INVESTIGATING THE CONTRIBUTION OF THE FRONTAL CORTEX IN EXECUTIVE CONTROL IN NORMAL VERSUS ABNORMAL AGING

by

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A thesis submitted to the Centre for Neuroscience Studies
In conformity with the requirements for
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The proportion of elderly individuals in society is increasing dramatically, leading to an increase in the prevalence of age-related neurological disorders that affect the function of the frontal lobes and overall movement control. This thesis aims to evaluate ‘executive control’ and the underlying brain changes in normal versus abnormal aging processes using saccadic eye movement tasks. Tasks performed by subjects that probe executive control consist of antisaccades (generate a voluntary eye movement after inhibiting an automatic movement to a visual stimulus), and memory-guided saccades (generate eye movements to three previous remembered visual stimuli in the same sequence they were presented). Both of these types of saccades require good functioning behavioural control, which is subserved by areas in the prefrontal cortex. This thesis specifically characterizes the changes in oculomotor control related to aging, Huntington’s disease, mild cognitive impairment (amnestic), and Alzheimer’s disease. We also specifically examine the neural mechanisms related to behavioural control in the antisaccade task in aging. Together, the conclusions drawn from this thesis reveal that specific areas in the prefrontal cortex are involved in executive dysfunction in both normal and abnormal aging, but the patient groups studied have provided new understanding that different underlying brain substrates may be altering function in the frontal cortical areas, such as the basal ganglia and the hippocampus.
Co-Authorship

The research in this thesis was conducted by Alicia Peltsch under the supervision of Dr. Douglas P Munoz and Dr. Angeles Garcia. Alicia Peltsch collected and analyzed all of the data in Chapters 2, 4, and 5 (except for a portion of control participants in Chapter 5 that were collected by Ian Cameron, and a portion of subjects in Chapter 4 that were collected by Melanie Schriber). Alicia Peltsch wrote all the first drafts of Chapters 2, 4, and 5, and was lead author on all subsequent drafts. Andrea Hoffman collected the data and wrote the first draft in Chapter 3, and Alicia Peltsch was lead author on all subsequent analyses and drafts. Dr. Nadia Alahyane designed the experimental paradigm in Chapter 5. Dr. Brian Coe assisted in the methodological designs in Chapter 5, and implemented the fMRI eye-tracking capability. Dr. Douglas Munoz co-conceived and assisted in the designs of Chapters 2, 3, 4, and 5. Dr. Giovanna Pari recruited participants with Huntington’s disease for Chapter 3. Alisha Hemraj and Dr. Angeles Garcia recruited participants with Mild Cognitive Impairment and Alzheimer’s disease for Chapter 4. Dr. Patrick Stroman assisted in the methodological design in Chapter 5. The entire Munoz lab provided editorial advice on the writing of all chapters.

Chapter 2 has been published in its entirety, and can be cited as:


Chapter 3 has been published in its entirety, and can be cited as:


Chapter 4 has been submitted for publication:

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Dedication

This thesis is dedicated to my late mother, Margaret Ellen Peltsch.

You have brains in your head.
You have feet in your shoes.
You can steer yourself
any direction you choose.
You’re on your own, and you know you what you know.
And YOU are the guy who’ll decide where to go.
-Dr. Seuss
Statement of Originality

I hereby certify that all of the work described within this thesis is the original work of the author. Any published (or unpublished) ideas and/or techniques from the work of others are fully acknowledged in accordance with the standard referencing practices.

Alicia Jane Peltsch

April, 2011
# Table of Contents

Abstract ............................................................................................................................................ ii  
Co-Authorship ............................................................................................................................... iii  
Acknowledgements ......................................................................................................................... iv  
Dedication ...................................................................................................................................... vi  
Statement of Originality ................................................................................................................. vii  
Table of Contents ............................................................................................................................. viii  
List of Figures ................................................................................................................................ xii  
List of Abbreviations and Symbols ................................................................................................. xv  
Chapter 1 ....................................................................................................................................... 1  
  General Introduction .................................................................................................................... 1  
    1.1 Statement of the research problem ...................................................................................... 1  
    1.2 A brief history of neuropsychology ................................................................................... 2  
    1.3 Executive functions and the brain ..................................................................................... 5  
    1.4 Normal versus abnormal aging ......................................................................................... 6  
      1.4.1 Normal aging ............................................................................................................. 6  
      1.4.2 Huntington’s disease ................................................................................................. 7  
      1.4.3 Alzheimer’s disease and Mild Cognitive Impairment .................................................. 7  
    1.5 Assessing neurological function: tools and techniques ....................................................... 8  
    1.6 Eye movement experiments: saccades .............................................................................. 9  
    1.7 Neurophysiology of saccadic eye movements .................................................................. 11  
      1.7.1 Motor generation ....................................................................................................... 11  
      1.7.2 Initiation and suppression ......................................................................................... 12  
    1.8 Objectives ......................................................................................................................... 14  
    1.9 References ....................................................................................................................... 16  
Chapter 2 Age-related trends in saccade characteristics among the elderly ............................. 24  
  2.1 Abstract ............................................................................................................................... 24  
  2.2 Introduction ........................................................................................................................ 25  
  2.3 Methods ............................................................................................................................. 28  
    2.3.1 Subjects ..................................................................................................................... 28  
    2.3.2 Experimental paradigm .............................................................................................. 29  
    2.3.3 Recording and apparatus ........................................................................................... 30
5.3.2 Paradigm ..................................................................................................................... 139
5.3.3 Eye tracking and visual display ................................................................................. 141
5.3.4 fMRI parameters ........................................................................................................ 141
5.3.5 Statistical analysis ....................................................................................................... 142
5.3.6 Behavioural analysis .................................................................................................. 143
5.3.7 Functional analysis ..................................................................................................... 143
5.4 Results .......................................................................................................................... 145
5.4.1 Behaviour .................................................................................................................. 145
Figure 5.3. The same subtractions as Figure 5.2 E, F, but comparing all three experimental
groups, with individual data points. ................................................................................. 147
5.4.2 fMRI ........................................................................................................................ 148
5.4.3 Antisaccades versus prosaccades ............................................................................. 149
5.4.4 Saccade preparation .................................................................................................. 151
5.4.5 Saccade execution ..................................................................................................... 154
5.5 Discussion ..................................................................................................................... 155
5.5.1 Aging controversies .................................................................................................. 156
5.5.2 Neural circuitry .......................................................................................................... 159
5.5.3 Limitations and future directions ............................................................................. 161
5.6 References .................................................................................................................... 162
Chapter 6 General Discussion ............................................................................................ 167
6.1 Clinical relevance ........................................................................................................ 170
6.2 Future directions .......................................................................................................... 171
6.2.1 Future directions for HD ......................................................................................... 173
6.2.2 Future directions for dementia ................................................................................ 173
6.3 Conclusions .................................................................................................................. 175
6.4 References .................................................................................................................... 175
List of Figures

Figure 1.1. The neural circuitry underlying saccadic eye movements 13
Figure 2.1. Saccade paradigm and individual eye traces 30
Figure 2.2. Distribution of SRTs for each age category 35
Figure 2.3. Onset versus offset of express saccade epoch 36
Figure 2.4. Pro- and anti-saccade results, individually plotted 37
Figure 2.5. Gap and anti effects individually plotted across age 39
Figure 3.1. Schematic of the basal ganglia in a healthy vs. AD brain 56
Figure 3.2. Saccade paradigms 57
Figure 3.3. Immediate pro- and anti-saccade task results, individually plotted 67
Figure 3.4. Errors in the delayed pro- and anti-saccade tasks 70
Figure 3.5. Cumulative SRT distributions 72
Figure 3.6. Delayed memory-guided sequential saccade task results 74
Figure 3.7. Mean correlations between saccade measures and HD disease severity 76
Figure 4.1. Pro- and anti-saccade paradigm and individual eye traces 97
Figure 4.2. SRT distributions for each experimental group 100
Figure 4.3. Cumulative SRT distributions 103
Figure 4.4. Prosaccade and antisaccade results in all three experimental groups 104
Figure 4.5. Prosaccade and antisaccade results in all three experimental groups 106
Figure 4.6. Mean gap and anti effect for each experimental group 109
Figure 4.7. Antisaccade results for aMCI patients and those who later converted to AD 112
Figure 5.1. fMRI experimental paradigm and representation of stimuli and timing 140
Figure 5.2. Behavioural results in fMRI setup 146
Figure 5.3. Behavioural subtractions in fMRI setup 147
Figure 5.4. fMRI contrast map for antisaccade minus prosaccade trials for younger vs. older subjects 149
Figure 5.5. fMRI contrast map for antisaccade minus prosaccade trials for younger vs. high-performing and low-performing older subjects 151
Figure 5.6. Contrasts maps for saccade preparation and saccade execution 152
Figure 5.7. Region of interest analysis for saccade preparation and execution 153
List of Tables

Table 1.1. Canadian life expectancy for males and females 1
Table 2.1. Composition of age-related groups 29
Table 2.2. Post-hoc analysis of mean differences between age groups 40
Table 3.1. Subject information 60
Table 3.2. Correlations between saccade measures and HD assessments 77
Table 4.1. Psychometric test scores 96
Table 4.2. Pearson correlations of saccade performance by age 110
## List of Abbreviations and Symbols

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>AC</td>
<td>anterior commissure</td>
</tr>
<tr>
<td>ACC</td>
<td>anterior cingulate cortex</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>BG</td>
<td>basal ganglia</td>
</tr>
<tr>
<td>BOLD</td>
<td>blood oxygen level dependent</td>
</tr>
<tr>
<td>cd/m²</td>
<td>candelas per square meter</td>
</tr>
<tr>
<td>CN</td>
<td>caudate nucleus</td>
</tr>
<tr>
<td>DLPFC</td>
<td>dorsolateral prefrontal cortex</td>
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<tr>
<td>DRS</td>
<td>Dementia Rating Scale</td>
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<tr>
<td>EOG</td>
<td>electrooculography</td>
</tr>
<tr>
<td>EPI</td>
<td>echo-planar imaging</td>
</tr>
<tr>
<td>FA</td>
<td>flip angle</td>
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<tr>
<td>FDR</td>
<td>false discovery rate</td>
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<tr>
<td>FEF</td>
<td>frontal eye fields</td>
</tr>
<tr>
<td>fMRI</td>
<td>functional magnetic resonance imaging</td>
</tr>
<tr>
<td>FOV</td>
<td>field of view</td>
</tr>
<tr>
<td>FWHM</td>
<td>full-width at half maximum</td>
</tr>
<tr>
<td>GPe</td>
<td>globus pallidus external segment</td>
</tr>
<tr>
<td>GPi</td>
<td>globus pallidus internal segment</td>
</tr>
<tr>
<td>L</td>
<td>left</td>
</tr>
<tr>
<td>LED</td>
<td>light emitting diode</td>
</tr>
<tr>
<td>LLBN</td>
<td>long lead burst neurons</td>
</tr>
<tr>
<td>MMSE</td>
<td>Mini-Mental Status Examination</td>
</tr>
<tr>
<td>MoCA</td>
<td>Montreal Cognitive Assessment</td>
</tr>
<tr>
<td>MP-RAGE</td>
<td>magnetization-prepared rapid gradient-echo</td>
</tr>
<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
<tr>
<td>ms</td>
<td>millisecond</td>
</tr>
<tr>
<td>ON</td>
<td>omnipause neurons</td>
</tr>
<tr>
<td>PD</td>
<td>Parkinson’s disease</td>
</tr>
<tr>
<td>PEF</td>
<td>parietal eye fields</td>
</tr>
<tr>
<td>PET</td>
<td>positron emission tomography</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>PFC</td>
<td>prefrontal cortex</td>
</tr>
<tr>
<td>R</td>
<td>right</td>
</tr>
<tr>
<td>s</td>
<td>second</td>
</tr>
<tr>
<td>SC</td>
<td>superior colliculus</td>
</tr>
<tr>
<td>SE</td>
<td>standard error of the mean</td>
</tr>
<tr>
<td>SEF</td>
<td>supplementary eye fields</td>
</tr>
<tr>
<td>SNr</td>
<td>substantia nigra pars reticulata</td>
</tr>
<tr>
<td>SNpc</td>
<td>substantia nigra pars compacta</td>
</tr>
<tr>
<td>SRT</td>
<td>saccadic reaction time</td>
</tr>
<tr>
<td>STN</td>
<td>subthalamic nucleus</td>
</tr>
<tr>
<td>TE</td>
<td>echo time</td>
</tr>
<tr>
<td>TMS</td>
<td>transcranial magnetic stimulation</td>
</tr>
<tr>
<td>TR</td>
<td>repetition time</td>
</tr>
<tr>
<td>UHDRS</td>
<td>Unified Huntington’s Disease Rating Scale</td>
</tr>
<tr>
<td>VLPFC</td>
<td>ventrolateral prefrontal cortex</td>
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~ approximately  
© copyright  
= equal  
> greater than  
< less than  
% percentage  
± plus or minus  
v.s. versus  
*, **, † to highlight statistical significance where described
Chapter 1

General Introduction

1.1 Statement of the research problem

A serious consequence to otherwise positive health care advancements is an aging society. Statistics Canada has reported a 1.3-fold increase in human life expectancy since the early 1920’s (Statistics Canada, 2010), likely due to the shift in demographics in which birth rates are lower and life expectancy is higher. As such, many individuals often live well into their 8th or 9th decade. It is not news to any of us over 30 years old that motor, sensory and cognitive processes start to slow down as we age.

<table>
<thead>
<tr>
<th>Life expectancy at birth, by sex, by province</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canada</td>
<td>59</td>
<td>61</td>
</tr>
<tr>
<td>1920 to 1922</td>
<td>59</td>
<td>61</td>
</tr>
<tr>
<td>1930 to 1932</td>
<td>60</td>
<td>62</td>
</tr>
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<td>1940 to 1942</td>
<td>63</td>
<td>64</td>
</tr>
<tr>
<td>1950 to 1952</td>
<td>66</td>
<td>71</td>
</tr>
<tr>
<td>1960 to 1962</td>
<td>68</td>
<td>74</td>
</tr>
<tr>
<td>1970 to 1972</td>
<td>69</td>
<td>76</td>
</tr>
<tr>
<td>1980 to 1982</td>
<td>72</td>
<td>79</td>
</tr>
<tr>
<td>1990 to 1992</td>
<td>75</td>
<td>81</td>
</tr>
<tr>
<td>2000 to 2002</td>
<td>77</td>
<td>82</td>
</tr>
<tr>
<td>2005 to 2007</td>
<td>76</td>
<td>83</td>
</tr>
<tr>
<td>Canada</td>
<td>76</td>
<td>83</td>
</tr>
<tr>
<td>Newfoundland and Labrador</td>
<td>76</td>
<td>81</td>
</tr>
<tr>
<td>Prince Edward Island</td>
<td>78</td>
<td>83</td>
</tr>
<tr>
<td>Nova Scotia</td>
<td>77</td>
<td>82</td>
</tr>
<tr>
<td>New Brunswick</td>
<td>78</td>
<td>83</td>
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<tr>
<td>Quebec</td>
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</tr>
<tr>
<td>Ontario</td>
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<td>Manitoba</td>
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<td>Saskatchewan</td>
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<td>Alberta</td>
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<td>83</td>
</tr>
<tr>
<td>British Columbia</td>
<td>79</td>
<td>84</td>
</tr>
</tbody>
</table>

Source: Statistics Canada, CANSIM, table 102-0512 and Catalogue no. 84-537-XIE.
Last modified: 2016-06-21.

