficacy of different treatments by comparing test results before and after treatment. The eye movement system is one system that could provide such a quantitative behavioural test. Furthermore, eye movements lend themselves to precise, non-invasive measurement and kinematic analysis. Finally, there is substantial understanding of the neural circuitry controlling the planning and execution of saccadic eye movements (Leigh & Zee, 1999; Moschovakis *et al.*, 1996; Munoz *et al.*, 2000; Munoz & Everling 2004).

Eye movements have been shown to be affected in PD patients. The eye movement circuitry involves the BG and PD pathophysiology results in deficits in visually-guided saccade suppression. This has been tested using the antisaccade task in which a subject looks away from a target, specifically to the target's opposite position. Previously, it has been demonstrated that PD patients make more direction errors while performing the antisaccade task compared to age-matched controls (Amador *et al.*, 2006; Briand *et al.*, 1999; Chan *et al.*, 2005). When healthy controls make errors in this task, it is correlated to significantly higher rates of microsaccades

during fixation (Watanabe *et al.*, 2013). These microsaccades can be detected using recent advances in camera technology and a bifocal lens which captures both eyes simultaneously (Engbert & Kliegl, 2002).

It has been postulated that microsaccades provide direct insight into volitional action preparation (Watanabe *et al.*, 2013) and therefore can be used to identify people with deficits in action preparation. In this thesis, I will record microsaccades in PD subjects as they perform saccadic tasks. I will investigate whether or not these subjects, who have known deficits in voluntary action preparation, exhibit greater rates of microsaccades and if these correspond to the PD subjects' increased error rate during the antisaccade task. Furthermore, a reduction in microsaccade suppression in PD patients would lend support to the claim that microsaccades can be used as an overt measure of voluntary action preparation (Watanabe *et al.*, 2013).

1.1 Clinical features and etiology

Due to the variable presentation of the disease, PD is currently characterized by the presence of motor symptoms with a slow onset and steady progression in frequency and severity (Calne, 1992). Considering the extensive list of differential diagnoses, the clinician must also consider family history of the disorder and the patient's response to dopaminergic treatment before ascertaining a diagnosis of PD (Calne, 2005). On initial clinical presentation, the three cardinal signs of PD are resting tremor, rigidity, and bradykinesia (Samii, 2008).

Resting tremor is the most common presenting sign, with approximately 70% of patients presenting with tremor at initial consultation (Hughes *et al.*, 1993). The tremor is a rhythmic, "pill-rolling" movement of the thumb and index finger that normally appears asymmetrically and at rest. Rigidity presents as increased resistance to passive stretch, clinically assessed through bilateral manipulations of the wrist and elbow joints (Samii, 2008). It can manifest as increased tone throughout the motion or "cogwheeling", a jerky movement similar to a ratchet. Bradykinesia, akinesia, and hypokinesia are grouped together as the final and often most

debilitating of the cardinal signs. Bradykinesia is defined as the slowing of voluntary movements and the absence of normal associated movements. For example, slow walking and reduced arm swing is a hallmark of bradykinesia. Akinesia is the absolute poverty of voluntary movements, such as in facial muscles resulting in a "masked face" expression. Lastly, hypokinesia refers to the slowness and smallness of movements often manifesting as micrographia (Berardelli *et al.*, 2001). Some consider postural instability as the fourth cardinal sign; however, it doesn't usually appear in the early stages of PD. In fact, postural instability is one of the initial symptoms of progressive supranuclear palsy, a neurodegenerative disease distinct from PD (Litvan *et al.*, 1996).

The presence of these cardinal signs can lead to a PD diagnosis; however, performing a post-mortem autopsy is the only means of confirming the diagnosis (Litvan *et al.,* 2003). The pathologist looks for dopaminergic cell loss in the SNc and the presence of Lewy-related pathology in neuronal populations. Lewy bodies, intracytoplasmic, eosinophilic intrusions, and Lewy neurites, nerve cell processes, both contain the α-synuclein protein as their primary structural component. The pathogenetic relevance of Lewy bodies in the surviving DA neurons and other affected regions in the CNS is unclear but their presence has been seen as critical in making the pathological diagnosis of PD (Warner & Schapira, 2003).

While the neural basis of PD is partially understood, the identity of the neurodegenerative insult remains unknown. If the initial cause was discovered, the mechanisms for cell death could be determined. Consequently, neuroprotective treatments could be developed to prevent neurodegeneration as opposed to our current dopamine replacement treatment options.

Although no exact cause has been pinpointed, several risk factors have been associated with the development of PD (Priyadarshi *et al.*, 2001). The major risk factor is aging; the majority of people are diagnosed after the age of 65 (Statistics Canada, 2013). PD is thought to be caused by an interaction between environmental and genetic factors. MPTP intoxication is a prototypical

example of how an exogenous toxin can present as PD (Langston et al., 1983). Other environmental factors include living in a rural area, drinking well water, farming, and exposure to pesticides. It is thought that a toxin could cause neurodegeneration through chronic exposure or a sudden exposure that initiates a deleterious cascade of events.

The results from genetic studies have provided insight into possibilities of the underlying pathogenesis of the disease. Synuclein deposition, mitochondrial dysfunction, or disorders of autophagy might all play a role in the development of PD (Jenner *et al.*, 2013). Of course, the heterogeneity within PD and related disorders means that finding one causal agent is unlikely. It is possible that different forms of PD have different pathogenesis. If so, more specific categories under the PD umbrella should be implemented to distinguish PD-like disorders from idiopathic PD. With the knowledge of the underlying cause of the symptoms, more specific treatment can be developed.

1.2 Saccades: a model of behavioural control

Saccades are quick, conjugate eye movements that move the foveae to a location in the visual field (Collewijn *et al.*, 1988; Westheimer, 1954). Saccades are a valuable behavioural output for studying motor control because they can be either sensory-guided or volitionally-directed. Sensory-guided saccades are directed to a stimulus that appears in the environment (Guiton *et al.*, 1985; Pierrot-Deseilligny *et al.*, 1991; Schiller *et al.*, 1987). For instance, a visually-guided saccade may be triggered after a sudden motion is detected in the visual field. In contrast, volitionally-directed saccades are eye movements initiated by an internal goal of the individual (Gaymard *et al.*, 1998; Hikosaka *et al.*, 2000). For example, a diligent worker suppresses the instinct to look at visual distractors and, instead, produces volitionally-directed saccades necessary for performing his/her work.

The saccadic eye movement system is commonly used as a model for studying cognitive function for several reasons (Leigh & Zee, 1999; Moschovakis *et al.*, 1996; Munoz *et al.*, 2000).

The eyes are rotated by the actions of only three orthogonal pairs of extraocular muscles. Furthermore, eye movement control circuitry does not have to compensate for variable loads and inertia present in larger skeletal muscle systems (Jones & Dejong, 1971). This circuitry is readily accessible to microelectrodes, facilitating electrophysiological recordings in awake, behaving non-human primates (Sparks, 2002). Since the entire circuit exists within the cranium, researchers can record from different neuronal components efficiently. The circuit's simplistic computational design and its accessibility to microelectrodes result in a well-understood sensory-to-motor transformation. The saccadic eye movement system can be easily measured in behavioural studies with the use of video-based eye tracking technology.

1.3 The oculomotor circuit

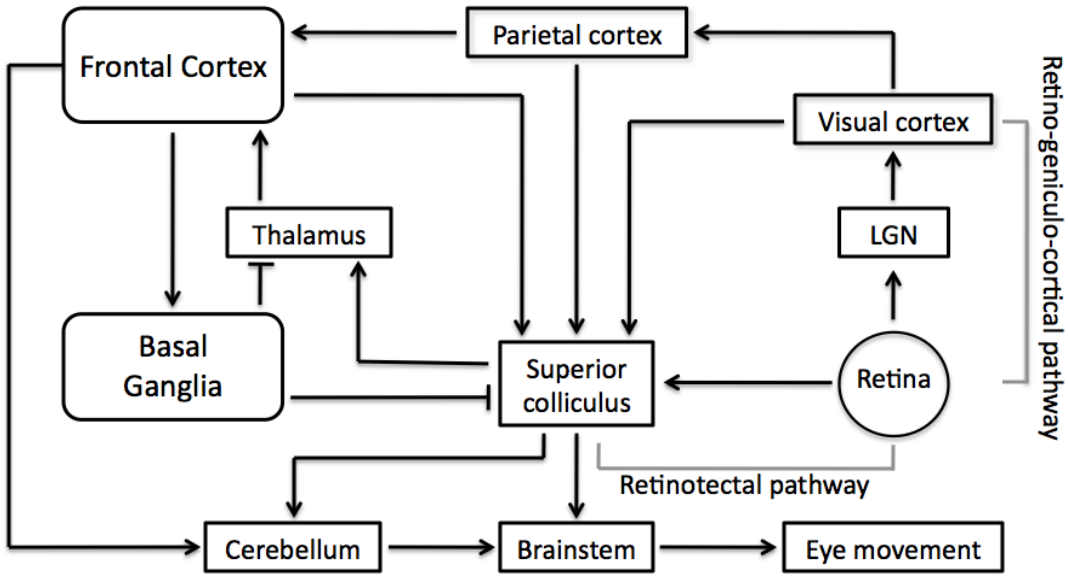


Figure 1.1: The saccadic eye movement system. Adapted from Munoz & Everling (2004).

Regions in the cerebral cortex, basal ganglia, thalamus, superior colliculus (SC), brainstem, and cerebellum contribute to the production and flexible control of saccades (Fig. 1.1) (Leigh & Zee, 1999; Moschovakis *et al.*, 1996; Pierrot-Deseilligny *et al.*, 1991; Scudder *et al.*, 2002). The consolidation of findings from lesion studies, behavioural testing, functional

neuroimaging, and primate neurophysiology has resulted in a well-understood sensory-to-motor transformation. This considerable body of knowledge makes the eye movement system a useful model for investigations into the function of the involved brain areas (Coe *et al.*, 2002; Dias & Segraves, 1999; Guitton *et al.*, 1985; Munoz & Everling, 2004).

Visual information enters the oculomotor circuit through the retina. The signal travels to the lateral geniculate nucleus (LGN) of the thalamus and to the visual cortex via the retino-geniculo-cortical pathway. The visual cortex projects to regions in the parietal cortex and the frontal cortex. Each cortical area, along with the retina itself via the retinotectal pathway, projects directly to the superior colliculus.

Frontal cortical regions involved in oculomotor functioning include the frontal eye field (FEF), the supplementary eye field (SEF), and the dorsolateral prefrontal cortex (DLPFC). The FEF is active during voluntary saccade execution. (Pierrot-Deseilligny *et al.,* 1991; Dias & Segraves, 1999; Rivaud *et al.*, 1994; Sommer & Tehovnik, 1997). The SEF performs roles of saccade sequencing and internally guided decision-making (Coe *et al.*, 2002). Finally, the DLPFC is important for executive function and suppressing visually-guided saccade responses (Guitton *et al.,* 1985; Pierrot-Deseilligny *et al.,* 1991). Overall, these frontal cortical areas signal the motor output regions to execute voluntary saccades.

Importantly, the frontal cortical oculomotor areas also project to the caudate nucleus (CN) of the BG. The BG as a whole act as an action selector by modulating signals from the frontal cortex (Watanabe & Munoz, 2011). The BG exert a strong, tonic inhibition and promote certain signals via disinhibition (Hikosaka *et al.,* 2000). Additionally, the BG exert control over the brainstem motor networks through projection to the SC from the substantia nigra pars reticulata (Snr) (Hikosaka & Wurtz, 1983; Hikosaka *et al.*, 2000; Jiang *et al.*, 2003; Niijima & Yoshida, 1982). The SC is a key station for saccade control, with retinal visual inputs mapped onto its superficial layer (Schiller & Stryker, 1972). Its intermediate layer possesses a motor

function (Sparks, 1986). With both sensory and motor functionalities, the SC can respond to visual signals and generate a saccade command to the reticular formation in the brainstem (Chimoto *et al.*, 1996; Rodgers *et al.*, 2006). Auditory and somatosensory information also converge onto the intermediate layer of the SC, triggering eye movements to non-visual stimuli (Munoz, 2002; Russo & Bruce, 1994; Stein & Meredith, 1993). Therefore, saccades can either be volitionally-generated, driven by internal goals and involving the frontal cortical areas, or triggered by salient stimuli in the environment. The role of the basal ganglia is to promote the appropriate action among these competing motor commands.

1.4 Basal ganglia thalamocortical networks

Along with the BG's control over brainstem motor networks, the BG contain different pathways to modulate incoming signals from the cortex (Fig. 1.2). The CN in the striatum receives input from cortical areas and the thalamus (Hikosaka *et al.*, 2000). It sends GABAergic projections directly to the SNr and indirectly through the external component of the globus pallidus (GPe). The GPe also communicates reciprocally with the subthalamic nucleus (STN) (Watanabe & Munoz, 2011). Via the direct pathway, the CN transiently inhibits the SNr, which reduces the tonic inhibition on the thalamus and excites the cortex (Hikosaka *et al.*, 2000). The indirect pathway is so named because it involves additional structures: the GPe and STN. Through the indirect pathway, increased activity of the striatum results in increased activity in the SNr. The indirect pathway results in inhibition of the thalamus and less excitation of the cortex (Takakusaki *et al.*, 2004). Therefore, the BG's role as an action selector is facilitated by the versatility offered by the antagonistic effects of the direct and indirect pathways.

A third pathway, known as the hyperdirect pathway, bypasses the striatum and connects the cortex directly to the STN (Hartmann-von Monakow *et al.*, 1978; Nambu *et al.*, 2000). This pathway provides shorter conduction times compared to signals through the direct and indirect

pathways. The hyperdirect pathway exerts powerful excitatory effects that activate the STN and, as a result, inhibit the thalamus and cortex (Nambu *et al.*, 2002).

The SNc plays an important role by projecting dopaminergic neurons into the striatum. Dopamine has different effects depending on the receptor it binds to (Hikosaka *et al.*, 2000). Neurons projecting to the SNr, the direct pathway, express D1-type receptors so DA binding will be excitatory. Neurons projecting to the GPe, the indirect pathway, express D2-type receptors so DA binding will be inhibitory. Since the actions of the direct and indirect pathways on the output of the basal ganglia are antagonistic, these different influences of the nigrostriatal dopaminergic neurons produce the same effect: a decrease in the inhibitory outflow of the BG and so greater cortical excitability (Gerfen *et al.*, 1990). In PD, dopamine depletion causes the dominance of the indirect pathway over the direct pathway, resulting in suppression of saccade initiation (Watanabe & Munoz, 2011).

It has been hypothesized that the direct, indirect, and hyperdirect pathways work in sequence in order to facilitate selection of a preferred motor plan. During the preparatory activity for a voluntary limb movement, a signal is transmitted through the hyperdirect pathway to suppress activity in the cortical and thalamic networks. Secondly, a signal from the direct pathway inhibits pallidal neurons in the SNr, which alleviates inhibition only on the selected motor plan. Finally, a signal travels through the indirect pathway to re-activate the SNr's inhibition on the thalamus. This sequential information processing encourages the initiation and execution of only the selected motor plan with the appropriate timing (Nambu *et al.*, 2002).

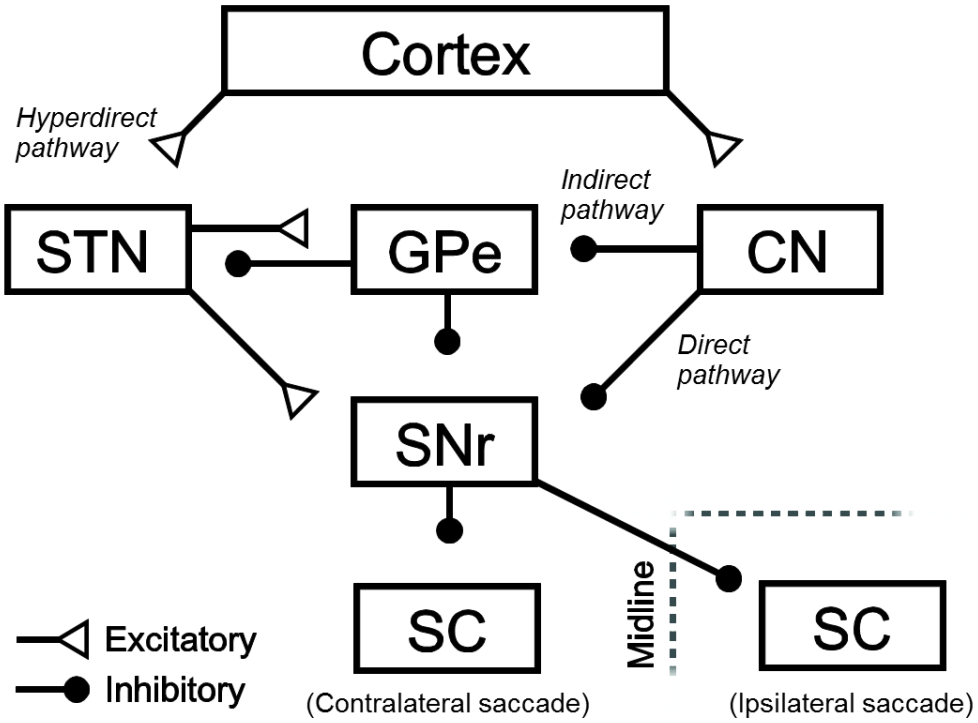


Figure 1.2: Neural structures involved in the basal ganglia control of saccadic eye movements. Adapted from Nambu *et al.* (2002). CN: Caudate nucleus; GPe: external segment of the globus pallidus; SC: superior colliculus; SNr: substantia nigra pars reticulata; STN: subthalamic nucleus

1.5 Pro- vs. antisaccade task: a test for basal ganglia function

Visually-guided and volitionally-directed saccades can be studied separately and compared using the prosaccade task and antisaccade task, respectively. In the prosaccade task, subjects are instructed to look towards a peripheral target once it appears. The prosaccade task requires an automatic response. Alternatively, in the antisaccade task, subjects are asked to look away from the target, specifically to the target's opposite position. Prior to each trial, the participant fixates on a spot at the centre of a visual screen termed the fixation point. The instructions for pro versus antisaccades are conveyed to the subject by the colour of the fixation dot at the start of each trial; a green fixation point indicates a prosaccade trial and a red fixation dot indicates an antisaccade trial (Fig 1.3).

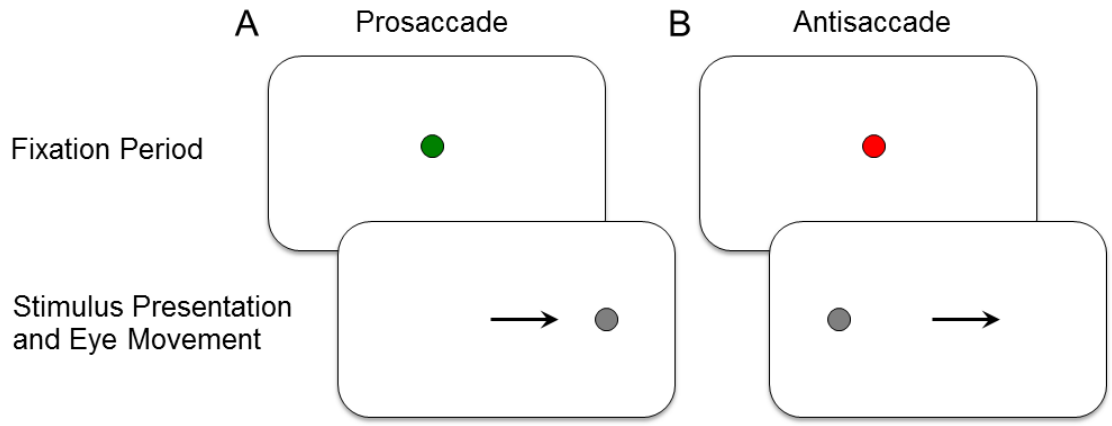


Figure 1.3: Schematic of prosaccade and antisaccade tasks. During the fixation period, a fixation dot is displayed on the screen, the colour of which indicates the task type. The fixation dot disappears and, simultaneously, a grey target dot appears either on the left or the right of the previously visible fixation dot. Correct saccadic eye movements are depicted by black arrows in the schematic.

Compared to prosaccades, the antisaccade motor plan takes longer to develop because a vector inversion is necessary to transform the representation of the visual stimulus location into an appropriate motor command. Therefore, the antisaccade task requires at least two additional processes: 1) the inhibition of the automatic, visually-guided saccade and 2) the generation of a volitionally-guided saccade (Munoz & Everling, 2004).

A quantitative model that describes this neuronal mechanism and predicts behaviour has only recently been proposed (Noorani & Carpenter, 2013). This model extrapolates upon the linear approach to threshold with ergodic rate (LATER) model of decision-making. An action is triggered once the decision signal reaches the threshold, the timing of which depends on a variable rate of rise (Carpenter & Williams, 1995). In order to account for the suppression of the visually-guided saccade, a LATER unit was added that cancels impending responses upon reaching threshold. This "stop" unit has been used successfully to model reaction time distributions and incidence of error trials in countermanding tasks, which require a subject to cancel responses if a "stop" signal is presented (Logan *et al.*, 1984). Therefore, the qualitative model for the antisaccade tasks involves three LATER units: two units (Pro and Anti) triggering

saccades in opposite directions and a "stop" unit to suppress the Pro unit (Noorani & Carpenter, 2013). The parameters for Pro and Anti units are identical while the directional inversion in the antisaccade is accounted for by a 50ms delay (Zhang & Barash, 2000).

Recently, neurophysiological recordings in behaving monkeys have identified three groups of putative projection neurons within the CN with roles in prosaccade and antisaccade control (Watanabe & Munoz, 2009). The first neuronal group is comprised of automatic saccade neurons (ANs), which generate sensory-driven saccades towards the stimulus via the direct pathway. In the antisaccade paradigm, this would facilitate a direction error saccade. The other two groups have greater activity prior to antisaccade initiation compared with prosaccade initiation. Both groups generate volitional saccades and are differentiated by their preferred directions and, correspondingly, their roles. The contralateral saccade-preferred volitional neurons (cVNs) facilitate correct antisaccades via the direct pathway. The conflict between the sensory-driven neurons and contralateral-preferred volitional neurons is resolved by a group of ipsilateral-preferred volitional neurons (iVNs). This group inputs on the SNr via the indirect pathway and suppresses direction error saccades. This model for an antisaccade paradigm is depicted in Fig. 1.4 (Watanabe & Munoz, 2011). This proposed model is substantiated by the LATER model (see above). Further investigation into the neuronal subsets within the CN could add insight into the determination of whether these neurons correspond to the units outlined by the LATER model.

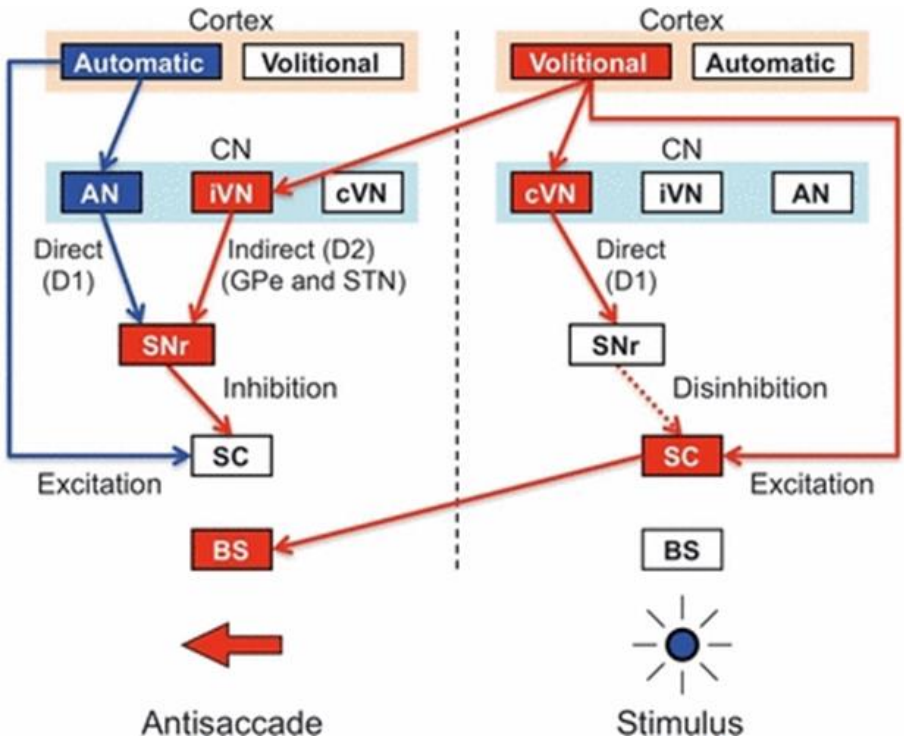


Figure 1.4: Basal ganglia model during the antisaccade paradigm (Watanabe & Munoz, 2011). ANs generate sensory-driven saccades towards the stimulus via the direct pathway. cVNs facilitate correct antisaccades via the direct pathway. The conflict is resolved by iVNs, which input on the SNr via the indirect pathway and suppress direction error saccades. AN: automatic (sensory-driven) neuron; BS: brainstem; CN: caudate nucleus; cVN: contralateral volitional neurons; D1: d1-like dopamine receptors; D2: D2-like dopamine receptors; GPe: external segment of the globus pallidus; iVN: ipsilateral volitional neurons; SC: superior colliculus; SNr: substantia nigra pars reticulate; STN: subthalamic nucleus

1.6 Eye movements affected by PD

It is well-established that saccadic eye movement abnormalities are found in clinical groups, including PD patients (Amador *et al.*¸ 2006; Briand *et al.*¸1999; Cameron *et al.*, 2010; Cameron *et al.*, 2012; Chan *et al.*, 2005; Hood *et al.*, 2007; Rivaud-Perchoux *et al.*, 2007). Hypometric saccade amplitudes are often reported despite normal saccadic velocity and amplitude relationships (Jones *et al*, 1971; Teräväinen & Calne, 1980). The major difference between PD and control subjects in eye movement performance is seen during antisaccade tasks. Antisaccade tasks (Fig. 1.3B) require subjects to make saccades away from visual targets (Hallett, 1978). Antisaccades are considered voluntary eye movements due to the requirements of

suppressing the automatic response to look at the visual stimulus and generating a voluntary movement in the opposite direction (Munoz & Everling, 2004). When the antisaccade task was applied to PD patients, it was determined that PD patients make more direction errors than control subjects (Briand *et al.,* 1999; Chan *et al.,* 2005). On correct antisaccade trials, PD patients had longer saccadic reaction times (SRTs) (Amador *et al.,* 2006; Cameron *et al.*¸2012; Chan *et al.*, 2005). These results align with the voluntary movement deficiencies that characterize PD.