Table 1.1. Top panel displays Canadian life expectancy for males and females in different time periods. Bottom panel displays life expectancy in each Canadian province. Notice that the life expectancy for females in Ontario was 83 years old in 2005 – 2007, up from 61 years old in 1920 – 1922.
Although most other human body systems and organs can now be repaired or maintained by medical technology, repair to the brain is limited. Consequently, age-related neurological problems have become considerably more prevalent. Diseases such as Alzheimer’s disease, Huntington’s disease, Parkinson’s disease, and many others, are on the rise. For example, the rate of Alzheimer’s disease is expected to triple in the next 45 years (Corbetta and Shulman, 2002).

The overlap between the neural circuitry controlling saccadic eye movements – fast eye movements directing the fovea to areas of interest – and the brain regions afflicted in neural disorders is striking. Patients with deficits in frontal, hippocampal or basal ganglia regions have been assessed with eye movements (Munoz et al., 2007; Garbutt et al., 2008). Saccade tasks in humans are mainly used to assess brain and cognitive function in healthy individuals versus patient groups with the goal of further understanding the disorder at hand. For example, specific saccade deficits are distinguishable between different neurodegenerative disorders, including Huntington’s disease (Blekher et al., 2006; Peltsch et al., 2008), Parkinson’s disease (Briand et al., 1999; Chan et al., 2005; Cameron et al., 2010; Mosimann et al., 2005), Alzheimer’s disease (Garbutt et al., 2008; Abel et al., 2002; Shafiq-Antonacci et al., 2003), Amyotrophic Lateral Sclerosis (Witiuk et al., 2011), and Progressive Supranuclear Palsy (Garbutt et al., 2008; Chen et al., 2010). The purpose of this thesis is to characterize saccade behaviour in normal versus abnormal aging, and to provide evidence that changes in the functioning of the frontal cortex underlie behavioural changes common to these populations.

1.2 A brief history of neuropsychology

Humans appear to have three primary desires throughout the lifespan. Simply put, these are to live, to love, and to learn. More specifically, humans have the desire or instinct to eat,
drink, and perform whatever actions make that necessary (live), to socialize, mate, and reproduce (love), and to continue developing and adapting to new environments (learn). Whether these desires are genetically-based or environmentally-driven, in order for all of them to be accomplished, the brain (arguably above all other structures) must be in good functioning order. Of course, this was only realized approximately 2000 years ago when a Roman physician (known as Galen) noticed that gladiators sustaining injury to arms, legs, or the torso retained their cognitive abilities, whereas those who sustained injury to the head did not. He then became one of the first scientists to use brain-damaged individuals to study the relationship between brain and behaviour (Rocca, 2003). Since that time, researchers and physicians have used many techniques to study the relationships between brain and behaviour. For the sake of brevity, three main populations – individuals with brain damage, individuals with intact brains, and animals – will be summarized here. Each method has its advantages and pitfalls, where the best method is usually dependent on the question at hand.

As mentioned above, Galen discovered that gladiators with head injuries had reduced mental capabilities. This led to further studies of patients with various types of brain damage; however, neuroscience as we know it did not take hold just yet. What followed many years later was the now debunked study of Phrenology – the idea that measurements of the skull would correspond to variations in the underlying brain tissue (Simpson, 2005). Naturally, although this idea remained popular throughout the 1800’s, it was later dismissed by mainstream science, unbelievably not until the early 20th century. However, modern localization of function may not have arisen had the idea that individuals had variations in brain tissue that corresponded to specific behaviours not been proposed. A many more years later in the mid 1950’s, Brenda Milner and colleagues (Scoville and Milner, 1957) found that the surgical removal of the
hippocampus, a structure within the medial temporal lobe, led to difficulties forming new long-term memories. One disadvantage with the lesion method however, is that each person who sustains a brain injury is quite different, and the lesions sustained are non-specific in extent and location (Banich, 1997).

Studying neurologically intact individuals has also provided plenty of information about the relationship between brain function and structure. Firstly, they act as an important control group for individuals with compromised brain function. But equally importantly, neuroscientists can assess neurologically intact individuals for the variations in anatomy between brains (and how those variations relate to function), and also to further understand on how brain structures work together under healthy conditions rather than with brain damage.

Finally, researchers also gain insight about brain-behaviour relationships from studying nonhuman animals, most notably monkeys, as they share closer structural organization to humans and can be trained to perform complex cognitive tasks. Research with animals can often be more straightforward than research with humans, as the environment can be more strictly controlled.

Using these three populations combined with various techniques such as electrical recording, neuroimaging, and clinical assessment, the study of neuroscience, or neuropsychology, has evolved as the years have progressed. Gaining knowledge from these populations has now allowed neuroscientists to study different psychiatric and neurological diseases with more insight into the affected brain areas. Although neurodegenerative disease is considerably different from blunt brain injury sustained in battle, the study of modern human brain conditions would not be possible without the extensive history of neuropsychology.
1.3 Executive functions and the brain

Executive functions have been loosely defined as a collection of brain processes responsible for planning, cognitive flexibility, abstract thinking, rule acquisition, initiating appropriate actions and inhibiting inappropriate actions, and selecting relevant sensory information (Stuss and Alexander, 2000) and allow humans to control behaviour in response to a variety of environmental cues to be able to perform complex mental tasks (e.g., driving a car or participating in sport). We are capable of reacting quickly and accurately to stimuli – but equally important, we are capable of not reacting to stimuli when that is the more appropriate response. The ability to voluntarily control behaviour and inhibit unwanted responses has been extensively studied, and falls within the realm of executive functions. These high-order cognitive skills are thought to rely primarily on the prefrontal cortex (PFC) (Miller and Cohen, 2001). Because good executive functioning primarily dictates our ability to live, love, and learn, the role of the frontal lobe and executive functions have been central to many research goals, and this has been studied using several tasks of different modalities. One of the most classic clinical examples of frontal cortex injury is the case of Phineus Gage. Discovering the role of the frontal cortex in executive functions is largely attributed to this famous case. Gage, a 26-year old mining foreman, was struck in the head with a ‘tamping iron’ in a mining accident. Although the iron entered below his left eye and went almost entirely through the entire orbitofrontal cortex, his physical recovery was profound. However, he was reported to have dramatically altered behaviour and mental capabilities, such that the physician reported:

“The equilibrium or balance, so to speak, between his intellectual faculties and animal propensities, seems to have been destroyed. He is fitful, irreverent, indulging at times in the
grossest profanity (which was not previously his custom), manifesting but little deference for his fellows, impatient of restraint or advice when it conflicts with his desires, at times pertinaciously obstinate, yet capricious and vacillating, devising many plans of future operations, which are no sooner arranged than they are abandoned in turn for others appearing more feasible. A child in his intellectual capacity and manifestations, he has the animal passions of a strong man. Previous to his injury, although untrained in the schools, he possessed a well-balanced mind, and was looked upon by those who knew him as a shrewd, smart businessman, very energetic and persistent in executing all his plans of operation. In this regard his mind was radically changed, so decidedly that his friends and acquaintances said he was "no longer Gage."

-Dr. John Martyn Harlow

1.4 Normal versus abnormal aging

1.4.1 Normal aging

Cognitive changes have been frequently reported in aging (Kirkwood, 2010; Healey et al., 2008; Bugg et al., 2007; Petersen, 2000). Presumably, this is due to the myriad of changes occurring in the aging brain across the lifespan. For instance, as humans age, neuronal losses are known to occur, such as the number and size of synaptic connections and changes in the branching of axons (Foster, 2002). It has also been suggested that aging is associated with a shift in timing, level of transmission, or connectivity between structures (Kumar and Foster, 2007). Whereas actual anatomical lesions may not be the cause of cognitive declines in aging, functional
lesions caused by modified synaptic connections and gradual cell loss could very well likely influence brain processing speed and ability. By determining the rates that specific eye movement parameters change with age, we can provide insight into whether eye movement testing is a feasible method to evaluate the aging process.

1.4.2 Huntington’s disease

Huntington’s disease (HD), a rare neurodegenerative disorder characterized by unwanted choreic movements (Young et al., 1986), behavioural disturbances, and sometimes dementia (Roos, 2010) that is the result of too many CAG repeats in the DNA sequence. The pathophysiology underlying HD selectively begins within the basal ganglia (an area in the brain devoted to movement control); specifically in the caudate and putamen (Purdon et al., 1994; Sharp and Ross, 1996), and often onset does not occur until greater than 30 years old. Examining the pattern of deficits in motor and cognitive control using eye movements in HD helped to elucidate the progression of atrophy in HD and may help provide a reliable indicator of HD progression.

1.4.3 Alzheimer’s disease and Mild Cognitive Impairment

Alzheimer’s disease (AD), a disorder in which memory plus at least one other cognitive function are impaired, is assessed in Chapter 4. Also in Chapter 4 we examine changes in amnestic Mild Cognitive Impairment (aMCI), a condition typically prodromal to AD in which the sole complaint is memory impairment (Corbetta et al., 1998; Dubois et al., 2007; Petersen et al., 2009). The purpose of assessing eye movement impairment in aMCI and AD was to determine if saccade tasks could measure executive dysfunction in aMCI patients similar to AD patients that
could then aid in the future tracking of conversion to AD. Early AD disease pathology is thought to begin in the medial temporal area including the hippocampus (Braak and Braak, 1991); however, hippocampal projections to frontal cortical areas could influence executive functioning. aMCI patients who are prodromal to AD should theoretically exhibit similar executive dysfunction. Studying very different diseases like HD and AD allowed us to investigate how differing underlying pathologies could potentially be influencing the frontal cortex, since both of these diseases involve executive function deficits.

1.5 Assessing neurological function: tools and techniques

Several different techniques are now available to assess neurological function in humans, some more invasive than others. Standardized clinical assessments are commonly used, where different tests probe different cognitive functions. For instance, it has been shown in HD that scores on the Unified Huntington’s Disease Rating Scale, a motor and functional checklist, correlate to disease severity (Peltsch et al., 2008). The same holds true for neuropsychological tests of AD such as the Dementia Rating Scale (DRS). This scale includes scores of attention, initiation/perseveration, conceptualization, and memory, and also assesses dementia severity (Schmidt et al., 2006). The difficulty with neuropsychological tests is the lack of objectivity and inter-rater reliability.

Neural recording techniques, such as electroencephalography (recording of electrical activity via the scalp) (Olejniczak, 2006), and in special circumstances live cellular recording or deep brain stimulation during surgeries (Lozano et al., 2010) can also provide insight into neural
functioning, and are a more objective measurement. These methods have excellent temporal resolution but can lack in spatial resolution.

Finally, neuroimaging techniques can provide images of the brain and also information about various neural properties, such as the connectivity between different regions. Although there are several forms of neuroimaging such as computed tomography (CT) and positron emission tomography (PET), magnetic resonance imaging (MRI), specifically functional MRI will be described for the purpose of this thesis. Blood oxygen level dependence (BOLD) is the MRI contrast of blood deoxyhemoglobin and was first discovered as useful for brain imaging in 1990 ((Ogawa et al., 1990). Simply put, the BOLD signal has been found to correlate to neural activity (Logothetis, 2003), as active neurons require glucose and oxygen to be delivered via the bloodstream. Since its discovery, the number of neuroscience studies employing fMRI has skyrocketed, such that when the term “fMRI brain” is searched in Pubmed, 10 6542 articles are returned. Using fMRI as a recording technique allows researchers to measure ‘neural activity’ via the BOLD signal, while an individual is performing a specific task. In this thesis, we used fMRI in conjunction with saccadic eye movement tasks.

1.6 Eye movement experiments: saccades

Eye movement experiments have been widely used by both clinicians and basic scientists to examine cognition (Leigh and Kennard 2004, Munoz and Everling 2004, Munoz et al 2007, Ramat et al 2007). Studying eye movement control holds several advantages over studying axial and limb movements: Firstly, the generation of eye movements is provided by motor neurons located within the cranium, so performance can be assessed without using body movements.
Secondly, several different types of eye movements exist, which differ based on their underlying physiological and anatomical substrates. Thirdly, many disease groups show distinct abnormalities in eye movements than can then provide insight into the pathology accompanying each disease. The type of eye movement used in this thesis is a saccadic eye movement. To reiterate, a saccade is a fast eye movement that directs the eye by moving the fovea, a region of highly concentrated photoreceptors, to fixate on an area of interest (Leigh and Zee 1999).

As a result of the advantages eye movements provide, saccade tasks are often used as a tool to study cognition (Leigh and Kennard 2004, Munoz and Everling 2004, Ramat et al 2007, Munoz et al 2007) in relation to specific brain changes, such as those accrued by disease, damage, or human development/aging. This is in part due to the ease and speed of deliverance, whereby the tasks are hands-/language-free, the ability to administer to a variety of populations, and the objective measurements provided (Kaufman et al., 2010). This thesis focuses particularly on two types of saccades: automatic and volitional. Volitional (goal-driven) saccades are made on command, even in the absence of an overt triggering stimulus. Examples of tasks that require voluntary saccades include the anti-saccade task (Hallett, 1978), and the memory-guided saccade task. In both of these tasks, subjects are required first to suppress a saccade toward a suddenly-appearing peripheral stimulus when it appears, and then generate a voluntary saccade. In the antisaccade the voluntary saccade is in the opposite location to the stimulus, and in the memory-guided saccade it is to the remembered location after the stimulus has disappeared. The basal ganglia and the frontal cortex are critical for voluntary saccade control (Hikosaka et al., 2000; Gaymard et al., 1998; Munoz and Everling, 2004; Pierrot-Deseilligny et al., 1991). Automatic saccades, such as in the prosaccade task, are automatic, externally-triggered saccades wherein the subject directs their saccade toward the sudden appearance of a peripheral stimulus. These
visually-triggered saccades are mediated by the intermediate layers of the superior colliculus, with inputs from the visual and parietal cortices (Munoz and Everling, 2004). The ability to perform the antisaccade task peaks in the early 20’s, after which latency increases with age (Munoz et al., 1998). Several cortical lesions can interfere with the ability to perform antisaccades, which will be discussed in the following section.