Interestingly, PD patients are generally found to exhibit normal or even superior saccadic performance when prosaccades, saccades towards visual targets, are required (Briand *et al.,* 1999). Previous studies have reported slightly faster SRTs in prosaccades for PD subjects compared to controls (Briand *et al.,* 1999; Cameron *et al.*, 2012; Chan *et al.,* 2005; Roll *et al.*, 1996). PD subjects also demonstrated increased proportions of express saccades (SRT of 90-140ms) (Cameron *et al.*, 2012; Chan *et al.*, 2005).

Considering the important, modulating role that the basal ganglia plays in saccadic eye movement control, it is not surprising that PD patients exhibit abnormalities in eye movement tasks. In normal physiological conditions, the action of DA in the SNc increases the excitation of the frontal cortex (Gerfen *et al.*, 1990). Dopamine promotes the voluntary saccade command to the motor output regions and suppresses involuntary saccades. In terms of the behavioural task, this would facilitate correct antisaccades.

In PD, the death of dopaminergic neurons in the SNc disrupts the prefrontal- basal ganglia circuitry resulting in deficits in voluntary saccade initiation as demonstrated by increased direction errors and slower SRTs in the antisaccade task in PD patients (Briand *et al.*, 1999; Amador *et al.,* 2006; Chan *et al.*, 2005). Superior prosaccade performance of PD patients indicates deficits in automatic response suppression (Chan *et al.*, 2005; Middleton & Strick, 2002). These effects can be attenuated through the administration of levodopa to PD patients;

SRT in the prosaccade task is increased and direction error rate in the antisaccade task is reduced (Hood et al., 2007).

Therefore, dopamine depletion in PD causes an imbalance between voluntary and involuntary movement. Impaired antisaccade performance coupled by enhanced prosaccade performance indicates reduced regulatory inhibition on visually-guided saccades. It is also possible that the neural networks in the PD brain have adapted in response to the basal ganglia's dysfunction. Because PD patients are slow to initiate voluntary movements, the brain may have reduced baseline response inhibition so as to promote all motor commands. In the oculomotor circuitry, this adaptive mechanism can be detected because structures such as the SC can be relied upon due to their direct visual-to-motor mapping. In order to determine the neural mechanisms underlying PD saccade abnormalities, we will focus on how PD subjects prepare for upcoming actions. Based on hypoactivation in the CN and cortical eye fields, it has been hypothesized that PD patients make antisaccade direction errors due to improper presetting towards the appropriate voluntary action (Cameron *et al.*, 2012). We will investigate action preparation in PD subjects through the measurement of microsaccades made during fixation.

1.7 Microsaccades

Microsaccades are small amplitude, ballistic fixational eye movements that occur at a rate of 1-2 per second (Engbert & Kliegl, 2002; Martinez-Conde *et al.*, 2013). Microsaccades are made involuntarily, but can be voluntarily suppressed without training (Bridgeman & Palca, 1980). Along with tremor and drift, microsaccades counteract retinal adaptation by displacing the retinal image during stationary viewing (Riggs *et al.,* 1953). Microsaccades restore faded vision during fixation for both foveal and peripheral targets (Martinez-Conde *et al.,* 2006; McCamy *et al.*, 2012). Other functions include fixation error correction (Otero-Millan *et al.*, 2011) and spatial resolution improvement during high-acuity tasks (Donner & Hemilä, 2007; Ko *et al.,* 2010).

Saccades and microsaccades both perform important roles in vision. Furthermore, recent studies have found a temporal connection between fixational saccade occurrence and saccade initiation (Rolfs *et al.*, 2006). Peripheral stimulus presentation induces a general decrease in the microsaccade frequency with a signature rise in rate occurring at approximately 350 ms after stimulus appearance (Engbert & Kliegl, 2002; Rolfs *et al.*, 2006). The functional similarities and temporal relationships between saccades and microsaccades suggest that they share a common generator and might even exist on the same continuum (Martinez-Conde *et al.*, 2013; Otero-Millan *et al.*, 2008).

In addition to their visual functions, microsaccades might reflect covert processes. Previous work has determined that microsaccades can reflect covert attentional states through their role in visual information processing (Engbert & Kliegl, 2002). Recently, Watanabe *et al.* (2013) has hypothesized that microsaccades reflect preparatory processes occurring prior to saccade execution. Voluntary saccade initiation requires the adoption of an appropriate task set, a rule about how to behave (Cameron *et al.*, 2012). It is possible that microsaccades can provide an overt measurement of the brain's presetting mechanisms.

1.8 Role of microsaccades in action preparation

In order for our behaviour to be predictive rather than reactive, voluntary actions are preceded by preparatory processes. These preparatory processes are regulated by networks in the cerebral cortex and the basal ganglia, and ready neural circuits based on environmental cues and internally-derived goals (Nachev *et al.*, 2008). Voluntary action preparation cannot be measured directly; however, cognitive models and neural recordings have provided inferential evidence into the existence of these processes.

As non-human primates prepare eye movements that indicate their choice between stimuli, FEF neurons associated with both stimuli increase their firing rates. Over time, cells associated with a preferred stimulus increase or maintain their activity whereas cells associated

with the non-preferrred stimulus decrease their activity (Schall, 2002; Thompson *et al.*, 1996). Since this build-up occurs whether or not a saccade is ultimately made, the activity is not likely part of the eye movement itself but rather it reflects a preparatory selection process (Thompson *et al.*, 1996). Furthermore, the growth of firing rates predicts reaction time with fast responses associated to rapid rises in activity (Smith & Ratcliff, 2004). Using fMRI, preparatory processing in the FEF was found to be greater in activation for correct antisaccade trials compared to direction error trials (Cameron *et al*., 2012).

Voluntary saccades, such as antisaccades, rely on sufficient presetting of a network of cortical regions and sub-cortical regions in preparation for saccade execution (Munoz & Everling, 2004). Without this presetting activity to favour volitional motor plans, it follows that there would be an increase in direction error rates in the antisaccade task. This behaviour is seen in PD, prompting investigations into the effects of BG pathophysiology on preparatory processing (Watanabe & Munoz, 2009; Watanabe & Munoz, 2010; Watanabe & Munoz, 2011). Cameron *et al.* (2012) observed hypo-activation of fMRI activation signals in motor areas involved in antisaccade generation during the preparatory stages of the saccadic response in PD. Since the frontal cortical regions are influenced by BG output, it is possible that BG pathophysiology in PD is responsible for this difference (Alexander *et al.*, 1986; Gerfen *et al.*, 1990).

The studies mentioned previously have relied on neural recordings, fMRI, and cognitive models to provide insight into covert preparatory processes. It has been hypothesized, however, that voluntary action preparation can be measured overtly by means of microsaccades. In order to determine the relationship between microsaccades and voluntary action preparation, Watanabe *et al.* (2013) measured microsaccades while participants performed prosaccade and antisaccade tasks. The prosaccade task is simply visually-guided whereas antisaccade task requires the suppression of a visually-guided saccade toward the stimulus and the facilitation of a voluntary saccade away from the stimulus. It was demonstrated that microsaccade occurrence was

suppressed by an antisaccade instruction, though this suppression was diminished when subjects made direction errors. This evidence led to the conclusion that the dual preparatory signals necessary for sufficient presetting prior to antisaccade execution can be read-out overtly by measuring microsaccades. In this thesis, I will test whether microsaccades can be used as an overt measurement of covert preparatory signals in PD.

1.9 Thesis objectives

The main objectives of this thesis are as follows. Firstly, eye movement abnormalities in PD found in previous studies will be verified. Specifically, I predict that PD subjects will exhibit slower SRTs and a greater percentage of direction errors in antisaccade tasks, faster SRTs and a greater percentage of express saccades in prosaccade tasks, and hypometric saccade amplitudes in comparison to controls (Amador *et al.*¸ 2006; Briand *et al.*¸1999; Cameron *et al.*, 2010; Cameron *et al.*, 2012; Chan *et al.*, 2005; Hood *et al.*, 2007; Rivaud-Perchoux *et al.*, 2007). Secondly, microsaccade behaviour will be investigated in order to determine if there are differences between PD and control subjects. It has been previously concluded that microsaccade activity reflects the covert preparatory processing necessary for antisaccade execution (Watanabe *et al.*, 2013). I hypothesize that microsaccade rates will be increased in PD, reflecting deficits in preparatory activity which correlate with poorer performance in the antisaccade task.

Finally, I will correlate error rate in the antisaccade task and microsaccade behaviour to measures of symptom severity and cognitive impairment. I anticipate that error rate will be positively correlated with symptom severity, as shown previously (Amador *et al.*, 2006). The progression of PD is due to further death of dopaminergic neurons resulting in difficulties initiating volitional movements, including volitional eye movements such as antisaccades. Also, error rate should be positively correlated with microsaccade rate. Microsaccade rate is suppressed by antisacccade instruction; however, this suppression is diminished prior to error trials (Watanabe *et al.*, 2013). I hypothesize that microsaccade rate will be positively correlated with

symptom severity. It has been suggested that microsaccades are mediated by the basal ganglia, specifically, the caudate nucleus (Watanabe *et al.*, 2013). Since PD involves the deterioration of the dopaminergic nigro-striatal system, it follows that disease advancement would result in abnormalities in microsaccade control.

Ultimately, I aim to investigate the relationship between microsaccades and preparatory activity by analysing microsaccade behaviour in PD. Deficits in antisaccade preparatory processes in PD subjects have recently been identified using fMRI (Cameron *et al.*, 2012). I seek to determine if these deficits are reflected in microsaccade behaviour. My thesis will support the role of microsaccades as a direct and non-invasive measurement of preparatory activity.

Chapter 2

Methods

2.1 Participants

All procedures were approved by the Human Research Ethics Board at Queen's University. All participants provided written informed consent and were compensated for their participation ($20).

For this study, 23 patients with PD (mean age = 66.4 years, SD ± 9 years) were recruited from the Movement Disorder Clinic at Kingston General Hospital. All patients were diagnosed with PD by neurologist Dr. G. Pari from the clinical presentation of at least 2 of the 4 major motor symptoms that characterize PD: tremor, akinesia/bradykinesia, rigidity, and postural instability. PD participants did not interrupt their medications for the study due to the fact that antisaccade deficits are present even while taking dopaminergic medications (Cameron *et al.*, 2012; Hood *et al.*, 2007). Nineteen of 23 participants were taking levodopa, 10 of which were additionally taking the controlled-release levodopa. 6 participants were taking a MAO-B inhibitor: 2 were taking rasagiline and 4 were taking amantadine. 4 participants were taking ropinirole and 15 participants were taking pramiprexole; both medications are dopamine receptor agonists. 3 participants were taking the COMT inhibitor entacapone. Lastly, one subject (PD Subject 13 from Table 1) had undergone deep brain stimulation. The mean levodopa equivalent dose was 705 mg (SD ± 510 mg) and the mean duration of symptoms was 76 months (SD ± 61 months). Dr. G. Pari conducted the United Parkinson's Disease Rating Scale (UPDRS) Parts I and II with each PD participant. The PD subjects self-assessed their activities of daily life for Part II and their motor symptoms were evaluated for Part III. In addition, Dr. G. Pari provided a disease severity score based on the modified Hoehn and Yahr staging (Fahn & Elton, 1987; Goetz *et al.*, 2004). The PD participant group had a mean UPDRS part II score of 11 (SD ± 5) and part

III score of 30 (SD ± 13). PD subjects were considered mild/moderate stage based on a mean Hoehn and Yahr score of 2.4 (SD ± 0.6). Clinical data and participant demographics are shown in Table 1.

For purposes of comparison to the PD subject group, data from 19 age-matched controls (mean age = 68.6 years, SD ± 7 years) were also collected. These subjects were spouses or friends of the PD participants or community members who responded to print advertisements. The control group did not differ significantly from the patient group in terms of age or years of education (Table 1).

Participants were tested without corrective lenses; however, all participants confirmed that the visual stimuli were easily visible. Participants with co-morbid neurological, psychiatric, or ophthalmic conditions, such as macular degeneration or cataracts, were excluded. Finally, the Montreal Cognitive Assessment (MoCA) was used as an evaluation of mental status. Although a score of 26 or higher is considered cognitively normal, a cut-off score of 24 was chosen due to the simplicity of the task instructions. Every subject corrected their errors by making a secondary saccade to the correct location, which confirmed their understanding.

Table 1: Clinical information of PD subjects.