1.7 Neurophysiology of saccadic eye movements

1.7.1 Motor generation

Several coinciding neural processes must take place for a saccade to be generated. Firstly, there are six extra-ocular motorneurons (MN’s) that encode the characteristics of the saccade via their temporal discharge to then cause the extra-ocular muscles to move the eyes. The MN discharge a burst of action potentials to move the eyes and a tonic discharge to keep the eyes stationary (Leigh and Zee, 2006). MN’s are innervated by excitatory and inhibitory burst neurons originating from the brainstem reticular formation. In addition to these two classes of neurons, long-lead burst neurons (LLBN’s) and omnipause neurons (ON’s) are also located in the brainstem reticular formation. LLBN’s project to the excitatory and inhibitory burst neurons causing them to discharge, while ON’s inhibit them. In order for a saccade to occur, the ON’s must be silenced and the LLBN’s must activate either the excitatory or inhibitory burst neurons to generate a saccade command to the MN’s. Once the saccade is complete, the ON’s are reactivated and the excitatory or inhibitory burst neurons are re-inhibited.
1.7.2 Initiation and suppression

The frontal cortex plays a crucial role in controlling saccades, as it receives direct projections from the visual cortex (Fig. 1.1). The frontal eye fields (FEF) interconnect with the parietal visual areas and these areas then connect to the supplementary eye fields (SEF), dorsalateral prefrontal cortex (dLPFC), parietal cortex, SCi and basal ganglia (Munoz et al., 2007; Thompson et al., 1997; Schall and Thompson, 1999). The FEF, parietal lobe and SCi have numerous connections between them, and contain neurons with similar firing patterns. The FEF, SEF and DLPFC all project directly to the SCi; and the FEF and SEF also project to the cerebellum and reticular formation. Initiation and suppression of saccades is thought to be mediated via projections from the frontal cortex (frontal/supplementary eye fields and dorsalateral prefrontal cortex (Munoz and Everling, 2004; Everling and Munoz, 2000; Hanes and Wurtz, 2001; Segraves and Goldberg, 1987), either directly to the superior colliculus and brainstem, or instead via the basal ganglia (Hikosaka et al., 2000; Hikosaka et al., 2006). Within the basal ganglia, there are two distinct pathways that output to the superior colliculus. The “indirect” pathway through the basal ganglia may mediate saccade suppression via increasing GABAergic inhibitory outflow from the substantia nigra pars reticulata to the superior colliculus, whereas the “direct” pathway may mediate saccade initiation via decreasing GABAergic connections focally within the superior colliculus (Hikosaka et al., 2006). Thus, dysfunction or degeneration of the frontal cortex and/or basal ganglia may influence the successful execution of saccades, and this can be tested experimentally. Previous lesion, fMRI and electrophysiological studies have provided insight into the neurological underpinnings of saccades.
There is some post-mortem evidence that extra-ocular eye muscles exhibit minor age-related changes (McKelvie et al., 1999). However, there is no evidence that HD, aMCI, and AD patients exhibit deficits in their extra-ocular eye muscles or motorneurons beyond that of normal aging, so this thesis will focus on the cortical and BG control of saccadic eye movements.
Studies of patients with frontal lobe lesions (Gaymard et al., 1998; Pierrot-Deseilligny et al., 1991; Guitton et al., 1985) and neuroimaging studies (Connolly et al., 2002; Ettinger et al., 2005; Ford et al., 2005; O'Driscoll et al., 1995; Sweeney et al., 1996) have revealed specific frontal regions involved in volitional saccade control, such as the DLPFC, FEF, and SEF. For instance, both monkey electrophysiology and lesion studies have shown that damage to the DLPFC, (Gaymard et al., 1998; Pierrot-Deseilligny et al., 1991; Guitton et al., 1985) led to difficulties in saccade inhibition (i.e., an increase in incorrect antisaccades – erroneous saccades to the stimulus), whereas lesions to the FEF led to increased antisaccade latencies (Gaymard et al., 1998; Rivaud et al., 1994). These lesions do not typically effect prosaccade generation.

Although the early pathology in HD, AD and aMCI patients does not typically begin in the frontal cortex, other areas can influence the functioning of the frontal cortex via projections to frontal structures. For instance, the basal ganglia is intimately involved in either boosting or suppressing frontal saccade signals, and is primarily effected by HD pathology. The hippocampus is one of the first structures thought to suffer pathological changes in AD and aMCI, and the hippocampus has known projections to frontal structures. The loss of input from the medial temporal area could in turn affect frontal regions (Buckner, 2004; Rabinovici et al., 2007).

1.8 Objectives

Because oculomotor recordings can be more sensitive and objective than clinical tests (Munoz and Everling, 2004; Leigh and Kennard, 2004; Leigh and Zee, 1982), we chose saccades tasks to assess executive functioning. The goal of this thesis is to characterize saccadic behaviour and brain function in normal and abnormal aging, and to show that changes in the frontal cortex,
or alterations in projections to the frontal cortex underlie the reduced executive control seen in these populations. We also aimed to determine whether or not aMCI and AD could be discernable by observed results on saccade tasks. We hypothesized that aMCI patients who would later convert to AD should show similar impairments to early stage AD patients.

The results from Chapter 2 show that the processes underlying automatic saccade generation are relatively resilient to age-related brain changes, whereas the processes underlying voluntary saccade control are not; the rate at which voluntary saccade parameters correlated with aging suggested that the mechanisms driving voluntary saccades degrade at a faster rate in the elderly. This was especially true for the inhibition of erroneous saccades, whereas initiating voluntary saccades was less affected. Following this, Chapter 3 reveals that HD patients have difficulties with both automatic and voluntary saccade control, but considerably more difficulty controlling voluntary saccades. Voluntary saccade measures also correlated to disease severity, indicating that saccade measurements could be useful in assessing motor and functional changes in HD. Chapter 4 reveals that again primarily voluntary saccade control was considerably more impaired in both aMCI and AD relative to normal aging. Finally, to map the changing behavioural control in aging onto the brain, Chapter 5 shows that the elderly recruit more fMRI signal than younger subjects in frontal oculomotor regions such as the DLPFC. In addition, the elderly individuals who performed at a higher level (i.e., similar to young adults with less than 1% direction errors) showed higher activation in the DLPFC than elderly individuals who performed at a lower level (generating an abnormally high percentage of erroneous responses). Taken together, these findings suggest that voluntary saccade tasks that measure executive function, such as the antisaccade task, provide a useful tool to assess neurological changes in both normal and abnormal aging. Diseases in which the underlying pathology in some way influences
the frontal cortex and therefore executive control could potentially be tracked using these tasks. Combined with fMRI, eye movement recordings provide an excellent measure of underlying functional changes in aging and disease, and can hopefully be used someday to track dysfunction and develop preventative methods against functional loss in disease.

1.9 References


Chapter 2

Age-related trends in saccade characteristics among the elderly

2.1 Abstract

Eye movement recordings are useful for assessing neurological disorders, the prevalence of which increases with age. However, there is little rigorous quantitative data on describing oculomotor changes that occur during healthy aging. Here, we measured the ability of 81 normal elderly subjects (60–85 years) to perform two saccadic eye movement tasks: a pro-saccade task, requiring an automatic response to look towards a stimulus and an anti-saccade task, requiring inhibition of the automatic response to instead initiate a voluntary saccade away from the stimulus. Saccadic ability decreased with age: the oldest subjects were slower to initiate saccades and they made more direction errors (i.e., erroneous pro-saccades) in the anti-saccade task. Intra-subject variability in reaction time also correlated positively with age in both saccade tasks. Voluntary saccade control, as assessed by the anti-saccade task, was far more affected by aging than automatic control, as assessed by the pro-saccade task, suggesting that the mechanisms driving voluntary and automatic saccade performance deteriorate at different rates in the aging brain, and therefore likely involves different neural substrates. Our data provide insight into deficits due to normal brain changes in aging as well as a baseline to evaluate deficits caused by neurological disorders common in this age range.
2.2 Introduction

The proportion of elderly individuals in society is increasing dramatically (Turcotte and Schellenberg, 2006), leading to an increase in the prevalence of age-related neurological disorders that affect the function of the frontal lobes and overall movement control (Gavrilov and Heuveline, 2003). In order to study these disorders most effectively, cognitive deficits due to normal brain changes in healthy aging first need to be understood. The eye movement system is an excellent model to assess brain function (Leigh and Kennard, 2004; Munoz et al., 2007, Ramat et al., 2007). The circuitry controlling saccadic eye movements is well understood and involves areas of the frontal and parietal lobes, basal ganglia, thalamus, visual cortex, superior colliculus, cerebellum, and brainstem reticular formation (Hikosaka et al., 2006; Leigh and Zee, 2006; Moschovakis et al., 1996; Munoz and Everling, 2004; Scudder et al. 2002; Wurtz and Goldberg, 1989). These structures contribute to specific components of saccadic behaviors, and altered saccade performance often gives insight into the etiology of various clinical disorders. Because there is overlap in the frontal cortical areas controlling the production of saccades and the areas involved in controlling various aspects of cognition, measuring saccadic eye movements can provide an important tool to assess cognitive functions subserved by the frontal lobes. These same areas are frequently degenerating as people age (Creasey and Rapoport, 1985; Kramer et al, 2007).

Saccadic eye movement tasks can be designed to probe simple sensory-motor processes as well as higher cognitive functions. Eye movement tasks can be used to dissect different components of the system. In a pro-saccade task, subjects are instructed to look towards an eccentric visual stimulus when it appears. This task has high stimulus-response compatibility and requires a simple, automatic response (Munoz and Everling, 2004). In the anti-saccade task
(Hallett 1978), subjects are instructed to look away from the eccentric stimulus in the opposite direction. The location of the stimulus and the saccade goal are dissociated in this task. Successful completion of the anti-saccade task requires additional stages of processing: suppression of the automatic pro-saccade to the stimulus, followed by voluntary initiation of the anti-saccade away from the stimulus (Munoz and Everling, 2004). The difference between pro-and anti-saccade latencies, the anti-effect, provides a measure of the time it takes for these additional processes. Fixation state can also be manipulated by introducing a gap period between disappearance of the fixation spot and the appearance of the stimulus (Saslow, 1967), which serves to reduce reaction times. A subset of these short-latency stimulus-driven saccades have latencies that approach the minimal afferent and efferent conduction times in the oculomotor system and have been called “express” saccades (for review, see Dorris et al. 1997; Fischer and Weber 1993). Express saccades have been identified as the first peak in a multimodal distribution of SRTs (Fischer and Boch, 1983; Fischer et al., 1997) and are often reported as minimal or absent in the elderly (Klein et al., 2000; Munoz et al., 1998). Here, we investigate in greater detail the occurrence of these short-latency stimulus-driven saccades in the elderly.

Our knowledge of the neural pathways underlying pro- and anti-saccade generation is under continual debate as new findings emerge. However, studies of patients with frontal lobe lesions (Gaymard et al., 1998; Guitton et al., 1985; Pierrot-Deseilligny et al, 1991; Rivaud et al, 1994) and recent neuroimaging studies of normal individuals (Connolly et al, 2002; Ettinger et al, 2005; Ford et al, 2005; O’Driscoll et al, 1995; Sweeney et al, 1996) have identified specific frontal regions (e.g., dorsolateral prefrontal cortex, frontal and supplementary eye fields) that are involved in voluntary saccade control. For example, lesions to the frontal eye fields (FEF) lead to increased anti-saccade latencies (Gaymard et al., 1998; Rivaud et al., 1994). Lesions to the
dorsolateral prefrontal cortex (DLPFC) lead to difficulties in saccade suppression (Gaymard et al., 1998; Guitton et al., 1985; Pierrot-Deseilligny et al., 1991). These lesions do not typically affect pro-saccade generation. Instead, lesions to the posterior parietal cortex and supplementary motor area influence the accuracy and timing of pro-saccade reaction times, respectively (Heide and Kömpf, 1998; Pierrot-Deseilligny et al., 1991). Therefore, contrasting performance on these tasks provides measures of frontal lobe function that can be applied to the elderly.

Numerous studies have described the effects of senescence on saccadic eye movement performance; but their conclusions are inconsistent. Many studies suggest saccade parameters such as reaction times, error rates, and metrics are correlated with aging (Abel and Douglas, 2007; Klein et al., 2000; Munoz et al., 1998a; Olincy et al., 1997; Shafiq-Antonacci et al., 1999), whereas others have shown no differences between elderly and younger subjects (Eenshuistra et al., 2004; Pratt et al., 2006). However, there is one broad consensus: the more automatic parameters such as pro-saccades latencies are at best, minimally influenced by aging (Abrams et al., 1998; Kaneko et al., 2004; Munoz et al., 1998a; Pratt et al., 2006), whereas more cognitively complex aspects of saccadic performance such as suppression and voluntary initiation of a goal-directed saccade (e.g., anti-saccades) are more strongly influenced by aging (Olincy et al., 1997; Shafiq-Antonacci et al., 1999). This suggests that the neural structures in the oculomotor system responsible for generating pro-saccades such as visual occipital cortex, parietal cortex, the brainstem burst generator, reticular formation, and superior colliculus (Munoz and Everling, 2004) may remain relatively uncompromised as people age compared to structures in the frontal and parietal cortices that are involved in complex cognitive function required in the anti-saccade task (Curtis and D'Esposito, 2003; Pierrot-Deseilligny et al., 2003).
The purpose of this study is to determine the rate at which various saccade parameters change between the ages of 60 and 85 years as assessed with pro- and anti-saccade tasks. If automatic processes are affected by aging, then pro-saccade latencies, and the gap-effect, including proportion of express saccades, should be altered. Alternatively, if voluntary processes are primarily affected with aging, then anti-saccade latencies, direction errors, and the anti-effect should be altered. It is hypothesized that performance decrements in the healthy aging will reflect the natural cognitive slowing and cerebral atrophy (Aizenstein et al., 2004; Creasey and Rapoport, 1985; Kramer et al, 2007) that occur over time. Elucidating the patterns of eye movement deficits in aging will help to determine both the feasibility of eye movement testing to evaluate the aging process and the aspects of the saccade system that are most resilient to the aging process.

2.3 Methods

2.3.1 Subjects

All experimental procedures were reviewed and approved by the Queen’s University Human Research Ethics Board. Eighty-one subjects ranging between 60 and 85 years of age were recruited into this study (Table 1). Subjects reported no known visual, neurological or psychiatric symptoms, and had normal or corrected to normal vision. All subjects provided informed consent and were compensated for their participation.
<table>
<thead>
<tr>
<th>Age Range (years)</th>
<th>Mean Age (± SD)</th>
<th>Number of Subjects</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>60-64</td>
<td>61.9 ± 1.4</td>
<td>16</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>65-69</td>
<td>67.0 ± 1.3</td>
<td>19</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>70-74</td>
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</tr>
<tr>
<td>75-79</td>
<td>77.2 ± 1.5</td>
<td>13</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>80-85</td>
<td>81.9 ± 1.7</td>
<td>14</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>81</td>
<td>52</td>
<td>29</td>
<td></td>
</tr>
</tbody>
</table>

Table 2.1. Composition of age-related groups.

2.3.2 Experimental paradigm

The experiment was conducted in one 40 minute session in which subjects performed one block of a pro-saccade task (120 trials; Fig. 2.1A), and two blocks of an anti-saccade task (120 trials per block; Fig. 2.1B). Subjects were seated alone in a dark room 100 cm away from a translucent visual screen. Visual stimuli consisted of red light emitting diodes (LEDs; central fixation point = 2.0 cd/m²; peripheral stimuli = 5.0 cd/m²). Between trials, the screen was diffusely illuminated (1.0 cd/m²) to reduce dark adaptation. Each trial began when the background illumination was turned off and after 250 ms of complete darkness, the fixation point (FP) appeared. After 1000 ms, one of two events occurred depending on the trial condition. In an ‘overlap’ condition, the FP remained illuminated while a peripheral stimulus appeared 20° to the left or right, and in a ‘gap’ condition, the FP disappeared for 200 ms before the peripheral stimulus appeared. In both conditions the stimulus remained illuminated for 1000 ms, after which all three LEDs were turned off and the background illumination came on for 500 ms. Stimulus location (left, right) and fixation condition (gap, overlap) were randomly interleaved within a block of trials. During the pro-saccade block (Fig. 2.1A), subjects were instructed to look towards the peripheral stimulus as soon as it appeared. During the blocks of anti-saccade trials (Fig. 2.1B), subjects were instructed to look in the opposite direction of the stimulus as soon as it appeared.
Figure 2.1. Left column: Saccade paradigm. Pro-saccade task (A) and anti-saccade task (B), including individual saccade traces and reaction time distributions (C-D) in the gap condition for a representative 67 year old. Right column: Individual saccade traces (E, F) and reaction time distributions (G, H) for a representative 80 year old.

2.3.3 Recording and apparatus

Horizontal eye movements were measured using direct current electrooculography (EOG) to obtain a continuous measure of eye position. The EOG signal was amplified (Grass P18 Amplifier) and low-pass filtered (50Hz). Horizontal eye position was digitized at a rate of 1 kHz using REX, ver 5.4 (Hays et al., 1982). Subjects were asked to direct their eyes between left/right peripheral and central stimulus locations in order to calibrate the signal. We then set the EOG amplification to 1 Volt = 10°. Within this range (± 20°) the horizontal eye position signal
was linear (Goldring et al., 1996) and the noise was < 2° in position and < 15 g/s in velocity (e.g., see traces in Fig. 2.1). Digitized data were stored on a hard disk, and analyzed off-line on a Sun Ultra 60 Spark station.

2.3.4 Data analysis

The onset and termination of each saccade was determined when eye velocity exceeded 30°/s. Trials were scored as correct if the first saccade after stimulus appearance was in the correct direction. Trials were classified as direction errors if the first saccade after stimulus appearance was in the wrong direction. Saccadic reaction time (SRT) was measured from stimulus appearance to onset of the first saccade.

For each subject, the following values were computed for the pro- and anti-saccade task from correct trials with latencies up to 1000 ms (broken down for both gap and overlap conditions): mean SRT for correct trials, coefficient of variation of SRT (SD/mean x 100) for correct trials, percentage of direction errors, and percentage of express saccades. Pro- and anti-saccades from 0 to 110 ms occurred at a 50% chance level (correct: incorrect), so saccades with SRTs < 110 ms were classified as anticipatory and were not included in measures of correct mean SRT (see Results 2.4.1, Fig. 2.2). Because saccadic latencies, including latency of express saccades, depend on stimulus intensity, size, and contrast (Bell et al. 2006; Carpenter 2004; White et al. 2006), we used anti-saccade direction error latencies (erroneous stimulus-driven pro-saccades) in combination with correct pro-saccade latencies to help identify the latency range wherein express saccades occurred. The first mode in the distribution of anti-saccade error latencies corresponded to both a decrease in correct anti-saccades, and the first mode in the
distribution of pro-saccades, reflecting the earliest stimulus-driven saccades that were no longer anticipatory. A binomial sign test determined the start and end of this stimulus-driven epoch (shaded grey boxes in Fig. 2.2) by measuring when the proportion of anti-saccade error trials (in 10 ms bins) significantly exceeded that of correct anti-saccade trials, for each age category (Fig. 2.2 F-G, black bars on negative ordinate). Then, the proportion of correct pro-saccades occurring during this epoch specific to each age category (i.e., express saccades) was measured for each age group.

2.3.5 Statistical analysis

For all tasks, statistical corrections for heterogeneity (Levene’s) and sphericity of variance (Greenhouse-Geisser) were made as needed, and outliers > ± 3 standard deviations of mean of each age category were removed (1-3 outliers per measurement for all age categories). Correlations were measured by pairing oculomotor parameters with age as a continuous variable where statistical significance was based on t-tests different from zero. To analyze differences with increasing age, the population was stratified into 5 groups by age as follows: 60-64, 65-69, 70-74, 75-79, and 80-85 (see Table 2.1 for group composition). Repeated measures ANOVAs were also used to evaluate the results from all elderly age groups. The independent variable used to carry out the ANOVAs was age (categorized into 5 year age increments; Table 2.1), and the repeated measures were experimental task (pro vs. anti-saccades), and fixation condition (gap vs. overlap). Values for right and left stimulus positions were not significantly different (paired t-test; p > .05), allowing the data to be collapsed across direction for each task.
2.4 Results

The results of this study can be summarized by four main points. 1) Saccadic reaction times (SRTs) increased with age; 2) intra-subject variability in SRT increased with age; 3) the proportion of direction errors increased with age; and 4) express saccades did not decrease with age (in contrast to previous literature).

2.4.1 Saccadic reaction time (SRT)

Figure 2.1 shows individual saccade traces in the gap condition for both pro-saccades (Fig.2.1A, E) and anti-saccades (Fig.2.1B, F) for a representative 67 year old (Fig.2.1A-D) and an 80 year old (Fig.2.1E-H). Reaction time distributions reveal that for these sample subjects, mean gap and overlap SRTs are greater in the 80 year old (Fig.2.1, G-H) than in the 67 year old (Fig.2.1, C-D). Individually, anti-saccade SRTs differed significantly from pro-saccade SRTs (anti-effect; anti SRT – pro SRT) in 89% of the subjects in the gap condition, and 77% in the overlap condition. Gap condition SRTs and overlap condition SRTs (gap effect; overlap SRT – gap SRT) differed significantly in 77% of subjects in the anti-saccade task, and 79% of subjects in the pro-saccade task. Subsequently, subjects were divided into five age groups (see Table 2.1).

Figure 2.2 shows the distribution of correct and error pro-saccade and anti-saccade latencies in the gap condition for the five different age groups studied. A change in both correct and incorrect latency distributions occurred between the eldest subjects (80-85 yrs) and the youngest subjects (60-64 yrs and 65-69 yrs) i.e., the distribution broadened, where an increased proportion of errors were seen as older subjects became slower to initiate correct anti-saccades. This same pattern held true in the overlap condition (not shown). The proportion of errors in
relation to the proportion of correct anti-saccades can be examined further to determine the occurrence of express saccades in the elderly.

The grey shaded boxes in Fig. 2.2 show the range of express saccades (short-latency pro-saccades) for each age group based on the binomial sign test between correct versus direction error anti-saccade latencies (see Methods 2.3.4). Surprisingly, the shortest SRTs in the error distribution (epoch onset) remained relatively fixed across age group (60-64: 120 ms; 65-69: 140 ms; 70-74: 130 ms; 75-79: 110 ms; 80-85: 130 ms), whereas the longest SRTs with more errors than correct responses changed with age group (epoch end; 60-64: 180 ms; 65-69: 180 ms; 70-74: 210 ms; 75-79: 210 ms; 80-85: 240 ms (r = .945, p = .015; Fig. 2.3)). This was consistent with a delay in the shortest SRTs for correct anti-saccades (Fig. 2.2F-J, upper histograms). Express saccade production is influenced by multiple factors and do not appear to be time-locked at the 90-135 ms reported in previous literature (Fischer & Weber, 1993; Munoz et al., 1998), so we used the epoch onset to epoch end latency range (grey shaded regions in Fig. 2.2) to determine the mean proportion of pro-saccades falling within these latencies (express saccades) for each age category (60-64 = 18%; 65-69 = 7%; 70-74 = 17%; 75-79 = 30%; 80-85 = 25%), which did not correlate with increasing age group. The express epoch onset did not change systematically across the ages of 60 – 85, but the epoch end did (Fig. 2.3). Furthermore, the proportion of fast, automatic saccades occurring per bin did not increase with aging (Fig. 2.2 F-J, lower histograms). Therefore, advancing age appears to differentially influence voluntary versus automatic processes.
Figure 2.2. A-E. Distribution of SRT in pro-saccade gap trials for each age category (correct responses on positive ordinate (light grey); incorrect responses on negative ordinate (medium grey). F-J. Distribution of SRT in anti-saccade gap trials. Darkened black bars on the negative ordinate represent 10 ms error bins statistically different from correct bins. Grey shaded boxes represent calculated express saccade epoch based on the error latencies (see Methods 2.3.4).
Correlation analyses were then performed to determine the rate at which pro- and anti-saccade latencies changed with age. SRTs increased with age in all four conditions; pro-gap ($r = .283$, $p = .011$); pro-overlap ($r = .222$, $p = .048$); anti-gap ($r = .501$, $p < .001$); and anti-overlap ($r = .420$, $p < .001$). However, age was a much stronger predictor of anti-saccade reaction times than of pro-saccade reaction times (Fig. 2.4 A, B). Furthermore, the rates (determined using a linear regression analysis) at which pro- and anti- SRTs changed with age also differed. Pro-SRTs increased at a rate of 2.0 ms/yr for gap, and 1.9 ms/yr for overlap conditions, whereas anti-SRTs increased at a rate of 5.0 ms/yr for gap and 4.7 ms/yr for overlap conditions. The anti-effect also correlated significantly with age for both gap ($r = .274$, $p = .014$) and overlap conditions ($r = .240$, $p = .032$). Conversely, the gap effect did not correlate with age either for pro- or anti-saccades (Fig. 2.5). The slope of the linear regression for the anti-effect (2.9 ms/year for gap, 2.8 ms/yr for overlap) differed significantly from that of the gap-effect (-.07 ms/year for pro, -.06 ms/yr for
These results suggest that the difference between pro- and anti-saccade latencies increases as people age, whereas the gap effect does not.

**Figure 2.4.** Pro- and anti-saccade results for both gap (open-faced circles) and overlap (solid circles) conditions, individually plotted (horizontal line = Pearson correlation, *p < .05) across age. A-B. Saccadic reaction time (SRT). C-D. Coefficient of variation (CV) of SRT (standard deviation/mean x 100). E-F. Percent direction errors (erroneous pro-saccades). * correlation across age, p < .05, ** p < .01. Outliers ±3 standard deviations from the mean have been removed.
Data was then grouped into five age categories to determine if age differences were specific to certain age ranges. A three-way ANOVA (age group x task (anti/pro) x condition (gap/overlap)) revealed significant differences between saccade tasks (anti vs. pro) and fixation conditions (gap vs. overlap) (Fig. 2.4 A, B). Pro-saccade reaction times were faster than correct anti-saccade reaction times in both gap and overlap conditions (the anti-effect; F (1, 75) = 130.05, p < .01). The gap condition produced shorter SRTs than the overlap condition in both pro- and anti-saccade tasks (the gap-effect; (F (1, 75) = 263.36, p < .01)). Furthermore, SRT increased with group age for all tasks (F (1, 75) = 5.57, p < .01). Specifically, group differences were most robust in the anti-saccade task, whereas in the pro-saccade task, only the eldest subjects (80-85 yrs) differed from the youngest subjects (60-64 yrs; see post-hoc comparisons, Table 2.2). The anti-effect increased for older age groups (Fig. 2.5) who became even slower to initiate voluntary anti-saccades than automatic pro-saccades (F (1, 75) = 2.85, p < .05). A trend analysis revealed that although there was a significant linear trend (p < .001) for SRT increasing with age in all conditions, there was also a significant non-linear trend (p = .007), indicating that the most severe decrement in performance occurred after the age of 70 years.
Figure 2.5. Gap effect and anti effect individually plotted across age (horizontal line = Pearson correlation, *p < .05). A. Anti effect = anti SRT – pro SRT for both gap (open-faced circles) and overlap (solid circles). B. Gap effect = overlap SRT – gap SRT for both pro (solid circles) and anti (open-faced circles). Horizontal line = Pearson correlation, *p < .05.

2.4.2 Intra-subject variability in SRT

To measure intra-subject variability for each of the four saccade conditions we computed coefficient of variation (CV) of SRT, the relative standard deviation expressed as a unitless proportion of each subject mean (see Methods 2.3.4). The amount of intra-subject variability was correlated positively with advancing age in all conditions; pro-gap (r = .455, p < .001); pro-overlap (r = .396, p < .001); anti-gap (r = .314, p = .005); and anti-overlap (r = .242, p = .031) (Figs. 2.4 C, D). Intra-subject variability increased in the pro-saccade task at a rate of 0.6 ms / yr.
for gap, 0.5 ms /yr for overlap, and 0.2 ms / yr for both gap and overlap conditions in the anti-
saccade task. A three-way ANOVA was performed to further investigate the pattern of increased
variability across age groups. As age categories increased, intra-subject variability in SRT also
increased ($F (1, 75) = 6.845; p < .001$). The CV was lower in the gap condition (22 ± 6) than in
the overlap condition (25 ± 5) for the anti-saccade task ($F (1, 73) = 16.62, p < .01$). The CV was
also higher in the pro-saccade task (29 ± 9) compared with the anti-saccade task (24 ± 6) ($F (1,
74) = 40.21, p < .01$), likely due to shorter reaction times in the pro-saccade task. A trend analysis
indicated that the CV increased ($p < .001$) with group age, and post-hoc analysis (Tukey HSD)
showed that the CVs of most age categories differed from those of others, especially in the pro-
saccade task (see Table 2.2 for post-hoc comparisons).