PD Subject	Sex	Age (years)	Education (years)	MoCA (/30)	Mo. since diagnosis	UPDRS Score Part II (/52)	UPDRS Score Part III (/108)	Hoehn-Yahr Stage (/5)	Medications/ Treatments	Levodopa Eq. Dose (mg)
1	m	63.5	12.0	27	59	12	41	2.0	L, L-CR	575
2	m	67.7	10.0	28	8	5	21	2.0	R, P	250
3	m	65.7	18.0	29	24	10	15	2.0	L, R	420
4	m	73.6	11.0	25	104	12	36	2.0	L, L-CR, P, A, S	1100
5	m	73.1	17.0	26	51	7	32	2.0	P, A	100
6	f	56.5	12.0	28	87	5	11	2.0	L, L-CR,P	775
7	m	50.9	12.0	28	12	6	17	1.5	P	125
8	m	78.9	19.0	26	148	15	43	3.0	L, L-CR, P, M, E	1248
9	m	74.7	17.0	25	167	14	47	3.0	L, S, E	1198
10	f	73.0	11.0	26	6	8	16	2.5	P	50
11	m	69.6	17.0	28	85	7	21	2.0	L, P	700
12	m	70.9	18.5	25	79	6	19	2.5	L, L-CR, P	1450
13	m	43.6	13.5	24	129	13	39	2.0	L, R, M, DBS	1160
14	f	63.7	17.0	28	60	11	26	2.5	L, L-CR, P	537.5
15	f	72.2	12.0	26	37	7	21	2.0	L, R	280
16	m	55.9	18.0	24	250	24	56	4.0	L, P, A	2080
17	m	63.1	16.0	27	64	19	53	3.0	L, L-CR	1250
18	f	83.6	13.0	26	2	11	41	3.0	L	250
19	m	70.3	18.0	26	29	3	22	2.0	L, P	350
20	m	62.8	12.0	28	158	18	37	3.0	L, L-CR, M	550
21	f	56.3	17.0	28	47	16	36	3.0	L, L-CR, P	725
22	f	68.9	15.0	30	55	9	11	2.0	L, P	475
23	f	69.0	17.0	30	78	8	26	2.0	L, L-CR, P	575
Mean ± SD (n= 23)	15 m; 8 f	66.4 ± 9.2	14.9 ± 2.9	27 ± 2	76 ± 61	11 ± 5	30 ± 13	2.4 ± 0.6	-	705 ± 510
Controls (n=19)	9 m; 10 f	68.6 ± 7	15.5 ± 3.0	27 ± 2	-	-	-	-	-	-

A: anticholinergic; DBS: deep brain stimulation; E: entacapone; Eq.: equivalent; L: levodopa; L-CR: levodopa controlled-release; M: amantadine; mg: milligrams; Mo.: months; MoCA: Montreal Cognitive Assessment; P: pramipexole; R: ropinirole; S: rasagiline; SD: standard deviation; UPDRS; United Parkinson's Disease Rating Scale

2.2 Eye tracking and visual display

Using a video-based binocular eye tracker (Eyelink 1000; SR Research Ltd.), horizontal and vertical gaze data were collected from each eye at a sampling rate of 500 Hz. A high-speed camera and infrared illuminator were mounted on the desk in front of the participant and used to track the pupils. The subject's head was stabilized with chin and forehead rest bars. The display screen was adjusted so the top of the display screen was aligned with the centre of the pupillary plane, as recommended in the Eyelink manual.

Eye-tracking experiments were completed in a darkened room located in Kingston General Hospital for all subjects except one control subject, for which the eye-tracking apparatus was set-up in their home. Stimuli were presented on a 17-in LCD monitor placed 58.5 cm from the subject's eyes. Calibration and validation were completed using a 9-point grid, immediately before each trial block. Data was collected as each participant performed the 3 trial blocks. Participants were encouraged to rest between the blocks.

2.3 Saccade paradigm

The experimental procedure is summarized in Fig. 1.3. Each trial began with the appearance of a green or red fixation point in the centre of the screen. After a fixation period of 1000ms or 2000ms, the fixation point disappeared with the simultaneous appearance of an eccentric white target at 10° horizontally to the left or right of centre. The visual stimuli were circles of 0.5˚ diameter presented on a black background. A photometer was used to measure and equalize the luminosity of the red, green, and white stimuli. Participants were instructed to look at the eccentric target if the fixation point was green (prosaccade) and look away from the eccentric target if the fixation point was red (antisaccade). The eccentric target appeared randomly on the left or right in equal proportions for prosaccade and antisaccade trials.

Participants performed 3 trial blocks each consisting of 120 trials. The first two blocks consisted of pseudo-randomized trials, with 30 prosaccade/left target, 30 prosaccade/right target, 30 antisaccade/left target, and 30 antisaccade/right target trials. The task type (prosaccade or antisaccade) and target location (left or right) were randomly interleaved. The first block had a fixation point duration of 1000ms and the second block had a fixation point duration of 2000ms. In the third trial block, the fixation point duration alternated from 1000ms to 2000ms in blocks of ten trials and only prosaccades were used. The target's location (left or right) was still pseudo-randomized. Results from the third trial block were only incorporated in the correlational analyses in order to increase the statistical power of the microsaccade rate calculations. Due to calibration issues, PD Subject 12 did not complete the third trial block so only results from the first two trial blocks were used in the rate calculations for this subject.

2.4 Saccade and microsaccade measurement

Saccade measurement relied on the eye position data, which was differentiated to produce instantaneous horizontal and vertical velocities. The baseline velocity was calculated by averaging the changes in displacement over time with an upper cutoff of 50°/sec. The mean baseline velocity represented the mean fixation velocity. The mean fixation velocity plus 1.5 times its standard deviation was used as the threshold velocity for saccade detection. Finally, the velocity had to remain above that threshold for 5 sample points for it to be considered a saccade. This approach ensured that noise causing spikes in velocity were not detected as saccades. SRT was calculated from the time that the peripheral target appeared to when the threshold velocity for saccade detection was held for at least 5 sample points

Microsaccades were detected using an algorithm similar to that of Martinez-Conde and colleagues (Martinez-Conde *et al.*, 2000). The eye position data were differentiated to produce instantaneous horizontal and vertical velocities. We then used two threshold values during the fixation period to determine when the eyes were stationary and when they were making a

microsaccade. In order to be considered a microsaccade, the instantaneous eye direction could not change more than 15^{o} (threshold 1) and the instantaneous eye speed was at least 8^{o}/sec (threshold 2). The speed threshold was chosen such that it gave the best main sequence as described by Zuber and colleagues (1965). Finally, a microsaccade was defined as a sequence in which the eye moved for a period of at least 6 sample points. From the eye movement data, the onset and termination of all microsaccades was calculated, along with the peak velocity, magnitude, and direction. An amplitude between 0.2-2° was necessary to be considered a microsaccade in order to exclude noise but include larger amplitude microsaccades (Brien *et al.*, 2009; Watanabe *et al.*, 2013).

2.5 Behavioural analysis

Analysis of behavioural data was completed using custom-made scripts in Matlab v8.0 (The MathWorks Inc., Natick, MA, USA). No differences were seen across rightward and leftward saccade trials; therefore all trials were pooled together. Correct trials were separated from incorrect trials on the basis of direction errors and anticipatory errors. The trial was marked as a direction error trial if a saccade was made to the visual target in the antisaccade task or to the location opposite to the target in the prosaccade task. Trials in which a participant made a direction error in the prosaccade or antisaccade task are known as error prosaccade trials and error antisaccade trials, respectively. Anticipatory errors have SRTs less than 90ms, which is faster than the conduction time of the shortest neural pathway from the retina to the eye muscles (Fischer & Boch, 1983). Trials in which subjects failed to respond were excluded from analysis.

Express saccades were defined as saccades with SRTs between 90-140ms, consistent with previous research (Chan *et al.*, 2005). An independent t-test was conducted to analyze express saccades in prosaccade trials. Express saccades in antisaccade trials were not analyzed because they cannot be generated due to the physiological pathways involved in this more complex task (Everling *et al.*, 1998). Cumulative SRT distributions were analyzed using a two-

sample non-parametric Kolmogorov-Smirnov test. Similarly to the saccade characteristics, the probability distributions shown are the average distributions of each subject pool.

Mean values of saccade characteristics were determined by finding the mean values of each participant and then calculating the mean for each subject group from these values. Using SPSS Statistics v20 (IBM, Chicago, IL, USA), 2x2 repeated measures analysis of variance (ANOVA) tests were performed on these means (mean SRT, mean amplitude, and percentage of direction errors). The variables used were Group with two levels (PD, controls) and Task with two levels (prosaccade, antisaccade).

The main sequence plots of microsaccades were fitted to a linear regression because it has been shown previously that peak velocities of microsaccades increase linearly with the log of their amplitudes but the relationship approaches linearity at low amplitudes (Otero-Millan *et al.*, 2008; Watanabe *et al.*, 2013; Zuber *et al.*, 1965). Then, a one-way ANOVA was performed to test for significant differences between the slopes of the fitted regression lines. Microsaccade directions were plotted in circular histograms termed rose plots. These plots were constructed by first calculating the percentage of microsaccades within each direction bin. The plots display the average percentage of each bin for the subject group. A Wilcoxon Rank Sum Test was used to analyze differences in the distributions of angles. The rose plots indicate microsaccade directions during fixation from 400ms until target appearance.

Microsaccade rate diagrams were plotted based on the mean microsaccade rates of subject groups calculated from the individual subject means. Differences in rates were analyzed using the Wilcoxon Rank Sum Test at every millisecond. The greyscale colour bar indicates levels of significance from $p = 0.20$ and below (e.g. Figs. 3.6 and 3.7).

Correlational analyses were conducted using the eye movement parameters of error rate on antisaccade trials and microsaccade rates in all three trial blocks. Data from the third trial block of prosaccades only was used in order to increase the statistical power of the analyses.

Microsaccade rate was calculated based on the final 400ms of fixation until target appearance. Linear regression analysis was conducted using Microsoft Excel 2010 v14.0 (Microsoft, Redmond, WA, USA).

Chapter 3

Results

3.1 Clinical evaluation and cognitive assessment

All of the 23 patients recruited for this study underwent a clinical evaluation of symptoms with a neurologist. Scores from the UPDRS Parts I and II, the Hoehn-Yahr scale, and medications for each participant can be found in Table 1. Most patients (17 of 19) fell within the range of 2.0-3.0 on the Hoehn-Yahr staging which indicates mild to moderate bilateral disability. The remaining two patients scored 1.5 and 4.0, corresponding to unilateral involvement and severe disability, respectively. All participants were cognitively evaluated using the MoCA. Mean MoCA scores, age, and years of education did not differ significantly between PD and control groups.

3.2 Saccade behaviour

Saccade behavior was analyzed to verify that previous eye movement abnormalities found in PD were reproduced in the current investigation. Cumulative SRT distributions were analyzed using the non-parametric Kolmogorov-Smirnov test to determine if quantitative differences in SRT distributions exist between PD and control subjects in prosaccade and antisaccade tasks (Fig. 3.1). Significant differences were found in SRT distributions between PD and control subjects for trials with 2000ms fixation periods (Fig. 3.1A + C) and 1000ms fixation periods (Fig. 3.1B + D). For 2000ms, differences were found in correct prosaccades ($K = 0.1429$, $p < .001$), correct antisaccades ($K = 0.0785$, $p = .0109$), and incorrect antisaccades ($K = 0.2592$, $p < .001$). Similarly for 1000ms, differences were found in correct prosaccades ($K = 0.1266$, $p < .001$), correct antisaccades ($K = 0.1669$, $p < .001$), and incorrect antisaccades ($K = 0.2411$, $p < .001$).

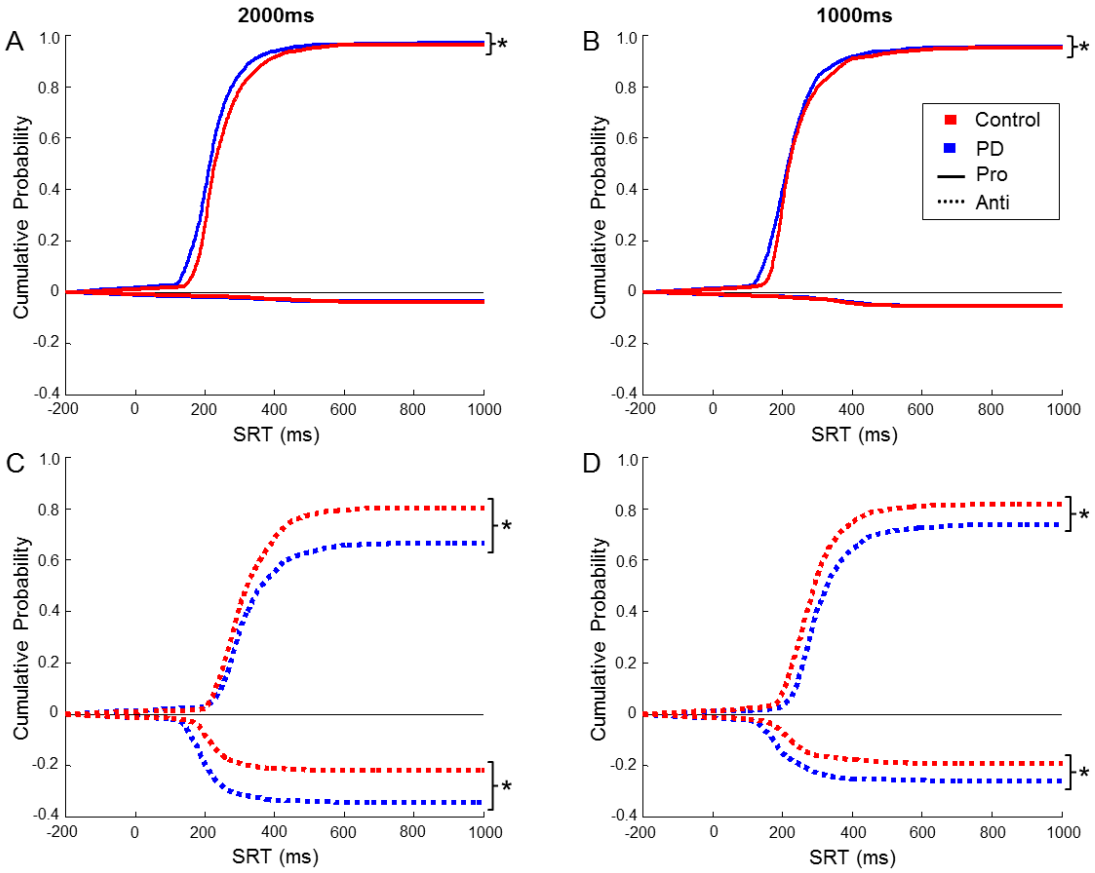


Figure 3.1: Cumulative distributions of saccadic reaction times (SRTs) for (A) prosaccade trials with 2000ms fixation periods, (B) prosaccade trials with 1000ms fixation periods, (C) antisaccade trials with 2000ms fixation periods, and (D) antisaccade trials with 1000ms fixation periods. Correct saccades are illustrated as positive proportions while direction errors are shown as negative proportions.