<table>
<thead>
<tr>
<th>Age Category</th>
<th>Age Category</th>
<th>PRO SRT (Sig.)</th>
<th>PRO CV (Sig.)</th>
<th>Direction Error (Sig.)</th>
<th>ANTI SRT (Sig.)</th>
<th>ANTI CV (Sig.)</th>
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<td>.106</td>
<td>.945</td>
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<tr>
<td>75-79</td>
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<td>.340</td>
<td>.797</td>
<td>.213</td>
<td>.842</td>
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<tr>
<td>80-85</td>
<td>.019*</td>
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<td>.181</td>
<td>.006*</td>
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<tr>
<td>65-69 vs. 70-74</td>
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<td>.991</td>
<td>.003*</td>
<td>.233</td>
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<td>.998</td>
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<td>.879</td>
<td>.015*</td>
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<td>.831</td>
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Table 2.2. Post-Hoc (Tukey) Analysis of Mean Differences between Age Groups
* the mean difference is significant at the .05 level.
2.4.3 Direction errors

As individuals aged, the percentage of direction errors increased significantly in the anti-overlap (r = .342, p = .002) and approached significance in the anti-gap (r = .219, p = .051) conditions (Fig. 2.4 F). A non-parametric correlation also showed that pro-gap direction errors also increased with age (r = .286, p = .011; Fig. 2.4 E). The anti-overlap errors increased at a rate of 0.5%/year – a considerable increase, considering that over a 20 year period, this would amount to approximately 10% increase in the proportion of anti-saccade direction errors. A three-way ANOVA was performed to investigate how direction errors were influenced for increasing age groups. The proportion of direction errors increased significantly with group age in the anti-but not in the pro-saccade task (F (1, 73) = 107.17, p = .035). A two-way interaction between task (pro vs. anti) and fixation condition (gap vs. overlap) revealed that subjects made more direction errors in the anti- versus pro-saccade task, and more errors in gap versus overlap conditions in the anti-saccade task (F (1, 73) = 3.64, p = .01; Fig. 2.4 E, F). In the anti-saccade task, post-hoc comparison revealed a significant difference only between age category 65-69 and age category 80-85 (Table 2.2). A trend analysis indicated a linear (p = .015) relationship between anti-saccade errors and group age.

2.5 Discussion

This study provides a detailed description of the changes in saccade parameters that occur with healthy aging (60 – 85 years). Saccadic reaction times, intra-subject variability, the range of express saccades, the “anti-effect” (mean latency difference between pro- and anti-saccades), and the proportion of direction errors were all sensitive to the effects of aging. Specifically, the generation of pro-saccades, a simple sensory-motor process, was minimally influenced by age
(revealed by pro-saccade SRT, express saccades, and the gap-effect – the mean latency difference between overlap and gap saccades). In contrast, processes required for voluntary saccade initiation (revealed by anti-saccade SRT and the anti-effect) and voluntary saccade suppression (revealed by anti-saccade errors) were more sensitive to aging. The rate at which these parameters changed correlated with age, such that from age 60 to 85 years, correct anti-saccade reaction times increased by about 110 ms, and error rates increased by approximately 11%. This suggests that the mechanisms driving anti- versus pro-saccades degrade at different rates in the elderly, implying that they are subserved by different underlying processes. These data support our hypothesis that the neurological changes related to healthy aging influence saccadic control, such that the ability to initiate voluntary saccades and inhibit automatic responses decreases with age.

2.5.1 Relation to previous literature

Previous studies on saccadic eye movements in senescence have used pro- and anti-saccade paradigms (Abel and Douglas, 2007; Abrams et al., 1998; Eenshuistra et al., 2004; Kaneko et al., 2004; Klein et al., 2000; Munoz et al., 1998a; Olincy et al., 1997; Pratt et al., 2006; Shafiq-Antonacci et al., 1999) and produced conflicting results. Many studies have identified increased error rates and reaction times in the anti-saccade task in the elderly (Abel and Douglas, 2007; Klein et al., 2000; Munoz et al., 1998a; Olincy et al., 1997; Shafiq-Antonacci et al., 1999). However, other studies found no differences between young (M = 19-22 yrs) and old (M = 62-66 yrs) populations in anti-saccade latencies and error rates (Eenshuistra et al., 2004; Pratt et al., 2006) or pro-saccade latencies (Pratt et al., 2006). These findings could be explained by the relatively young mean age of the elderly populations used in both studies (e.g., 62 years), which does not capture differences that occur later in life. Examining older populations reveals deficits
within healthy aging such as slower latencies, increased variability and direction errors, especially between age category 65-69 and age category 80-85 (similar to Klein et al., 2000). A large subject base with many trials from each participant allowed us to clarify many age effects that previously have been masked by the amount of between-subject variability in the elderly. Contrary to Pratt et al. (2006), our results revealed that the anti-effect (the increased time required to inhibit the automatic pro-saccade and initiate the voluntary anti-saccade (Munoz & Everling, 2004; Olincy et al., 1997)) correlated with aging (Fig. 2.5 A), revealing that the time it takes to process a voluntary movement lengthens with age.

The gap-effect did not correlate with increased aging (Fig. 2.5 B). Controversy remains surrounding the nature of the gap-effect. Some studies suggest that the introduction of a gap between fixation removal and stimulus appearance allows attention to disengage before shifting to the new stimulus location (Harwood et al., 2008; Pratt et al., 2006). However, the gap effect has also been linked to low-level warning effects (Ross and Ross, 1980). In our studies, the gap-effect was not modulated with task (i.e., consistent across both pro- and anti-saccade tasks), nor was it influenced by aging. Therefore, we speculate that this mechanism of reduced SRT produced by the advanced disappearance of the fixation point is not governed by high-level cognitive processes. Similarly, pro-saccade latencies only changed minimally with age, further suggesting that these more automatic behaviors are subserved by different mechanisms than voluntary saccade initiation and saccade suppression. The automatic processes are less affected by aging than the voluntary processes.

Short-latency pro-saccades (express saccades) reflect the shortest afferent-to-efferent conduction times in the oculomotor system (Fischer and Weber 1993; Dorris et al., 1997).
Express saccades in humans were reported to range from 75-140 (Fischer et al., 1997; Fischer and Ramsperger, 1984; Fischer and Weber, 1993; Munoz et al., 1998). Previously, it has been reported that elderly individuals make significantly fewer express saccades (Klein et al., 2000; Munoz et al., 1998). However, the previous criteria for defining this range did not take into account that SRT can be influenced dramatically by factors such as stimulus intensity (Bell et al., 2006, and are therefore not locked to a specific time. Here, we used the distribution of correct and error SRTs to define the express saccade epoch. Using the SRT distributions to calculate express saccade ranges for each age group revealed that the proportion of express saccades occurring in our elderly population was similar to those previously reported in younger populations. Although Klein et al. (2000) reported minimal express saccades in the elderly, the SRT distributions had errors corresponding to bimodal peaks in the pro- SRT distributions occurring at latencies of approximately 110-200ms. This is very similar to what we have calculated in the present study (Fig. 2.2 F-J). Therefore, it appears that elderly subjects are indeed capable of generating express saccades, but they are delayed in onset. The onset of the range of express saccades in the elderly is delayed from 90 ms to at least 110 ms after stimulus onset. The persistence of short-latency errors in the anti-saccade task up to 200 ms in the elderly suggests that the increase in the duration of this range (gray shaded boxes in Fig. 2.2) may be due to a weaker voluntary saccade initiation signal which subsequently delays initiation of the correct anti-saccade. Therefore, in addition to a minimal delay in onset of the express saccade epoch, it appears that elderly subjects also require more time to process voluntary initiation signals as reflected by the increase in the end of the express epoch. Although the saccades occurring within this epoch are too slow to fit previous criteria for express saccades (Fischer and Weber, 1993), the onset of the express epoch remained stable across the age groups studied, suggesting that these saccades are subserved by the same mechanism as traditional express saccades, but occur at slightly longer latencies (i.e.,
the entire distribution is shifted to the right in the elderly). In addition, the minimal changes in pro-saccade latencies suggest that visual and motor processing is not as impaired as cognitive processing in the elderly.

2.5.2 Saccade circuitry

The dissociation between automatic and voluntary saccade behaviors provides insight into how aging influences the brain circuitry underlying these mechanisms. Lesions studies have demonstrated that frontal areas such as frontal eye fields (FEF), supplementary eye fields (SEF), and dorsolateral prefrontal cortex (DLPFC) are important for voluntary saccade initiation and suppression of automatic saccades (Guitton et al., 1985; Pierrot-Deseilligny et al., 1991; Rivaud et al., 1994), whereas the supplementary motor area and the posterior parietal cortex are more important for automatic saccade generation (Heide & Kömpf, 1998; Pierrot-Deseilligny et al., 1991). Electrophysiological studies have also confirmed that neurons in the FEF (Everling and Munoz, 2000), SEF (Schlag-Rey et al., 1998), and DLPFC (Johnston and Everling, 2006) are modulated by task instruction and appear to be selectively recruited for the anti-saccade task. Therefore, we expect that altered input from any one of these areas could influence the oculomotor circuitry downstream, leading to increases in SRT and error rates. Therefore, the ability to initiate movement voluntarily and suppress unwanted or automatic saccades is reflective of good frontal function (Everling and Fischer, 1998; Munoz and Everling, 2004). The stronger correlation between aging and voluntary saccade performance suggested that the structures related to automatic saccade initiation are not as susceptible to age-related declines as those related to voluntary saccade initiation. It is known that the frontal cortex is more susceptible to natural neural degeneration that occurs in the cerebral cortex with age (Buckner et al., 2000;
Creasey and Rapoport, 1985; Kramer et al, 2007; Raemaekers et al., 2006). The fact that aging minimally influenced pro-saccade performance and the gap effect, but systematically increased voluntary saccade latencies, the anti-effect, and the quantity of direction errors, supports the notion that different underlying mechanisms and structures are responsible for the generation of automatic versus volitional saccades (Hikosaka et al., 2006; Munoz and Everling, 2004; Raemaekers et al., 2006), and that the structures involved in executing voluntary saccades are less resilient to the aging process.

2.6 Conclusions

This is the first study to quantify precisely the rate at which saccade control declines across the elderly, and to show that the latency range of express saccades lengthens in the elderly. It appears that aging is selective for specific aspects of the oculomotor circuitry (Shafiq-Antonacci et al., 1999) such that automatic saccade processing declines with age, but at a much slower rate than the voluntary control of saccades. We speculate that the natural neural degeneration that occurs with age influences the input coming from the frontal cortex to the saccade generating system, and is thus responsible for these changes. This corresponds to the age-related decline in cognitive functions associated with these frontal structures, such as focused attention, task switching, and working memory (Eppinger et al., 2007; Grady et al., 2006; Kray et al., 2005). Appreciation of these normative saccade parameters will be useful in assessing age-related frontal lobe function/dysfunction and as a comparison to age-related neurological disorders such as Mild Cognitive Impairment, Alzheimer’s disease, and Parkinson’s disease.
2.7 Acknowledgements

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Chapter 3
Saccadic impairments in Huntington’s disease

3.1 Abstract

Huntington’s disease (HD), a progressive neurological disorder involving degeneration in basal ganglia structures, leads to abnormal control of saccadic eye movements. We investigated whether saccadic impairments in HD ($N = 9$) correlated with clinical disease severity to determine the relationship between saccadic control and basal ganglia pathology. HD patients and age/sex-matched controls performed various eye movement tasks that required the execution or suppression of automatic or voluntary saccades. In the “immediate” saccade tasks, subjects were instructed to look either toward (pro-saccade) or away from (anti-saccade) a peripheral stimulus. In the “delayed” saccade tasks (pro-/anti-saccades; delayed memory-guided sequential saccades), subjects were instructed to wait for a central fixation point to disappear before initiating saccades towards or away from a peripheral stimulus that had appeared previously. In all tasks, mean saccadic reaction time was longer and more variable amongst the HD patients. On immediate anti-saccade trials, the occurrence of direction errors (pro-saccades initiated toward stimulus) was higher in the HD patients. In the delayed tasks, timing errors (eye movements made prior to the go signal) were also greater in the HD patients. The increased variability in saccadic reaction times and occurrence of errors (both timing and direction errors) were highly correlated with disease severity, as assessed with the Unified Huntington’s Disease Rating Scale, suggesting that saccadic impairments worsen as the disease progresses. Thus, performance on voluntary saccade paradigms provides a sensitive indicator of disease progression in HD.
3.2 Introduction

Huntington’s disease (HD) is a progressive neurological disorder characterized by changes in motor performance, cognitive functions, and personality (Huntington Study Group 1996). The pathophysiology underlying HD is strikingly selective, with atrophy affecting structures within the basal ganglia; most specifically, the caudate and putamen (Purdon et al. 1994; Sharp and Ross 1996). The hallmark symptom of HD is the purposeless, involuntary, choreic movements seen even in the early stages of the disease (Folstein 1989; Young et al. 1986). Oculomotor impairments are also among the first manifestations of HD (Leigh et al. 1983), probably due to the intimate involvement of the basal ganglia with the saccadic control circuit (Hikosaka et al. 2006; Hikosaka et al. 2000). Voluntary control of initiation and suppression of saccades is thought to be mediated via projections from the frontal cortex, directly to the superior colliculus (Everling and Munoz 2000; Hanes and Wurtz 2001; Segraves and Goldberg 1987), and via the basal ganglia (Hikosaka et al. 2000; Hikosaka et al. 2006). Within the basal ganglia, there are two distinct pathways that output to the superior colliculus (Fig. 3.1A). In an intact system, the two pathways are able to work sequentially to suppress planned eye movements -- the “indirect” pathway maintaining inhibition on the superior colliculus to suppress the movement until a trigger to initiate a move occurs, wherein the direct pathway ramps up to selectively disinhibit the SC and produce the desired saccade (for review, see Hikosaka et al. 2000). Therefore, the “indirect” pathway through the basal ganglia may mediate saccade suppression via increasing GABAergic inhibitory outflow from the substantia nigra pars reticulata to the superior colliculus, whereas the “direct” pathway may mediate saccade initiation via decreasing GABAergic connections focally within the superior colliculus (Hikosaka et al. 2006).
Figure 3.1. Schematic of the basal ganglia (A) in a healthy brain versus (B) early stage Huntington’s disease. Voluntary saccades are triggered by excitatory projections to the caudate from the frontal cortex via the indirect pathway. The CN, in turn, phasically inhibits the substantia nigra (SNr), which tonically inhibits the superior colliculus (SC). Thus, excitation of the CN could lead to disinhibition of the SC and facilitate the generation of voluntary saccades. Saccade initiation may be mediated by attenuating the inhibitory pathway to the SC via the direct pathway of the basal ganglia. GPe/GPi = Globus Pallidus (external/internal); STN = Subthalamic Nucleus. Pointed arrows denote excitatory projections, and rounded arrows denote inhibitory projections.