The saccadic behavioural results of prosaccade and antisaccade trials in PD and control subjects are illustrated in Fig. 3.2. Trials with 1000ms and 2000ms fixation periods were collapsed. Analysis of variance for SRT (Fig. 3.2A) demonstrated a significant main effect of Task ($F(1,40)=136.295$, $p<.001$), with subjects having a faster SRT on prosaccades than antisaccades. There was no main effect of Group [$F(1,40) = 0.001$, ns] but there was a significant interaction of Task with Group [$F(1,40) = 6.317$, $p<0.02$]. The PD and control groups separately exhibited faster SRTs on prosaccades compared to antisaccades (using LSD = 37.49). PD subjects

tended towards faster SRT in prosaccade trials and slower SRT in antisaccade trials compared to controls but these differences were not significant.

The express saccade epoch was defined as 90-140ms, consistent with previous literature (Chan *et al.*, 2005) (Fig. 3.2B). An independent t-test revealed that the PD group made a significantly greater proportion of express saccades on prosaccade trials compared to controls ($t(23)= -2.817$, $p=.01$).

Analysis of variance of direction errors (Fig. 3.2C) showed a main effect of Task ($F(1,40)=61.117$, $p<.001$) but no main effect of Group ($F(1,40)=3.758$, ns). There was a significant interaction of Task with Group ($F(1,40)=4.704$, $p<0.05$). Both PD and control groups separately had a higher error percentage on the antisaccade task compared to the prosaccade task. There was no significant difference in error rate between groups on the prosaccade task; however, PD subjects had a higher error rate compared to controls in the antisaccade task. Direction errors were also compared between 1000ms and 2000ms fixation duration antisaccade trials for PD and control groups. There was a main effect of Group ($F(1,40)=4.222$, $p<.05$), as expected, but also a main effect of Fixation Duration ($F(1,40)=8.070$, $p<.01$) indicating that both PD and control groups make more direction errors in 2000ms antisaccade trials compared to 1000ms trials. There was no significant interaction of Fixation Duration with Group ($F(1,40)=3.010$, ns).

For saccadic amplitude (Fig. 3.2D), analysis of variance demonstrated a significant main effect of Group ($F(1,40)=9.072$, $p<.01$) with PD subjects producing hypometric saccadic amplitudes compared to controls, as shown previously (Jones & Dejong, 1971). However, there was no main effect of Task ($F(1,40)=1.424$, ns).

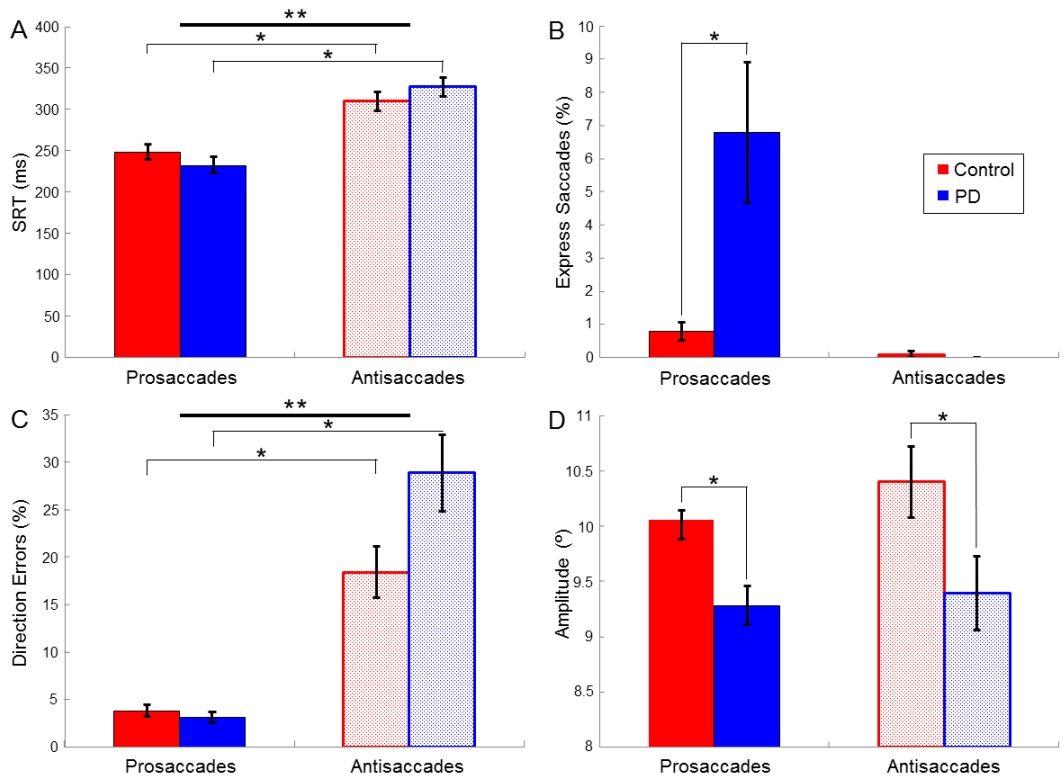


Figure 3.2: Saccade characteristics of PD and control subjects for prosaccades and antisaccades. (A) Mean saccadic reaction time (SRT) on correct trials. (B) Mean percentage of express saccades (90-140ms) on correct trials. (C) Mean percentage of direction errors on antisaccade trials. (D) Mean saccadic amplitude on correct trials. Targets were 10° from the fixation point. Error bars represent the standard error of the mean (SE). Results from trials with 1000ms and 2000ms fixation periods were collapsed.

3.3 Microsaccade characteristics

Advancements in eye movement detection technology have permitted the recording of microsaccades during fixational periods of eye movement tasks. Microsaccade main sequences for PD and control subjects are shown in Figure 3.3. Consistent with previous studies, the peak velocities increased linearly with their amplitudes (Watanabe *et al.*¸ 2013; Zuber *et al.*, 1965; Otero-Millan *et al.*, 2008). There was a slight but significant difference between the slopes of PD (86.30 ± 0.11) and control subjects (90.99 ± 0.01) ($F(1,35825)= 123.94$, $p<.001$), indicating that PD microsaccades have a lower peak velocity than control microsaccades per saccade amplitude.

Figures 3.4 and 3.5 contain circular histograms displaying the distribution of microsaccade directions. Horizontally rightward and vertically up microsaccades were designated as 0° and 90°, respectively. PD and control subjects both showed a strong horizontal bias, as described previously (Watanabe *et al.*, 2013). Error prosaccade trials are not shown due to insufficient data. Microsaccade directional distributions were not different between PD and control subjects for correct prosaccade (2000ms: p=0.252, ns; 1000ms: p=0.2448), correct antisaccade trials (2000ms: p=0.6138, ns; 1000ms: p=0.5485, ns), and error antisaccade trials (2000ms: p=0.4356; 1000ms: p=0.1588, ns) (Fig. 3.4 and Fig. 3.5).

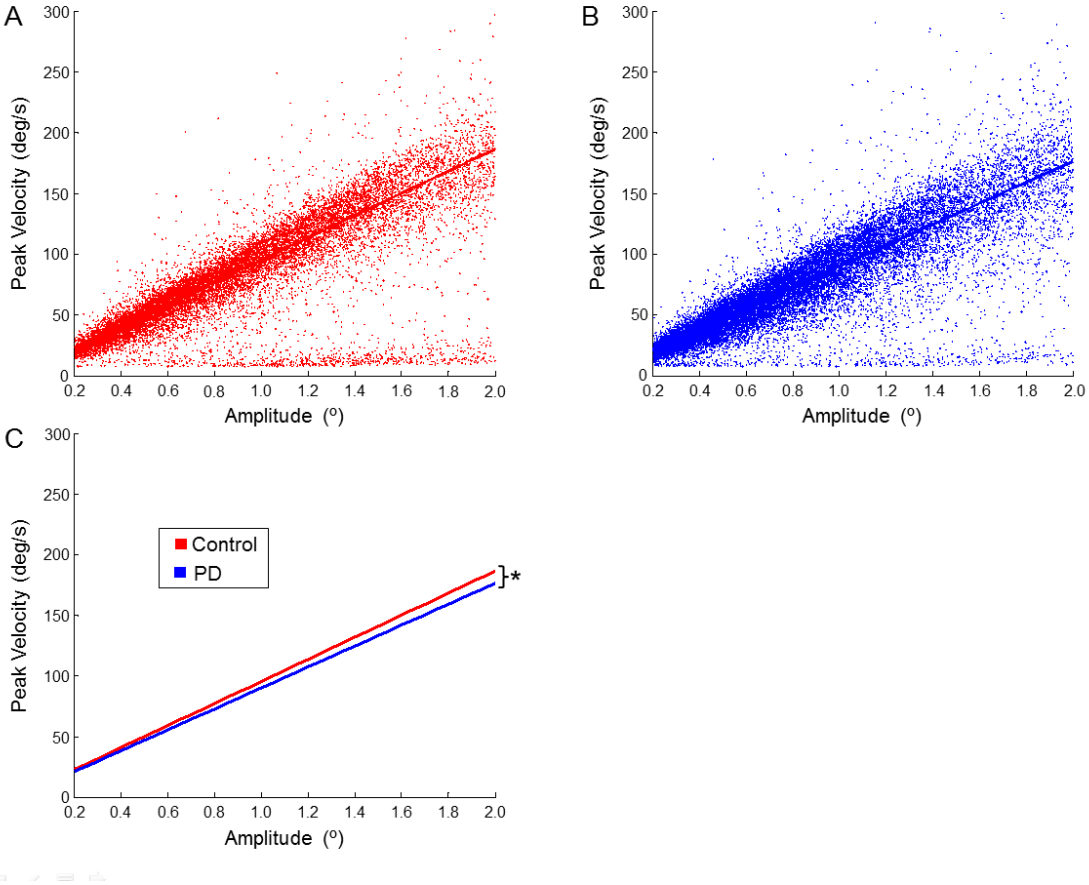


Figure 3.3: Microsaccade main sequences for (A) control subjects and (B) PD subjects. (C) Regression lines for control and PD subjects. Slope ± confidence interval for controls is 90.99 ± 0.01 and 86.30 ± 0.11 for PD.

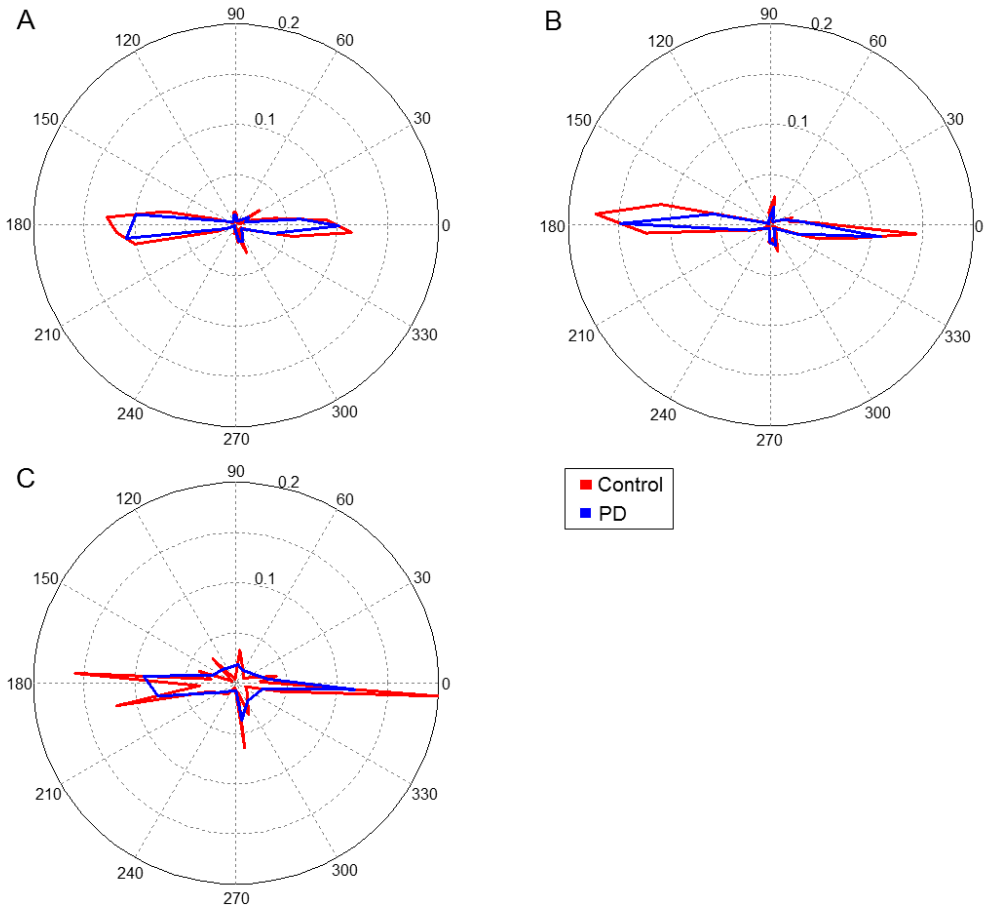


Figure 3.4: Rose plots (circular histograms) of microsaccade directions for trials with 2000ms fixation periods. Comparison of PD and control subjects for microsaccades preceding (A) correct prosaccade trials, (B) correct antisaccade trials, and (C) error antisaccade trials. Proportions of microsaccades during the fixational epoch of 400ms until target appearance are displayed in 50 bins of direction.