The saccade system provides an excellent tool for assessing and contrasting various neurological disorders because the circuitry spans almost the entire neuraxis (Leigh and Kennard 2004; Leigh and Zee 1999; Munoz 2002). Several saccadic eye movement tasks have been developed to assess clinical populations. The immediate pro-saccade task is often used to test the ability to generate automatic, visually triggered saccades. In this task (Fig. 3.2A), subjects are
required to look immediately toward a visual stimulus when it appears. This visual input to the oculomotor system arises from retino-geniculo-cortical and retinotectal pathways that can bypass both the basal ganglia and frontal cortex. As such, automatic saccades should not be altered by the pathophysiology found in HD. However, previous studies have found deficits in the initiation of automatic saccades in HD, such as longer reaction times and increased duration of saccades (Lasker et al. 1987; Lasker and Zee 1996; Winograd-Gurvich et al. 2003).

Figure 3.2. Saccade paradigms: A. Immediate pro-saccade task. B. Immediate anti-saccade task. C. In the overlap condition, the central FP remained on when the S appeared. D. In the gap condition, the FP disappeared 200ms before the appearance of the S. E. In the delayed version of the pro-/anti-saccade task, the S appeared while the FP remained illuminated and subjects were instructed to refrain from initiating a saccade until FP disappearance. F. In the delayed memory-guided sequential saccade task, three stimuli (S1, S2, S3) were presented sequentially for 100 ms each in three of the four quadrants of the visual field. Subjects were instructed to move their eyes after FP disappearance to the remembered location of each S in the correct order of their appearance. FP = fixation point; S = stimulus; SRT = saccadic reaction time.
Voluntary (goal-driven) saccades are made on command, even in the absence of an overt triggering stimulus. Examples of tasks that require voluntary saccades include the anti-saccade task (Fig. 3.2B), delayed saccade task (Fig. 3.2E), or memory-guided sequential saccade task (Fig. 3.2F). In all of these tasks, subjects are required to first suppress a saccade toward the stimulus light when it appears, and then generate a voluntary saccade. The basal ganglia and frontal cortex are critical for voluntary saccade control (Gaymard et al. 1998; Hikosaka and Wurtz 1985b; Munoz and Everling 2004). As expected, deficits of saccadic suppression and voluntary saccade initiation have been demonstrated in HD patients (Blekher et al. 2004; Blekher et al. 2006; Bollen et al. 1986; Lasker et al. 1987; Lasker and Zee 1996; Leigh et al. 1993; Tian et al. 1991; Winograd-Gurvich et al. 2003).

The motor impairments seen early in HD (O’Walker 2007) are suggestive of a problem in voluntary motor control, likely resulting from the initial degeneration of striatal efferents to the external Globus Pallidus (GP_e; indirect pathway) of the basal ganglia (Crossman et al. 1988; Jackson and Crossman 1984; Mitchell et al. 1989). Impairments in suppressing unwanted saccadic eye movements in HD patients may result from reduced inhibitory outflow from the basal ganglia to the superior colliculus (Fig. 3.1B). As the disease progresses, the choreic movements generally tend to diminish and are replaced by increased rigidity and bradykinesia -- perhaps the result of atrophy spreading to the direct pathway (Globus Pallidus internal (GP_i) efferents), occurring at a later stage (Storey and Beal 1993). If the direct pathway is compromised, decreased excitation from thalamus to cortex and increased inhibitory projection to the superior colliculus will likely lead to difficulties initiating saccades.
To investigate the symptomatic changes in motor control seen in early versus late stages of HD, we first examined both automatic and voluntary oculomotor behaviors. It is important to determine how the saccadic impairments in HD change as the disease progresses in order to further understand the patterns of atrophy in HD. We hypothesize that neural degeneration occurring in HD will lead to specific deficits in the generation of both voluntary and automatic saccades, and that these deficits will increase with worsening disease severity, possibly due to alterations in the direct and indirect pathways of the basal ganglia (Fig. 3.1). Clinically, changes in the pattern of deficits in saccadic eye movement tasks may provide a reliable indicator of HD progression.

3.3 Methods

3.3.1 Subjects

All experimental procedures were reviewed and approved by the Queen’s University Human Research Ethics Board. Nine patients with Huntington’s disease (HD) aged 37 - 69 years participated in this study, along with nine age- and sex-matched controls (Table 3.1). The HD subjects met clinical criteria for the diagnosis of HD based on a genetic test, and were referred by a neurologist (co-author G. P.). All subjects provided informed consent and were compensated for their participation. Control and HD participants were not asked to interrupt their medications (see Table 3.1) during the recording sessions. Subjects wore corrective lenses if needed throughout all experiments.
<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Sex</th>
<th>UHDRS (Motor/Functional)</th>
<th>Co-morbid Symptoms</th>
<th>Medication</th>
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</table>

M = male; F = female  * Patient 9 did not complete the memory guided sequential saccade task

Table 3.1. Subject information.

HD subjects were scored on the motor and functional checklist sub-components of the Unified Huntington’s Disease Rating Scale (UHDRS) to assess disease progression (Huntington Study Group 1996). The motor assessment was performed by a neurologist (co-author G. P.). The motor component has standardized ratings of oculomotor function, dysarthria, chorea, dystonia, gait, and postural stability. The total motor impairment score is the sum of all the individual motor ratings. Higher scores on the motor scale indicate more severe motor impairment. The functional assessment was carried out by an experimenter (co-author A.H). It
includes a checklist regarding the subject’s ability to perform common daily activities. Higher scores on the functional scale are also indicative of more severe impairment.

3.3.2 Experimental paradigms

The experiments were conducted in two separate 45 minute sessions and were identical to those described previously (Chan et al. 2005; LeVasseur et al. 2001). In the first session, subjects performed one block of an immediate pro-saccade task (120 trials), two blocks of an immediate anti-saccade task (120 trials per block), and one block of a randomly interleaved delayed pro-/anti-saccade task (96 trials). Task instruction (pro-saccade or anti-saccade) was indicated by the color of the central fixation point (FP). In the second session conducted 1 - 4 days later, subjects performed 96 trials of a delayed memory-guided sequential saccade task. In all tasks, subjects were given approximately 20 practice trials.

Immediate and delayed pro- and anti-saccade tasks

Subjects were seated alone in a dark room 100 cm away from a translucent visual screen. Visual stimuli consisted of red and green light emitting diodes (LEDs). The FP and peripheral stimuli were produced by LEDs (red central FP = 2.0 cd/m²; green central FP = 1.0 cd/m²; red peripheral stimuli = 5.0 cd/m²). Between trials, the screen was diffusely illuminated with background illumination (1.0 cd/m²) to reduce dark adaptation. In the immediate pro-saccade task (Fig. 3.2A), subjects were instructed to look towards the peripheral stimulus as soon as it appeared. In the immediate anti-saccade task (Fig. 3.2B), participants were instructed to look in the opposite direction of the stimulus as soon as it appeared. Each trial began when the background illumination was turned off and after 250 ms of complete darkness, the FP appeared.
After 1000 ms, one of two events occurred depending on the trial condition. In an ‘overlap’ condition (Fig. 3.2C), the FP remained illuminated while a stimulus appeared 20° to the left or right, and in a ‘gap’ condition (Fig. 3.2D), the FP disappeared for 200 ms before the peripheral stimulus appeared. In both conditions the stimulus remained illuminated for 1000 ms, after which all LEDs were turned off and the background illumination came on for 500 ms. Stimulus location (left, right) and fixation condition (gap, overlap) were randomly interleaved within a block of trials.

In the delayed pro-/anti-saccade task (Fig. 3.2E) the pro- and anti-saccade trials were randomly interleaved within a block of trials. The color of the central FP provided the instruction for either a pro-saccade (red FP) or anti-saccade (green FP). Participants were instructed to delay any eye movement until the disappearance of the FP, which occurred at a randomized time (200, 400, 600, 800 or 1000 ms) after stimulus appearance. All other experimental details were in concordance with the above description.

**Delayed memory-guided sequential saccade task**

Eight HD subjects completed the task in session 2. Subject 9 did not return for this session. The delayed memory-guided sequential saccade task (Fig. 3.2F) was performed in a different laboratory and subjects were seated 60 cm from a computer screen. Stimuli were presented on a viewSonic 17PS monitor using an S3 VGA card. The visual display had a resolution of 640 X 480 pixels, refreshed at 60Hz. Subjects initiated the trials with a button press, and each trial subsequently began with the appearance of a central white FP on a black background. Participants were instructed to maintain fixation at the FP while three green
eccentric stimuli (S1, S2, S3) were flashed sequentially in three of the four quadrants of the visual field (100 ms each with no temporal gap between stimuli). Within each quadrant, a stimulus was flashed randomly at one of 25 preset locations, which were centred at 8° of visual angle from the FP and evenly spaced within a 5 x 5 grid that ranged from 5° of visual angle at the location nearest the FP to 11° at the location farthest from the FP. Subjects were instructed to wait until the disappearance of the FP before looking to the remembered stimulus locations in the same sequence as the stimuli appeared. The interval between the disappearance of S3 and the disappearance of the FP varied randomly (0, 600, 1200 and 1800 ms). The sequence in which the stimuli appeared and the exact stimulus location within each quadrant varied randomly between trials, and there was equal probability of the stimulus appearing in each quadrant.

3.3.3 Recording and apparatus

Immediate and delayed pro-/anti-saccade tasks

Horizontal eye movements were measured using direct current electrooculography (EOG) to obtain maximal temporal resolution. The EOG signal was amplified (Grass P18 Amplifier) and low-pass filtered (50Hz). Horizontal eye position was digitized at a rate of 1 kHz using REX, ver 5.4 (Hays et al. 1982). Digitized data were stored on a hard disk, and analyzed off line on a Sun Ultra 60 Spark station.

Delayed memory-guided sequential task

Eye position data were collected using a video-based eyetracker (Eyelink; SR Research Ltd, Toronto, Canada) that was mounted on the subject’s head (with head movements restrained
using a chin rest). The eyetracker used infrared cameras to track the movements of the pupils, measuring vertical and horizontal eye position and pupil size with a sampling rate of 240 Hz. It also provided spatial information about head position for gaze data.

3.3.4 Data analysis

Immediate and Delayed Tasks

The onset and termination of each saccade was determined when eye velocity exceeded 30°/s. Saccades were scored as correct if the first movement after stimulus appearance was in the correct direction, and if it occurred after the disappearance of the FP in the delayed saccade paradigm. Saccades were classified as direction errors if the first saccade after stimulus appearance was in the wrong direction and as timing errors if they occurred before disappearance of the FP in the delayed saccade paradigm. Combined timing-direction errors were classified as a third type of error.

In the immediate pro- and anti-saccade tasks, saccadic reaction time (SRT) was measured from the time of stimulus appearance to the onset of the first saccade. In the delayed pro-/anti-saccade task, SRT was measured from the time of FP disappearance to the onset of the first saccade. Saccades with SRTs < 90 ms were classified as anticipatory (Munoz et al. 1998) and were treated as errors. Mean SRT was computed from correct trials with latencies between 90 ms and 1000 ms.

For every subject, the following values were computed for the immediate pro- and anti-saccade task: mean SRT for correct trials, coefficient of variation of SRT (SD/mean x 100) for correct trials, and the percentage of direction errors. Each of these measures was broken down for both gap and overlap conditions. In the delayed pro-/anti-saccade task, the percentage of timing errors and combined timing-direction errors were also measured.
Saccade metrics were analyzed for correct trials in the immediate pro-saccade task only. The average number of saccades required to reach the stimulus and the amplitude of the first saccade were calculated. Peak velocity and duration were quantified for primary saccades that fell between 18 and 21° in amplitude.

**Memory-Guided Sequential Saccade Task**

The Eyelink system identified saccades when peak velocities exceeded 30°/s, acceleration was greater than 9500°/s² and there was motion greater than 0.15° from the position of fixation before saccade onset. Movement accuracy to each stimulus was measured by calculating the distance between each stimulus location and the closest eye fixation. Eye movement sequences not executed in the same order as stimulus sequences were classified as sequence errors. Eye movements occurring prior to disappearance of the FP were classified as timing errors. These movements were further analyzed to determine the direction and timing of the first saccade in which each timing error was made. The percentage of timing and sequence errors was calculated for each subject.

For all tasks, the appropriate statistical corrections for heterogeneity and sphericity of variance were made as needed (Greenhouse-Geisser). Mixed design ANOVA’s were used to compare the results from all HD subjects with all age- and sex-matched controls. The variables used in all tasks to carry out the ANOVAs were subject group (HD vs. controls) and either stimulus delay (0, 600, 1200, 1800 ms), or stimulus location (S1, S2, S3), respectively. Correlations were made by pairing an individual HD subject with their UHDRS score, where statistical significance was based on t-tests different from zero. Values for right and left stimulus positions were not significantly different (p > .05), allowing the data to be collapsed across direction for each task.
3.4 Results

Immediate Pro- and Anti-Saccade Task

3.4.1 Saccadic Reaction Time

In both pro-saccade and anti-saccade tasks, reaction times in the HD group were dramatically increased compared to controls (Fig. 3.3A). These findings are confirmed in the statistical analysis. Mean SRT was longer for HD patients than for controls across all tasks and conditions (F(1, 16) = 26.02, p < .01). Mean SRT for pro-saccades was shorter than for anti-saccades (the anti-effect (anti SRT - pro SRT); F(1, 16) = 9.36, p < .01), however, there was no interaction between subject group and experimental task (F(1,16) = .347, p = .56); the anti-effect was present for both control and HD subjects. Mean SRTs were shorter in gap trials than in overlap trials (the gap effect; F(1, 16) = 9.32, p < .01). A planned comparison revealed that this gap effect was largely due to the control subjects in both the pro-saccade task (t(8) = 5.539, p < .01) and the anti-saccade task (t(8) = 4.52, p < .01). Among HD subjects, the gap effect was significant only in the pro-saccade task (t(8) = 2.32, p < .05).
Figure 3.3. Immediate pro- and anti-saccade task results in controls (diamond) and HD subjects (squares), individually plotted (horizontal bar = mean) across task (pro-/anti) and condition (gap/overlap). A. SRT. B. Coefficient of variation of SRT (standard deviation/mean x 100). C. Percent direction errors (erroneous pro-saccades). * HD - control, p < .05.

3.4.2 Coefficient of Variation

Intra-subject variability in SRT was analyzed using the coefficient of variation (CV). This measure is a unitless quantity that indicates the variability around the mean in relation to the size of the mean. CV was elevated in HD subjects compared to controls across all tasks and conditions (Fig. 3.3B), \(F(1, 16) = 23.73, p < .01\). In addition, HD subjects had slightly higher CV in the gap task than in the overlap task \(F(1, 16) = 13.39, p < .05\).
3.4.3 Direction Errors

HD subjects made many more direction errors than control subjects (Fig. 3.3C) \( F(1,16) = 17.45, p < .01 \). More direction errors occurred in the anti-saccade task than in the pro-saccade task \( F(1, 16) = 27.07, p < .001 \). Upon closer inspection, the increase in direction errors from the pro- to the anti-saccade task was considerably larger for HD subjects than for controls, resulting in a group x task interaction \( F(1, 16) = 14.83, p < .01 \). Post-hoc analysis indicates that in the anti-saccade task, HD subjects performed with a higher proportion of errors than controls in both gap \( (t(8.56) = 3.94, p < .01) \) and overlap \( (t(8.475) = 4.14, p < .01) \) conditions.