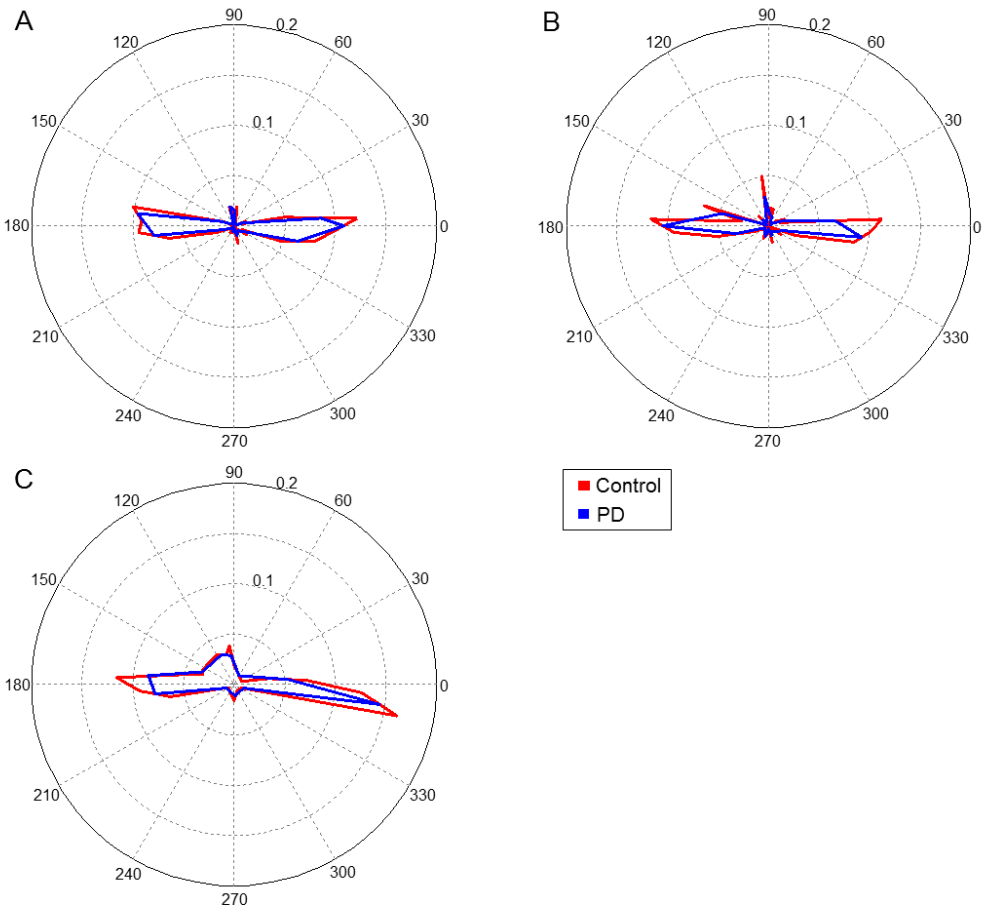


Figure 3.5: Rose plots (circular histograms) of microsaccade directions for trials with 1000ms fixation periods. Comparison of PD and control subjects for microsaccades preceding (A) correct prosaccade trials, (B) correct antisaccade trials, and (C) error antisaccade trials. Proportions of microsaccades during the fixational epoch of 400ms until target appearance are displayed in 50 bins of direction.

3.4 Microsaccade rates

The temporal dynamics of microsaccade occurrence during the fixation period is displayed in Figs. 3.6, 3.7 and 3.8. Figure 3.6 compares PD and control microsaccade rates for all trials separately for 1000ms and 2000ms fixation periods. In the trials with 1000ms fixation periods, the microsaccade rates of PD subjects only diverted from controls around 600ms prior to target appearance. However, rates were consistently higher in PD subjects compared to controls in trials with 2000ms fixation periods (Fig. 3.6A).

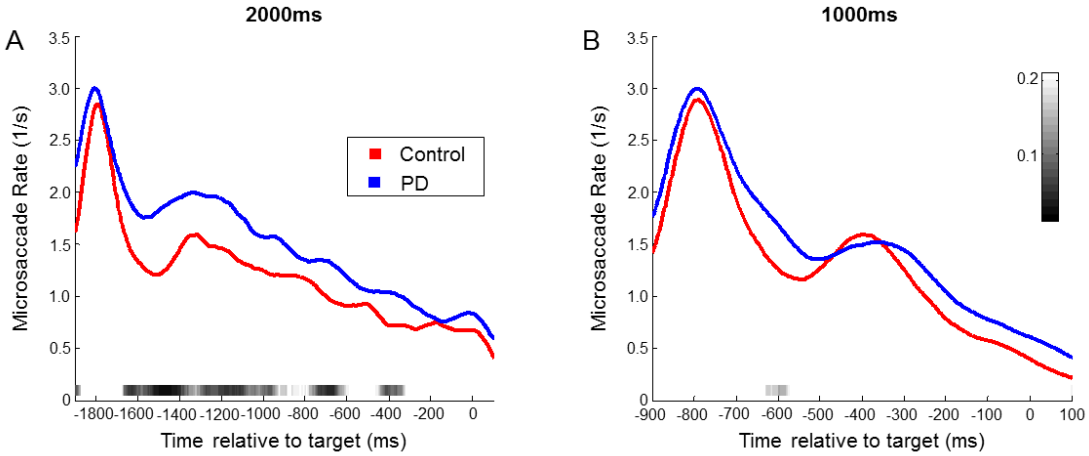


Figure 3.6: Time courses of microsaccade rates of PD and control subjects in (A) all trials with 2000ms fixation periods, (B) all trials with 1000ms fixation periods. The greyscale colour bar indicates microseconds at which microsaccade rates are divergent with varying levels of significance($p \leq 0.20$).

Figure 3.7 includes only correct trials and separates prosaccade trials from antisaccade trials for 2000ms and 1000ms fixation periods. As in Figure 3.6B, the microsaccade rate of PD subjects was higher than controls around 600ms to target appearance in the trials with 1000ms fixation periods (Fig. 3.7B + D). The microsaccade rate for PD subjects also remains higher in correct prosaccades for 100ms after target appearance (Fig. 3.7B). In trials with 2000ms fixation periods, the microsaccade rate was consistently higher in PD subjects compared to controls. This trend was most apparent in the range of 1600ms to 1000ms until target appearance for correct prosaccades and 1600ms to 1400ms until target appearance for correct antisaccades.

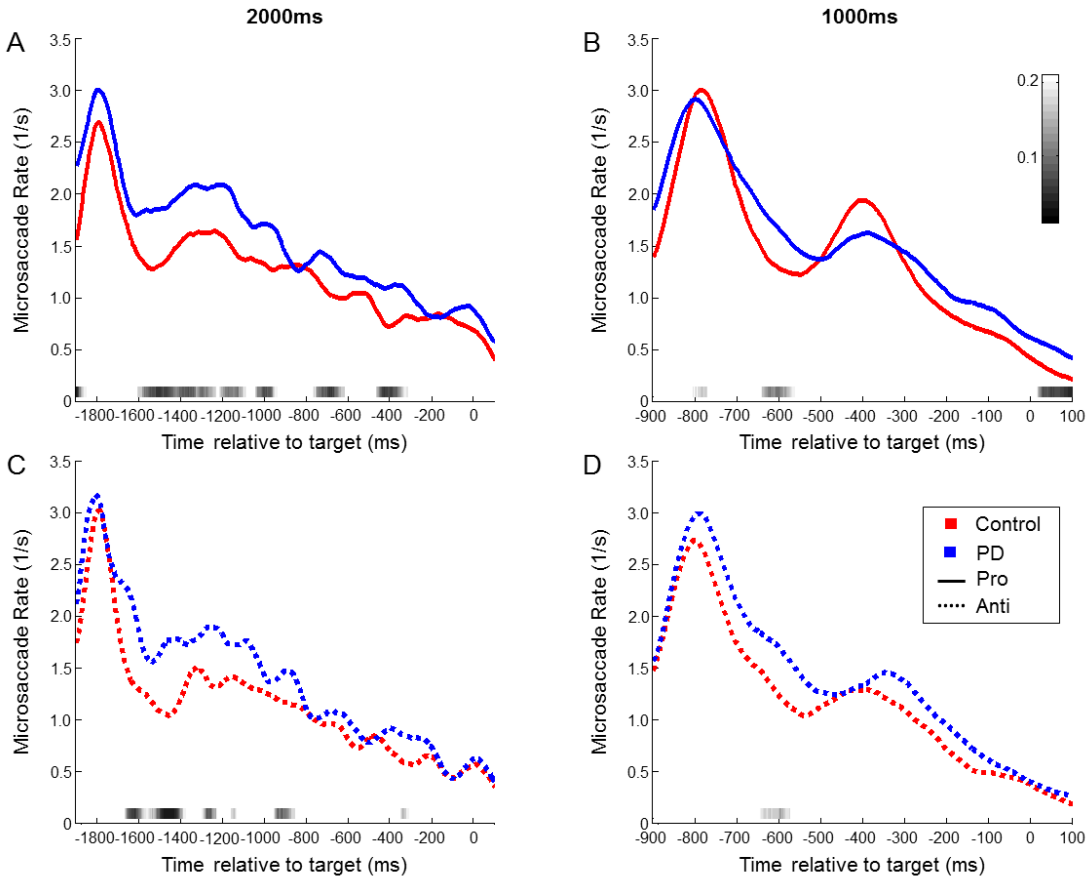


Figure 3.7: Time courses of microsaccade rates of PD and control subjects in (A) correct prosaccade trials with 2000ms fixation periods, (B) correct prosaccade trials with 1000ms fixation periods, (C) correct antisaccade trials with 2000ms fixation periods, (D) correct antisaccade trials with 1000ms fixation periods. The greyscale colour bar indicates microseconds at which microsaccade rates are divergent with varying levels of significance($p \leq 0.20$).

In Fig. 3.8, microsaccade rates for error antisaccade trials are compared to correct antisaccade trials separately with 2000ms and 1000ms fixation periods for PD and control subjects. This comparison was completed based on a study by Watanabe *et al.* (2013) which demonstrated a higher rate of microsaccades prior to error trials compared to correct trials. Despite these previous findings, our analysis did not show consistent results.

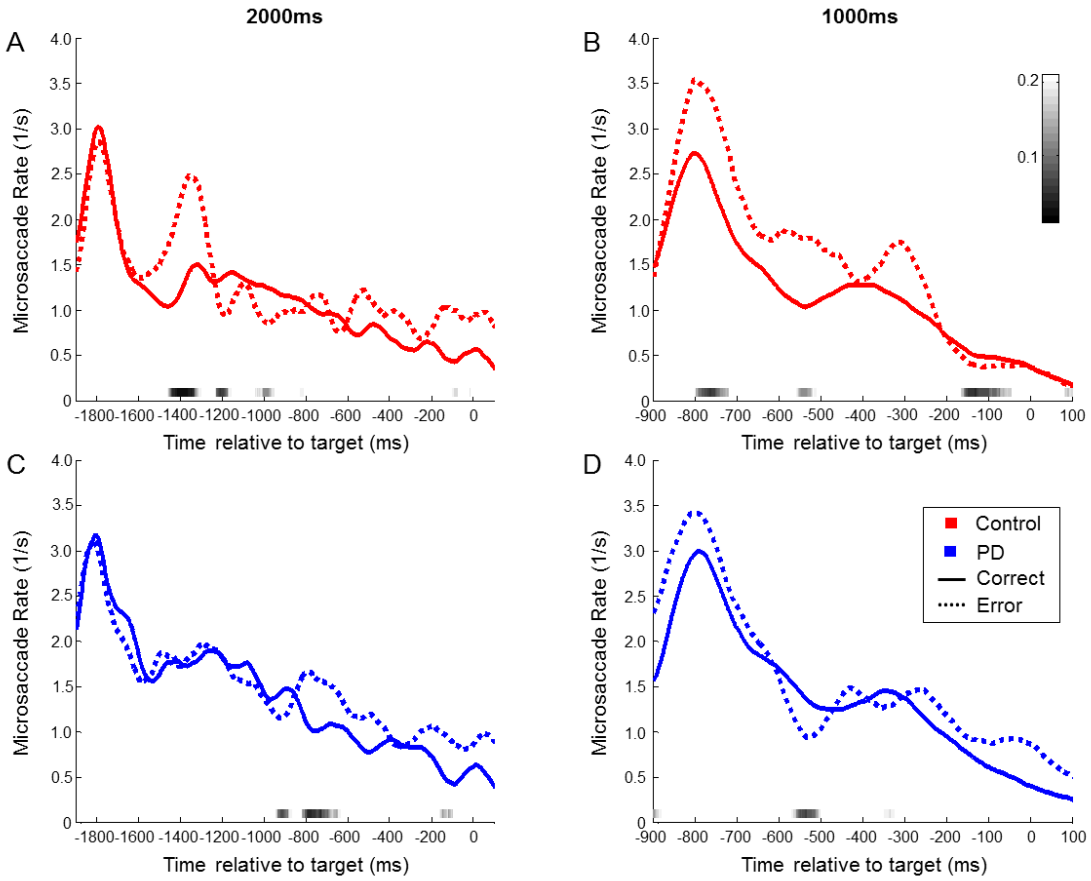


Figure 3.8: Time courses of microsaccade rates comparing correct and error antisaccade trials in (A) trials with 2000ms fixation periods (control subjects), (B) trials with 1000ms fixation periods (control subjects), (C) trials with 2000ms fixation periods (control subjects), (D) trials with 1000ms fixation periods (PD subjects). The greyscale colour bar indicates microseconds at which microsaccade rates are divergent with varying levels of significance ($p \leq 0.20$).

3.5 Correlational analysis

Correlational analyses were conducted in order to determine if significant relationships exist between saccadic measurements and cognitive test scores or symptom assessment scores. Error rate on the antisaccade task was positively correlated with Hoehn-Yahr scores ($r(23)=0.599$, $p=.0025$) and UPDRS scores ($r(23)=0.510$, $p=.013$) (Figs. 3.9A + B). This result indicates that deficiencies in behavioural control might result from advanced disease. Correlational analysis between error rate and microsaccade rate was completed in order to determine if preparatory activity levels, measured by microsaccade rate, are related to deficits in behavioural control (error

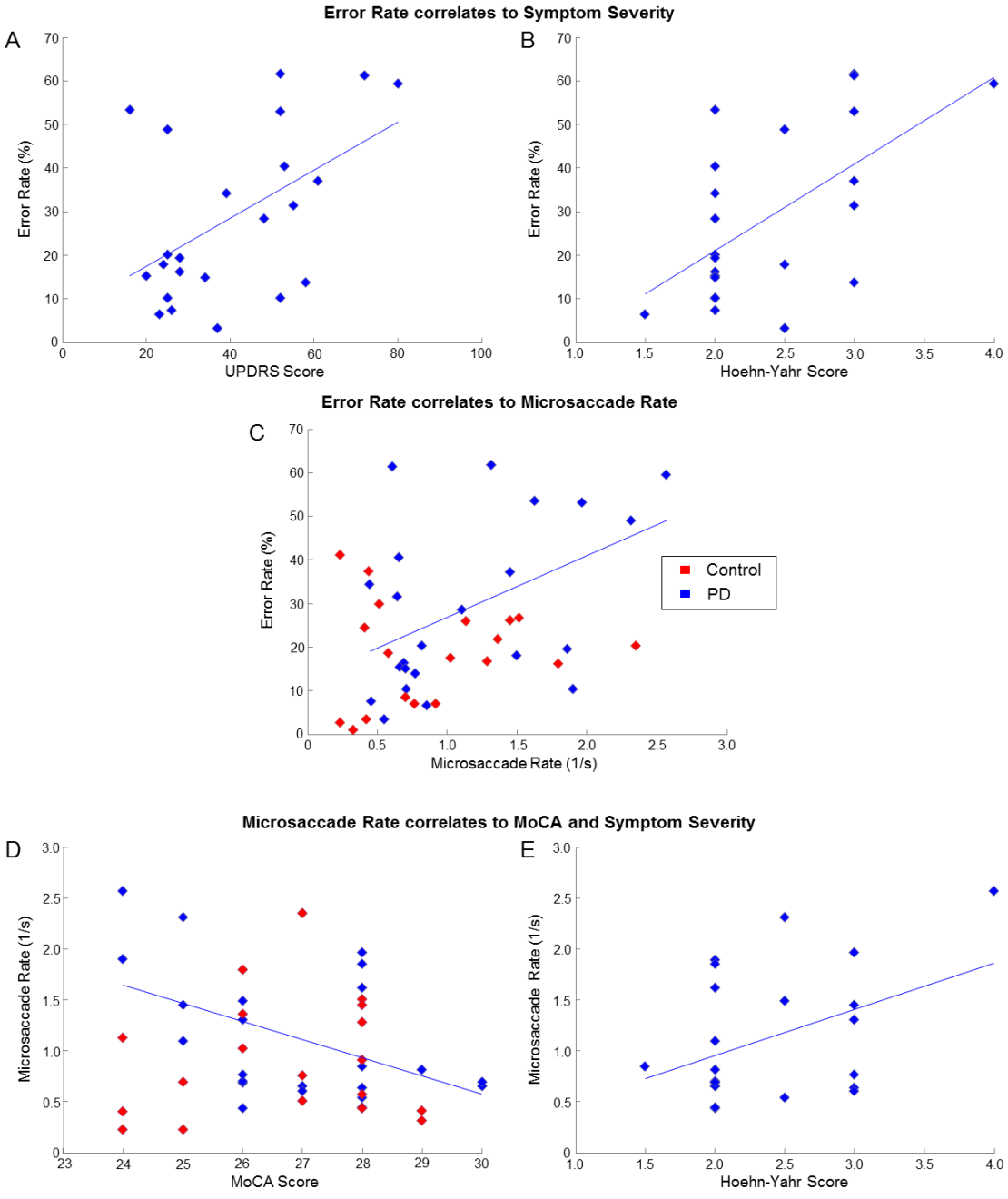
rate). Error rate was positively correlated to microsaccade rate for PD subjects (r(23)=0.464, p=.026) but not for control subjects (r(19)=0.101, ns).

Correlational analyses with microsaccade rate was completed to add insight into what could be affecting microsaccade rate. Microsaccade rate was negatively correlated with MoCA scores for PD subjects (r(23)= -0.480, p=.020), but not control subjects (r(19)=0.379, ns) (Fig. 3.9D). Therefore, PD subjects with low MoCA scores had higher rates of microsaccades but, in contrast, MoCA scores did not have an effect on microsaccade rate in control subjects. Furthermore, microsaccade rate was positively correlated to Hoehn-Yahr scores (r(23)=0.418, p=.047) (Fig. 3.9E) suggesting that microsaccade rate, like error rate, might be affected by advanced disease. Finally, there was a significant negative correlation between MoCA scores and UPDRS (r(23)=0.475, p=.022) (not reported in Table 2). Lower MoCA scores may be associated with more advanced disease as measured by the UPDRS due to the cognitive decline associated with PD (Amador *et al.*, 2006).

Table 2: R-values for correlations between saccade measurements and cognitive or symptom assessments.

	MoCA		Hoehn-Yahr	UPDRS	Microsaccade Rate (1/s)	
Error Rate (%)	0.290	0.273	**0.599***	**0.510***	0.101	**0.464***
Microsaccade Rate (1/s)	0.102	**0.480***	**0.418***	0.198	-	

Significant correlations are bolded in red for control subjects (none found) and blue for PD subjects. MoCA: Montreal Cognitive Assessment; UPDRS: United Parkinson's Diagnostic Rating Scale


Error Rate correlates to Symptom Severity
A
Error Rate (%)
UPDRS Score
B
Error Rate (%)
Hoehn-Yahr Score
Error Rate correlates to Microsaccade Rate
C
Error Rate (%)
Microsaccade Rate (1/s)
Control
PD
Microsaccade Rate correlates to MoCA and Symptom Severity
D
Microsaccade Rate (1/s)
MoCA Score
E
Microsaccade Rate (1/s)
Hoehn-Yahr Score

Chapter 4

Discussion

The objective of this study was to investigate microsaccade behaviour in PD subjects using the prosaccade and antisaccade tasks. Our hypothesis was that PD subjects would have elevated microsaccade rates, reflecting deficits in preparatory activity in terms of impaired advanced saccade suppression and correlating with poorer performance in the antisaccade task. The PD subject group exhibited the same eye movement abnormalities that are routinely observed in PD: a greater percentage of direction errors in the antisaccade task, a greater percentage of express saccades in prosaccade trials, and hypometric saccade amplitudes compared to controls (Amador et al.¸ 2006; Briand et al.¸1999; Cameron et al., 2010; Cameron et al., 2012; Chan et al., 2005; Hood et al., 2007; Rivaud-Perchoux et al., 2007). We also examined the spatial distribution of microsaccades and the temporal modulation of microsaccade rate during the instructed fixation period prior to stimulus appearance. We found that microsaccades were primarily horizontal in direction and the rate of microsaccade occurrence decreased over time from the start of fixation to target onset, as reported in Watanabe et al., 2013. The rate of microsaccades tended to be higher in PD compared to controls, especially in the 2000ms fixation period (Fig. 3.6). Importantly, we correlated the error rate and microsaccade rate to measures of disease symptom severity and cognitive impairment. The results indicated that error rate on the antisaccade task was positively correlated with symptom severity. Error rate was also positively correlated to microsaccade rate in PD subjects, which indicates that a lack of suppression of microsaccades corresponds to increased error rate. The positive correlation between microsaccade rate and Hoehn-Yahr score suggests that PD pathology affects microsaccade suppression. Ultimately, these results indicate that diminished preparatory activity in PD as previously determined through the use of fMRI can also be observed by means of microsaccade measurement. Microsaccades reflect preparatory

activity by indicating deficits in advanced saccade suppression. Their non-invasive measurement provides a promising alternative to scanning in the study of preparatory activity in humans.

4.1 Saccade control abnormalities in PD

The antisaccade deficits we observed in PD in the current study were similar to those described previously (Amador et al., 2006; Briand et al.,1999; Cameron et al., 2010; Cameron et al., 2012; Chan et al., 2005; Hood et al., 2007; Rivaud-Perchoux et al., 2007). PD subjects performed a greater number of direction errors in the antisaccade task (Fig. 3.2C). Direction error rate on the antisaccade task was found to be positively correlated to Hoehn-Yahr and UPDRS scores (Table 2; Fig. 3.9A + B) but not correlated to MoCA scores (Table 2). PD subjects tended to have slower SRTs in correct antisaccade trials (Fig. 3.1C + D; Fig. 3.2A) whereas previous studies found significant results (Amador et al., 2006; Briand et al., 1999; Chan et al., 2005). These results indicate that PD subjects have impaired control over voluntary movement. Significant positive correlations between error rate and measures of disease progression suggest that PD pathology is responsible for voluntary saccade deficits.

In contrast, PD subjects exhibited superior performance in the prosaccade task. PD subjects only tended towards faster SRTs in prosaccade trials in this investigation (Fig. 3.2A) but several studies have replicated this result, which suggests slight enhancement in PD subjects (Briand et al., 1999; Cameron et al., 2012; Chan et al., 2005; Roll et al., 1996). Other previous findings support the result of superior performance of PD subjects in visually-guided tasks. PD subjects off their medications executed fewer direction errors in prosaccade trials compared to controls (Cameron et al., 2012) and PD subjects had shorter SRTs on a reflexive visual-orientating task (Briand et al., 2001).

Impaired antisaccade performance coupled with enhanced prosaccade performance indicates an imbalance between voluntary and involuntary movement in PD. This trade-off is apparent in the visual tasks described above, as well as in other cognitive tests. In the Stroop task,

subjects are presented with colour or neutral words and must verbally identify the font colour. PD subjects performed poorly due to difficulty with suppressing the reflexive response to read the words (Henik et al., 1993). A model by Sereno and Holzman (1995) suggests that the impairment of the voluntary saccadic system results in the enhancement of the involuntary saccade system. The model proposes that voluntary saccades and involuntary saccades are controlled by two separate attentional systems, and the voluntary eye movement system tonically inhibits the involuntary system. Although this "tonic inhibition" model originally accounted for eye movements in schizophrenia, it fits the saccadic eye movement abnormalities commonly observed in PD (Chan et al., 2005). The disinhibition of involuntary movement may serve an adaptive purpose. PD neural circuitry may compensate for the slow initiation of voluntary movement by reducing saccadic response inhibition.

4.2 General characteristics of microsaccades

Before comparing microsaccade results between PD subjects and controls, it is prudent to examine the general characteristics of microsaccades. Like saccades, microsaccades are binocular and conjugate (Ditchburn & Ginsborg, 1953; Krauskopf et al., 1960; Lord, 1951). The main sequence of microsaccades (Fig. 3.3) depicts a velocity-amplitude relationship consistent with saccade main sequences, as shown previously (Otero-Millan et al., 2008; Watanabe et al., 2013; Zuber et al., 1965).