3.4.4 Saccade Metrics

Saccade metrics were analyzed for the immediate pro-saccade task only by comparing subject group (HD vs. control) and condition (gap vs. overlap). Measurements were based on the first saccade made after the appearance of the stimulus during correct trials only. The first saccade to stimulus amongst HD subjects was slightly smaller in amplitude \( (18.05^\circ \pm 0.51) \) compared to control subjects \( (19.33^\circ \pm 0.20) \), \( F(1, 8) = 4.77, p = .06 \). There were no differences between HD subjects \( (1.29 \pm 0.07) \) and controls \( (1.19 \pm 0.05) \) in the mean number of saccades required to reach the stimulus \( F(1, 8) = 1.53, p > 0.2 \). Among saccades that were 18-21° in amplitude, HD subjects made saccades that were of a longer duration \( (134 \pm 14 \text{ ms}) \), \( F(1, 8) = 9.55, p < .02 \) and lower peak saccadic velocity \( (316 \pm 26 \, ^\circ/\text{s}) \), \( F(1, 8) = 7.14, p < .03 \) compared to control subjects \( (90 \pm 4 \text{ ms}; 391 \pm 22 \, ^\circ/\text{s}) \).
Delayed Pro- and Anti-Saccade Task

3.4.5 Saccadic Reaction Time

In the delayed tasks, mean SRT was increased amongst HD subjects when compared to controls ($F(1, 8) = 4.89, p < .05$); however, no group x task interaction was noted. In other words, although HD subjects had slower SRTs than controls, this did not vary as a function of task type, as it did in the immediate saccade task. To investigate this further a post hoc pairwise comparison revealed that only control subjects demonstrated the anti-effect in the delayed tasks ($t(8) = 5.39, p = 0.001$).

3.4.6 Errors

There were several parameters of performance in the delayed pro- and anti-saccade tasks that were impaired in HD subjects. Figures 3.4A and B show that the percentage of incorrect trials was dramatically elevated in HD patients at all delay periods, for both pro- and anti-saccades, ($F(1, 16) = 32.40, p < .001$), and the overall percentage of incorrect trials was greater in the anti-saccade trials than in pro-saccade trials ($F(1, 8) = 6.56, p < .05$). However, in both pro- and anti-saccade tasks, the number of errors increased as the delay interval increased systematically from 200ms – 1000ms ($F(2, 16) = 11.28, p < .01$). In addition, HD patients made more errors in the anti-saccade task than in the pro-saccade task, a difference not observed in controls (group x task interaction; $F(1, 16) = 8.12, p < .05$).
Errors were then segregated into three types to examine the ability of HD patients to delay saccade responses: timing errors (saccades made in the correct direction, but before disappearance of the FP); direction errors (correctly delayed saccades, but in the wrong direction); and combined timing-direction errors (saccades made in the wrong direction before disappearance of the FP) (Fig. 3.4 C,D). Patients with HD made proportionally more timing errors ($F(1, 16) = 9.40, p < .01$), direction errors ($F(1, 16) = 9.33, p < .01$), and combined timing-direction errors (Fig. 3.4C,D; $F(1, 16) = 33.40, p < .001$). More direction errors ($F(1,16) = 9.23, p < .05$) and combined timing-direction errors ($F(1, 16) = 20.17, p < .001$) were made on anti-
saccade trials, and more timing errors were made on pro-saccade trials \( F(1, 16) = 16.83, p < .01 \). All types of errors were affected by delay duration in both controls and HD patients. More specifically, as the delay interval increased, both the percentage of timing errors \( F(2, 16) = 18.53, p < .001 \) and combined timing-direction errors increased \( F(3, 16) = 9.85, p < .001 \). However, the opposite relationship was observed for direction errors. As the delay interval increased, the percentage of direction errors decreased \( F(3, 16) = 20.08, p < .001 \). In addition, the increase in error rate in the anti-saccade task compared to the pro-saccade to was greater for HD subjects than for control subjects \( F(1, 16) = 8.12, p < .05 \).

Because HD patients had profound difficulty delaying responses, we examined whether HD patients could delay their responses at all over long periods of time. To do this, cumulative distributions of SRTs were constructed for both control and HD subjects from correct trials of the immediate pro-saccade task (overlap condition) and timing errors in the delayed pro-saccade trials in which the delay interval was greater than or equal to 600ms (Fig. 3.5). In these two conditions, the stimulus display was identical -- the FP and the stimulus were both visible during the 600ms period shown, and only the task instruction differed. If no timing errors were made in the delayed pro-saccade task, the cumulative distribution of SRTs in the first 600ms of the delay period would be flat (i.e., at zero until after 600ms). If the subjects had absolutely no ability to delay their eye movements, the curve for the delayed pro-saccade trials should be indistinguishable from that produced in the immediate pro-overlap task, where the subject was instructed to make a pro-saccade immediately upon the appearance of the stimulus.
The dotted lines in Fig. 3.5 reveal that control subjects made nearly 100% of their saccades by 600 ms in the immediate pro-overlap task, and suppressed nearly all saccades (~90%) in the pro-delayed task until after FP disappearance (i.e., >600 ms). The area between the two dotted curves in Fig. 3.5 provides a measure of the ability of control subjects to follow the task instruction and delay their response. HD subjects were significantly impaired in their ability to delay, as can be seen by the much smaller shaded area between the two solid curves. Thus, although the HD subjects did have some ability to suppress eye movements until the disappearance of the FP (i.e., shaded region between solid lines in Fig. 3.5); they were dramatically impaired relative to controls.

Figure 3.5. Cumulative distributions of SRT in the immediate pro-overlap trials (correct responses) and the delayed pro-saccade trials (timing errors) with a delay of 600ms of greater. Solid lines = HD data; dotted lines = control data.
Delayed Memory-Guided Sequential Saccade Task

The delayed memory-guided sequential saccade task was used to assess saccadic suppression and spatial working memory. HD subjects produced considerably fewer correct trials than control subjects ($t(8) = 4.463, p < .01$) in which the correct three-stimulus sequence and proper maintenance of fixation (until FP disappearance) were accomplished (Fig. 3.6A). HD subjects also made more timing errors than control subjects ($t(7) = 3.82, p < .01$). HD patients appeared to make more sequence errors than control subjects (not significant; $t(14) = 1.663, p = .12$), and did make more combined timing-sequence errors than control subjects ($t(8) = 4.01, p = .003$).

Movement inaccuracy was assessed by calculating the visual angle between each stimulus and the closest eye fixation, and plotted as degrees of displacement from the stimulus. Among all the trials (Fig. 3.6B), control subjects fixated significantly closer to the stimuli ($3.0 \pm 0.3^\circ$) than HD patients ($6.8 \pm 1.0^\circ$) ($F(1, 6) = 15.49, p < .01$). Similarly, control subjects ($2.9 \pm 0.3^\circ$) moved closer to stimuli than HD subjects ($6.2 \pm 0.8^\circ$) on the subset of trials in which only correctly delayed saccades were analyzed (Fig. 6C) ($F(1, 7) = 20.99, p < .01$). Finally, on the subset of trials that included only correct timing and correct sequence (Fig. 3.6D), a similar trend was observed ($F(1, 5) = 5.48, p = 0.07$). Note that two HD patients had zero trials in which they maintained fixation until the FP disappeared and carried out the correct sequence. Consequently, only six HD subjects were included in this analysis, reducing statistical power.
Figure 3.6. Delayed memory-guided sequential saccade task results. A. Percent of correct and incorrect trials (± standard error) for HD (filled bars) and control (empty bars) subjects. Mean inaccuracy of saccades (± standard error) to the remembered location of the first, second and third stimuli in the sequence for (B) all 96 trials, (C) the subset of trials in which subjects did not make an anticipatory error (i.e., maintained proper fixation), and (D) those trials in which subjects maintained proper fixation and completed the correct sequential movement to the three stimuli. E. Direction of the first stimulus attended when making an anticipatory timing error (i.e., percent of anticipation trials that subjects first attended each of the three stimuli (± standard error)). Squares = HD data; triangles = control data. * HD - control, p < .05.

Errors during the delay period may distinguish between an inability to suppress a movement to the most recently presented eccentric sensory stimulus or an inability to suppress a planned motor sequence. If subjects were unable to suppress movement to the most recently
presented visual stimulus during the delay interval, then timing errors should be directed more often to the last of the successive flashes. In contrast, if subjects were unable to suppress the appropriate motor plan, the first saccade of the timing error would be directed to the location of the first stimulus. Figure 3.6E reveals that both control and HD subjects tended to make their first saccade to the first stimulus (S1) more often ($F(1, 9) = 32.78, p < .001$). However, patients with HD made saccades to S1 (48.1 ± 4.3%) less often than control subjects and then showed a gradual decline in the proportion of errors to the remaining stimuli, such that in HD, performance to S1 differed from S2 ($t(7) = 4.54, p = .003$) and S3 ($t(7) = 4.18, p = .002$) whereas control subjects moved at S1 significantly more (71.1 ± 8.5%) than to S2 or S3, with no difference of probability of movement between these later points ($F(1, 8) = 9.49, p < .01$). This suggests that timing errors resulted from a failure to suppress a planned motor program. Confirming this, no difference was observed between the mean SRT of control and HD subjects ($F(1, 8) = 0.21, p > 0.5$), or between the mean SRT of saccades to each of the three stimuli ($F(2, 21) = .44, p = .65$).

3.5 Correlations to Disease Severity

Identification of a simple behavioural measure that correlates with disease progression may be useful to track clinical changes in HD patients and to predict pre-symptomatic disease onset. Several of the saccadic parameters we measured correlated with measures of disease severity (Table 3.2), as assessed by motor and functional subcomponents of the UHDRS. Figure 3.7B and D illustrate that as disease severity worsened, HD subjects showed more variability of their SRTs (Table 3.2, $p < .01$). The frequency of direction errors correlated with disease severity in both the immediate pro- ($p < .05$) and anti-saccade tasks (Fig. 3.7A, C and Table 3.2; $p < .01$). In the delayed anti-saccade task, the rate of combined timing-direction errors strongly correlated
with disease severity (Table 3.2; p < .01). In the delayed memory-guided saccade task, the percentage of total errors increased with disease severity (Table 3.2), however, accuracy reaching the stimuli did not correlate with disease progression (Table 3.2, p < .05). Of the saccade metrics, saccade amplitude and duration in HD subjects were correlated with disease severity (Table 3.2, p < .05). Mean SRT in HD subjects did not correlate with disease severity, regardless of task (Table 3.2, p > .05).

**Figure 3.7.** Mean correlations between saccade measures and disease severity in HD subjects, as assessed by the Unified Huntington’s Disease Rating Scale (UHDRS). Direction errors (A, C) and coefficient of variation (B, D) correlating with the motor (A, B) and functional scores (C, D) of the UHDRS are shown for both the pro-saccade task (solid lines), and the anti-saccade task (dashed lines). See Table 3 for values.
### Table 3.2. Correlations (Pearson r values) between various measures of saccadic behaviors and combined motor and functional assessment scores in individuals with HD.

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<td>.111</td>
</tr>
<tr>
<td>Percent combined</td>
<td>.556</td>
<td>.930**</td>
</tr>
<tr>
<td><strong>DELAYED MEMORY-GUIDED SEQUENTIAL SACCADE TASK</strong></td>
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<tr>
<td>Percent correct</td>
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<td>.415</td>
</tr>
<tr>
<td>Total % errors</td>
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<td>.930**</td>
</tr>
<tr>
<td><strong>SACCADE METRICS</strong></td>
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<tr>
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<td></td>
</tr>
<tr>
<td>Duration</td>
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<td></td>
</tr>
<tr>
<td>Velocity</td>
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</tr>
</tbody>
</table>

(* = p < .05;  ** = p < .01)

#### 3.6 Discussion

We examined impairments in saccadic eye movements amongst individuals with HD and we identified multiple deficits in automatic and voluntary saccade control. Consistent with previous studies (Blekher et al. 2004; Blekher et al. 2006; Lasker et al. 1987; Leigh et al. 1983; Tian et al. 1991), we have confirmed that HD subjects have the following eye movement abnormalities: 1) Difficulties initiating voluntary saccades, identified as increased SRT in the immediate (Fig. 3.4) and delayed pro- and anti-saccade tasks. 2) Reduced ability to suppress inappropriate automatic saccades (Figs. 3.3-3.6). 3) An inability to hold and execute complex motor plans involving spatial working memory (Fig. 3.6). 4) Atypical saccade metrics. 5) Correlations between voluntary saccade measures and HD disease severity. We discuss the
implications of these findings in relation to known pathophysiology in HD patients and their relation to disease severity.

### 3.6.1 Relation to Brain Pathology

Based on the evidence presented above, the view commonly held that HD is a disorder involving primarily the indirect pathway of the basal ganglia (i.e., the pathway responsible for suppressing saccades; see Fig. 1B), is incomplete. Although previous research has suggested that most of the atrophy in HD is localized to the indirect pathway (Albin et al. 1990; Johnson et al. 2001; Reiner et al. 1998; Vonsattel et al. 1985), with relative sparing of the direct pathway (i.e., damage to which would likely result in increased inhibition to the superior colliculus, and difficulty initiating/generating saccades), the results from the present study, along with anatomical and physiological evidence (Storey and Beal 1993) suggest that this differentiation of the pathology is an oversimplification of the circuit. In its later stages HD may also be associated with cell loss in areas that receive basal ganglia outputs, including the thalamus, subthalamic nucleus, substantia nigra, cerebellum, cortex, and brainstem (Johnson et al. 2001; Kassubek et al. 2005; Macmillan and Quarrel 1996). We have shown deficits in tasks requiring the generation of both automatic and voluntary saccades, albeit greater deficits seen in tasks requiring saccadic suppression (e.g., anti-saccades, delayed saccades, and memory-guided saccades -- all of which elicited performance changes correlated with disease severity). Considering that suppression deficits correlate with disease severity (Fig. 3.7, Table 3.2), and initiation deficits do not (Table 3.2), it can be inferred that the areas in the basal ganglia involved in suppressing unwanted saccades are more susceptible to the pathological changes that occur as disease severity worsens. This parallels other findings (Blekher et al. 2006) wherein similar anti-saccade and memory-
guided saccade tasks were most effective in differentiating impairments in pre-diagnostic HD gene carriers versus nongene carriers. However, the memory-guided saccade task that we employed required subjects to remember the location of three stimuli that could appear anywhere within three of the four visual quadrants (each with 25 different locations), a task more challenging than those previously employed (Blekher et al. 2006; Lasker et al. 1987; Lasker et al. 1988). Consequently, deficits were much more pronounced; recall that two HD patients were unable to perform a single correct trial. Taken together, our findings support prior pathophysiological findings, which suggest that although the striatal-globus pallidus (external) portion of the indirect pathway in the basal ganglia is where degeneration initially occurs, the direct pathway is also compromised in HD, perhaps at a later stage of disease progression (Albin et al. 1990; Berardelli et al. 1999; Storey and Beal 1993). Both localized and widespread cell loss in HD patients would eventually lead to deficits in generation of both automatic and voluntary saccades, as demonstrated in this study.