The similarity of physical and functional properties between saccades and microsaccades indicate that they likely share a common generator (Martinez-Conde et al., 2013; Otero-Millan et al., 2008). Indeed, a continuous representation of saccade directions and amplitudes exists in the SC with the smallest amplitude saccades represented in the rostral pole (Munoz & Wurtz, 1993; Robinson, 1972). Inactivation of the rostral SC results in decreased microsaccade rates (Hafed et al., 2009). Equivalent microsaccade activity has also been found in all brainstem neural

populations associated with saccades except for inhibitory burst neurons, which have yet to be investigated for connections to microsaccades (Martinez-Conde et al., 2013).

The temporal relationship between saccades and microsaccades also suggest a common generator (Hafed & Ignaschchenkova, 2013). Microsaccade occurrence before stimulus appearance prolonged pro- and antisaccade reaction times, possibly due to competitive interactions between different saccade commands (Rolfs et al., 2006; Watanabe et al., 2013). Microsaccade rates must decline prior to saccades execution so saccades are not delayed and the task is completed efficiently. It is possible that the mechanism that facilitates saccade initiation completes this role by suppressing microsaccades.

If microsaccade rates only reflected saccade facilitation, microsaccade rates prior to prosaccades should be lower than rates preceding antisaccade trials considering the faster SRTs in the prosaccade task. Instead, microsaccade rates were shown previously to be lower prior to correct antisaccade trials than correct prosaccade trials (Watanabe et al., 2013). Therefore, microsaccades might also be reflecting saccade suppression mechanisms. Correct antisaccade performance requires the suppression of automatic visually-triggered saccades (Munoz & Everling, 2004) and this process is reflected in the lower microsaccade rates preceding antisaccades. Higher microsaccade rates were also found prior to error antisaccade trials compared to correct antisaccade trials (Watanabe et al., 2013) demonstrating that a lack of saccade suppression, as reflected in increased microsaccade rates, results in a direction error. In the current study, we did not reproduce this result (Fig. 3.8) due to several methodological differences such as using older adults as opposed to healthy, young adults. However, error antisaccade rates were positively correlated with microsaccade rates in PD subjects (Table 2; Fig. 3.9C).

Microsaccade rates were higher prior to prosaccades compared to antisaccades and prior to error trials compared to correct trials, suggesting that microsaccades not only reflect saccade

facilitation but also saccade suppression. Because saccades and microsaccades likely share a common generator (Otaro-Millan et al., 2008), it is possible that microsaccade output during fixation provides insight into the mechanisms responsible for saccade preparation.

4.3 Microsaccades in PD

The slope of the main sequence of PD microsaccades was significantly lower than that of control subjects (Fig. 3.3). Hypometric amplitudes were found in PD saccadic eye movements (Fig. 3.2D) (Jones & Dejong, 1971) so it is likely that microsaccades exhibit the same deficit, especially if microsaccades and saccades exist on a continuum with a common generator. No differences were found in microsaccade distribution between PD and controls (Fig. 3.4) indicating that the same spatial areas are being sampled by each group. Microsaccades trigger spike rate increases in the primary visual cortex and the lateral geniculate nucleus (Martinez-Conde et al., 2000; Martinez-Conde et al., 2002). By evoking transient responses in visual neurons, microsaccades create illusory motion in a static pattern thereby increasing visibility (Otero-Millan et al., 2012). Indeed, this discrete sampling strategy could be superior to smooth visual scanning for information processing (Martinez-Conde et al., 2000). The primarily horizontal distribution of microsaccades may indicate the location of subjects' covert attention to the two possible target locations in this task. The location of covert attention might bias the direction of microsaccades due to extensive overlap between attention control and saccade generation neural circuitry (Corbetta et al., 1998; Engbert & Kliegl, 2003; Hafed & Clark, 2002).

Figure 3.6 demonstrates the same overall trend of microsaccade occurrence over time for PD subjects and controls. As stated in the previous section, microsaccades are suppressed as the time of target appearance approaches presumably to optimize the saccade control system for the upcoming eye movement. While the average microsaccade rate of PD subjects tended to be higher compared to controls in trials with 1000ms fixation periods, the difference was significant in the trials with 2000ms fixation periods. Therefore, PD subjects exhibit diminished suppression

of microsaccades, a deficiency highlighted by a longer fixation period. It might be that the 1000ms trial block does not provide enough time during fixation for the infrequent incidence of microsaccades to significantly differ between groups.

One could reason that the enhanced discrepancy between PD and control microsaccade rates in the 2000ms fixation duration trial block is explainable by an increased error rate in the 2000ms fixation duration block compared to the 1000ms block. Watanabe et al. (2013) demonstrated that microsaccade rates were increased prior to error trials compared to correct trials. As seen in Figure 3.1, more errors were made in the 2000ms fixation duration trial block compared to the 1000ms block. As expected, PD subjects made more errors than controls but there was no significant interaction. Because PD and control groups both increased their direction error rates in the 2000ms trial block, the enhanced discrepancy between group microsaccade rates cannot be explained by an increased difference in error rate.

Although microsaccade rates between correct and error antisaccade trials did not differ significantly, a positive correlation was found between microsaccade rate and antisaccade error rate for PD subjects (Table 2 + Fig. 3.9C). This suggests that PD subjects who made more microsaccades had a greater error rate even though the microsaccade rate did not differentiate prior to correct and error trials. Microsaccade rate was also negatively correlated to MoCA score for PD subjects only, which is likely due to the cognitive deficits associated with PD (Amador et al., 2006; Dubois & Pillon, 1996; Gill et al., 2008). Indeed, a positive correlation was found between microsaccade rate and Hoehn-Yahr score (Table 2; Fig. 3.9E)

Separating correct trials by task demonstrated higher microsaccade rates for PD subjects versus controls in both prosaccades and antisaccades (Fig. 3.7). The diminished rate of decline of microsaccade rate in PD subjects indicates that the mechanism that suppresses microsaccades is operating abnormally regardless of task.

4.4 The role of the basal ganglia

The prosaccade and antisaccade tasks are routinely used to test flexible control over behaviour (Everling & Fischer, 1998; Hallett, 1978). The prosaccade task is visually-guided because the stimulus location matches the saccade goal. In contrast, the antisaccade task decouples the stimulus location and saccade goal (Munoz & Everling, 2004). In the case of the antisaccade task, the frequent incidence of direction errors (Fig. 3.2B) and differences in SRT distribution (Fig. 3.1) indicate that there are two incompatible motor plans in competition (Everling & Fischer, 1998; Munoz & Everling, 2004).

The anatomical pathways within the BG support its role as an action selector (Watanabe & Munoz, 2009; Watanabe & Munoz, 2011). Neurons within the CN of the BG receive input from frontal cortical regions and give rise to the direct and indirect pathways through their GABAergic projections to the SNr and GPe, respectively. The direct pathway results in a disinhibition of the SC whereas the indirect pathway inhibits the SC (Hikosaka et al., 2000; Parent & Hazrati, 1994; Smith et al., 1998). The counteracting effects of these pathways permit the modulation of SC activation thereby exerting control over the initiation of saccadic eye movements.

Cortical signals encoding visually-guided responses and volitional responses input into the CN (Alexander et al., 1986; Hikosaka et al., 2000). Since only one action can be produced, the BG modulates the incoming signals to generate the desired response. The antisaccade task is commonly used to test BG function due to the conflict presented between motor plans encoding visually-guided responses (direction errors) and voluntary responses (correct antisaccades) (Briand et al., 1999; Munoz & Everling, 2004; Watanabe & Munoz, 2009).

It has been recently suggested that 3 groups of neurons within the CN perform this conflict resolution task (Watanabe & Munoz, 2009). Cortical signals encoding the visually-guided response trigger activity within CN sensory-driven neurons, which would generate a

direction error in the antisaccade task. Cortical signals encoding the volitional antisaccade response trigger activity in two groups of volitional neurons in the CN. Contralateral saccade-preferred volitional neurons generate the correct antisaccade response, which requires additional time to invert the visual stimulus saccade vector into the appropriate motor command hence the slower SRT in correct antisaccade trials compared to correct prosaccade trials (Fig. 3.2A) (Munoz & Everling, 2004). The remaining group of CN neurons, ipsilateral saccade-preferred volitional neurons, resolves the conflict between the previous groups by suppressing direction error saccades via the indirect pathway. Because the antisaccade motor plan requires more time to generate, the prosaccade motor plan must be suppressed while the antisaccade motor plan is generated in order to perform a correct antisaccade. A lack of suppression of the prosaccade motor plan results in a direction error. This architecture matches the model proposed by Noorani & Carpenter (2013), which accounts precisely for the SRT distributions and error responses found in subjects performing the antisaccade task.

Along with cortical inputs, the CN also receives input from the SNc. The SNc decreases the inhibitory outflow of the BG by facilitating the direct pathway and inhibiting the indirect pathway (Albin et al., 1989; Delong, 1990; Gerfen et al., 1990). In PD, dopaminergic neuronal death in the SNc results in a dominance of the indirect pathway over the direct pathway (Kravitz et al., 2010; Watanabe & Munoz, 2011). Diminished BG output results in less excitation of the cortex so volitional motor plans are not facilitated. Dopamine depletion explains the deficits in visually-guided saccade suppression observed in PD. Indeed, administering levodopa to PD subjects increases SRT in the prosaccade task and reduces error rate in the antisaccade task (Hood et al., 2007).

If microsaccades reflect action preparatory processes, it follows that the BG would be involved. While the specific mechanism that triggers microsaccades is currently unknown, several models place the SC in a centralized role (Hafed et al., 2009; Otero-Millan et al., 2011;

Rolfs et al., 2008). SC fluctuations that trigger microsaccades are attributed to neural noise and fixation error (Otero-Millan et al., 2011). Higher rates of microsaccades in PD subjects compared to controls suggest that the BG can induce microsaccades. The BG is a major input into the SC, sending GABAergic projections to the SC's intermediate layer (Fig. 1.1) (Hikosaka & Wurtz, 1983; Mohler & Wurtz, 1976; Sparks, 1986). The activity within the direct and indirect pathways during fixation reflects preparatory mechanisms of saccade facilitation and suppression, respectively. These signals could cause the fluctuations in the SC which result in changes in microsaccade rates.

Indeed, errors in antisaccade trials do not originate in the motor execution of the voluntary eye movement. Rather, the CN and select cortical areas exhibit hypoactivation during the preparatory phase (Cameron et al., 2012). PD subjects were less efficient at establishing a voluntary task set in networks controlling voluntary movement. Therefore, sufficient action preparation is crucial to voluntary saccade performance. We suggest that the higher microsaccade rates observed in PD patients correspond to deficits in task set establishment and provide an overt measure of saccade preparatory processes (Watanabe et al., 2013).

4.5 Limitations and future directions

There are several limitations associated with recruiting eligible participants from an elderly clinical population such as PD. At advanced age, it is challenging to find subjects who are cognitively normal without ophthalamic conditions. In the current study, the MoCA cut-off score was lowered below cognitively normal acceptability at the risk of confounding the results in order to include more participants. Although a score of 26 or higher is considered cognitively normal, individuals with scores of 24 to 26 were included for analysis. Importantly, every subject corrected their errors by making a secondary saccade to the correct location, which confirmed their understanding. PD participants were not asked to interrupt their medications because eye movement abnormalities are observable while participants are on medications (Cameron et al.,

2012; Hood et al., 2007). However, testing PD participants off-medication has been shown to enhance PD saccadic abnormalities and alter fMRI activation patterns (Cameron et al., 2012). It is possible that microsaccade rates would be increased if PD subjects interrupted their medications prior to behavioural testing. PD itself sets its own challenges on finding significant results. PD subject groups show increased intra-subject variability (Chan et al., 2005) compared to control groups.

This study provides preliminary results connecting microsaccade occurrence with BG dysfunction. Expanding this investigation to other major patient centres will increase the sample size and overcome the limitations of recruitment and intra-subject variability listed above. Eye-trackers are now being placed in several hospitals in Ontario to capture more PD patients using this study's behavioural paradigm. Furthermore, the portability of the eye-tracker will permit the participation of PD patients who live remotely.

Behavioural studies that include larger sample sizes and at-risk populations would increase the statistical significance of our microsaccade results. Probing microsaccades in other disorders involving BG dysfunction with known saccade abnormalities, such as Huntington's Disease (Lasker et al., 1987; Leigh et al., 1983) and Progressive Supranuclear Palsy (Pierrot-Deseilligny et al., 1989) would add insight to the BG's role in microsaccade suppression. Neurophysiological recordings from BG nuclei in parkinsonian monkeys could help target the specific nucleus triggering microsaccades. Overall, future research into microsaccade rates will determine whether microsaccades adequately reflect action preparation. If so, microsaccades can be a non-invasive measurement of cognitive mechanisms such as task set preparation and decision making. Clinically, microsaccades may be used as a potential biomarker to detect BG dysfunction.

4.6 Summary and conclusions

The results of this study indicate that PD subjects have increased microsaccade rates compared to controls during saccadic preparation. Higher microsaccade rates were correlated to increased error rates on the antisaccade task and higher Hoehn-Yahr scores indicating disease severity. Our results support the hypothesis that microsaccades can be used as a non-invasive measure of action preparation in terms of saccade facilitation and suppression (Watanabe et al., 2013). To our knowledge, this study is the first to investigate the effect of BG dysfunction on microsaccades in the PD population. We have demonstrated that the BG plays a pivotal role in the generation and suppression of microsaccades. Future studies are required to identify the specific neural correlates within the BG that give rise to microsaccades. Overall, microsaccades have the potential to be useful overt measurements of action preparation and BG dysfunction in clinical populations.

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