The pattern of neural degeneration in HD and how it affects saccadic control can be summarized as follows. First, supporting evidence from electrophysiological and lesion studies suggests that the frontal (Everling and Munoz 2000; Gaymard et al. 1998) and supplementary (Amador et al. 2004) eye fields play an important role in the execution of voluntary saccades, both of which have projections through the basal ganglia. Therefore, we would expect that altered input to one or all of these structures would make it more difficult to excite the superior colliculus and initiate a voluntary saccade. Secondly, it is suggested that frontal lesions may be implicated in saccade initiation deficits (Tian et al. 1991), or that abnormalities in the substantia nigra (pars reticulata), superior colliculus, or the brainstem itself cause the elevated latencies in automatic saccades (Hikosaka and Wurtz 1985a; Hikosaka and Wurtz, 1985b). Reduced excitability from
the direct pathway of the basal ganglia to the saccade generating circuit (via compromised frontal structures) could lead to reduced ability to initiate movement. Alternatively, increased sensory-processing delays are also known to occur in HD subjects (as assessed by auditory and visual event-related potentials (Goodin and Aminoff 1992)), which may also slow saccadic reaction times. Thirdly, the prefrontal cortex has been implicated in executive functions such as spatial working memory (Miller and Cohen 2001), and is critical for suppression of erroneous automatic saccades in the anti-saccade task (Condy et al. 2007; Guitton et al. 1985; Pierrot-Deseilligny et al. 1991). Damage to the frontal cortex may also lead to decreased inhibition of the superior colliculus (via the basal ganglia or the direct projection from cortex to the subthalamic nucleus (Hikosaka et al. 2000)), which can influence saccadic control (i.e., intra-subject variability), and the ability to generate and execute a correct motor plan (via a global over-excitation of the saccade generating circuit). Finally, changes in saccade metrics can also be accounted for by changes occurring in the basal ganglia and frontal cortex. Control of saccade amplitude has been associated with a feedback loop through the basal ganglia to the superior colliculus (Kimmig et al. 2002), which is perhaps responsible for the decreased amplitude in saccades of patients with Parkinson’s disease (Chan et al. 2005). However, the specific pattern of degeneration in the basal ganglia of HD patients instead influenced the velocity and duration of saccades. This region-specific atrophy in the basal ganglia and frontal cortex may account for the progressively more hypometric saccades seen in HD patients as the disease progresses. In addition, slowed saccade velocities are often attributed to abnormalities in the brainstem reticular formation (Leigh and Zee 1999; Scudder et al. 2002). Slowed saccades are also found in patients with lesions of frontal eye fields and superior colliculus (Gaymard et al. 1998; Pierrot-Deseilligny et al. 1991), and monkeys with reversible inactivation of the frontal eye fields (Dias and Segraves 1999; Sommer and
Tehovnik 1999) and superior colliculus (Hikosaka and Wurtz 1985; Lee et al. 1988); the major inputs to the premotor circuit in the brainstem reticular formation (Munoz 2002).

3.6.2 Relation to Disease Severity

Detection of a simple behavioral measure that correlates with functional disease progression is important to track clinical changes in HD patients and could be used as a tool in controlled clinical trials to assess improvements in motor and functional abilities during therapeutic interventions. Eye movement deficits were correlated to motor and functional capacities of HD patients, as assessed by the UHDRS (Table 3.2). A decline in HD subject’s ability to perform these saccadic eye movement tasks can provide an important index of disease progression. Most of these progressive deficits observed in HD can be linked to degeneration of the basal ganglia and the frontal lobes (Berardelli et al. 1999; Tsai et al. 1995). The ability of subjects with HD to suppress saccadic eye movements progressively worsened with the degree of disease severity (Table 3.2, Fig. 3.7) – suggesting a more rapidly advancing neural degeneration in the indirect (inhibitory) pathway of the basal ganglia and frontal cortex over the course of the disease that directly affects saccadic suppression. This is due to alterations in basal ganglia modulation (i.e., striatal atrophy leading to increased excitation to frontal cortex (via thalamus) and decreased tonic inhibitory outflow to the superior colliculus; see Fig.3.1B). This implies that worsening voluntary saccadic dysfunction provides insight into the spreading pathophysiology.

A further, perhaps speculative, implication of these findings is that because neural degeneration in HD begins in the basal ganglia, and saccadic suppression appears to be affected directly by these changes, measures of saccadic suppression specifically, may be an effective
of disease onset in pre-symptomatic HD patients. Comparable studies (Blekher et al. 2006; Smith et al. 2000) observed that fully diagnosed HD patients were more severely impaired than pre-symptomatic HD gene carriers, who were more impaired than controls in measures of movement control (oculomotor and reaching, respectively), findings similar to our study. Recall that certain oculomotor impairments we found (inability to suppress incorrect automatic saccades, and drastically reduced precision and accuracy), changed with disease severity, supporting that by the time of diagnosis, the associated neuronal degeneration has already advanced (Aylward et al. 2004). Therefore, eye movement testing – the immediate anti-saccade task in particular – may prove to be a useful early marker of symptomatic disease onset in HD.

Although many of the observations discussed above involve the basal ganglia and/or frontal cortex, we cannot be sure that these deficits solely reflect dysfunction in these structures due to the complex involvement and interaction of other brain structures in HD, many of which also experience cell loss in the later stages of the disease. However, understanding the progressive changes in oculomotor behaviours may provide future insight into the neural pathophysiology in HD. Since worsening saccade performance provides a strong indicator of disease severity in HD, saccadic eye movements could be a useful and sensitive clinical tool in assessing motor and functional changes in individuals with HD.

3.7 Acknowledgements

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3.8 References


Chapter 4
Saccade deficits in amnestic Mild Cognitive Impairment resemble mild Alzheimer’s disease

4.1 Abstract
Alzheimer’s disease (AD) is a cognitive disorder of progressive memory loss and executive dysfunction. Amnestic Mild Cognitive Impairment (aMCI) is considered a prodromal stage of AD and is characterized by isolated memory loss. However, little is known about the progression from aMCI to AD. Previous studies have found impairments in executive function (e.g., reduced inhibition and selective attention) in mild-stage AD and aMCI; however, executive dysfunction in aMCI is often missed by standardized clinical tests. Using simple eye movement tasks that probe executive function, we expected to observe similar impairments in aMCI and mild AD. We measured the ability of 26 aMCI patients (63 – 90 years old), 26 mild AD patients (61 – 87 years old), and 76 healthy elderly participants (60 – 85 years old) to perform two specific saccadic eye movement tasks: a prosaccade task (make an automatic saccade toward an eccentric stimulus) and an antisaccade task (inhibit automatic saccade and initiate a voluntary saccade to the opposite location; processes requiring good executive functioning). Antisaccade, but not prosaccade performance was selectively impaired in both patient groups relative to controls: both patient groups were slower to initiate correct antisaccades and made more direction errors (i.e., erroneous prosaccades) on antisaccade trials. aMCI and mild AD patients differed from each other only in the variability of their response times in the antisaccade task: AD patients were significantly more variable than both aMCI and healthy elderly. Importantly, aMCI patients who converted to AD within 3 years later were more likely to generate more anticipatory
saccades and direction errors in the antisaccade task than those who did not convert. Our results support the hypothesis that the effects of aMCI/AD disease pathology on executive function and thus saccade behavior may be distinguishable from that of healthy aging. The antisaccade task may also be useful for objectively detecting subtle executive dysfunction in aMCI that is not yet clinically significant using standardized neuropsychological tests.

4.2 Introduction

Patients with amnestic mild cognitive impairment (aMCI) exhibit memory deficits similar to those seen in mild Alzheimer’s disease (AD). However, unlike AD, aMCI patients do not typically exhibit deficits in executive functioning (i.e., behavioral control, cognitive flexibility, abstract thinking) on standard clinical tests (Devanand et al., 1997), but do show executive function impairment on certain psychometric tasks such as tests of perceptual speed (Bennett et al., 2002) and response inhibition (Traykov et al., 2007). Deficits in executive functions, assessed with tests such as the Wisconsin Card Sorting Task, are present in mild AD (Balota and Faust 2001; Takeda et al., 2010), and are predictive of AD development (Chen et al., 2000). This suggests frontal executive dysfunction may occur prior to AD diagnosis.

Although aMCI is thought to lead to AD in a large proportion of patients (Corbetta et al., 1998; Dubois et al., 2007; Petersen et al., 1999), it is clinically difficult to determine which aMCI patients are at risk for conversion to AD. Because the prevalence of AD is continuously rising (Smetanin et al., 2010), it is important to develop non-invasive methods that would allow for earlier detection. Here, we use saccadic eye movement tasks in aMCI and AD groups to probe executive control, which relies on various regions in the frontal cortex (Banich 2009; Miller and
If aMCI patients perform similarly to elderly controls, saccade tasks may be useful in differentiating aMCI from AD. On the other hand, if aMCI patients exhibit executive deficits similar to AD patients, saccade tasks may prove useful for identifying mild AD in aMCI patients prior to clinical manifestation.

Saccadic eye movement tasks are commonly used to assess sensory, motor, and cognitive function in neurological disease (Leigh and Kennard 2004; Munoz and Everling 2004; Munoz et al., 2007; Ramat et al., 2007) because they are non-invasive, hands/language-free, and because of the extensive knowledge of the brain circuitry controlling saccades (Hikosaka et al., 2006; Leigh and Zee 2006; Moschovakis et al., 1996; Munoz 2002; Scudder et al., 2002; Sparks 2002; Wurtz and Goldberg 1989). Eye movement control is sensitive to normal aging (Abel and Douglas 2007; Klein et al., 2000; Munoz et al., 1998; Olincy et al., 1997; Peltsch et al., 2009), and a variety of saccade deficits are distinguishable between different neurodegenerative disorders, including Parkinson’s (Briand et al., 1999, Cameron et al., 2010; Chan et al., 2005; Mosimann et al., 2005), Huntington’s (Blekher et al., 2006; Peltsch et al., 2008), Progressive Supranuclear Palsy (Chen et al., 2010; Garbutt et al., 2008), Alzheimer’s diseases (Abel et al., 2002; Garbutt et al., 2008; Shafiq-Antonacci et al., 2003), and Amyotrophic Lateral Sclerosis (Witiuk et al., 2011) because these pathologies alter different components of the eye movement circuitry controlling the behavior.

To investigate saccade characteristics in aMCI, AD, and normal aging, we employed tasks ideal for testing executive function. In the prosaccade task, subjects are asked to look towards a peripheral visual stimulus; this task involves a fast, automatic response that does not require optimal executive functions. In the antisaccade task (Hallett 1978), the presentation of
stimuli is identical, however, additional executive processing is required to perform the task: subjects are instructed to look away from the peripheral stimulus, requiring suppression of the automatic prosaccade, followed by voluntary initiation of the antisaccade to the opposite side. The ability to inhibit unwanted saccades and voluntarily initiate movement is reflective of good function of areas in the frontal oculomotor circuit (Everling and Fischer 1998; Munoz and Everling 2004).

Previous studies have reported antisaccade deficits in AD such as increased latencies, error rates, difficulty maintaining fixation, and increased variability in SRT (Carter et al., 1983; Grundman et al., 2004; Hebert et al., 2003; Kabani et al., 2002; Schewe et al., 1999). However, it is not known to what extent saccade deficits are present in aMCI, or whether such findings can distinguish aMCI from AD or from normal aging. Brain deterioration in normal aging is known to influence saccade performance (Abel and Douglas 2007; Klein et al., 2000; Munoz et al., 1998; Olincy et al., 1997; Pelsch et al., 2009; Yang and Kapoula 2006).

AD pathology in the medial temporal lobe, evident many years prior to symptom onset (Seahill et al., 2002; Smith 2002), may influence frontal function due to loss of input from the medial temporal areas (Smith 2002). Because mild AD pathology affects frontal regions (Buckner 2004; Rabinovici et al., 2007), and frontal executive functions are impaired in mild AD (Balota and Faust 2001; Takeda et al., 2010), it is expected that AD patients will exhibit impaired voluntary saccade control, especially in the antisaccade task. We also expect that the similar brain and behavioral changes seen in aMCI (Morris et al., 2001) will elicit subtle but similar alterations in antisaccade performance in aMCI patients, despite maintaining good executive function in some standard clinical tests. Our goal here is to determine if the antisaccade task can measure
executive dysfunction in aMCI patients that may aid in the future tracking of executive impairments and conversion to AD.

4.3 Methods

4.3.1 Subjects

All experimental procedures were reviewed and approved by the Queen’s University Human Research Ethics Board in accordance with the Tri Council Policy. aMCI patients (N = 26; ages 63-90; 46% male), and mild AD patients (N = 26; ages 61-87; 38% male) were recruited by co-author AG into this study. Patients with AD or aMCI were diagnosed according to NINCDS-ADRDA (McKhann et al., 1984) or Petersen’s criteria (Petersen et al., 1999), respectively, by a geriatrician (co-author AG). Elderly controls (N = 76; ages 60-85; 30% male), were recruited via posters or often were patient spouses. All subjects reported no visual or neurological symptoms other than aMCI or AD, and had normal or corrected to normal vision. In order to reduce contamination of the control sample by cases of preclinical AD, controls underwent the same rigorous neuropsychological testing (see 4.3.2) under direct supervision of co-author AG. The experiment was conducted in one 120 minute session in which subjects performed a battery of neuropsychological tests followed by the two saccade tasks.

4.3.2 Neuropsychological testing

Neuropsychological assessment consisted of both screening tests and tests specifically designed to assess frontal function. Tests included: the Mini Mental Status Examination (MMSE, (Folstein et al., 1975)); the Mattis Dementia Rating Scale (DRS, (Mattis 1988)), a test designed
for the detection of dementia, that includes sub-scores in the areas of Attention, Initiation/Perseveration, Construction, Conceptualization, and Memory (the combination of the sub-scores of memory and initiation, correctly classifies 98% of the subjects (Monsch et al., 1995)); the California Verbal Learning Test (CVLT, (Delis et al., 2000)), a verbal memory and learning test that compiles 27 outcomes that can be reduced to 3 by factor analysis. The CVLT has been standardized by age, sex, and years of education providing an excellent tool for accurate testing and scoring of verbal memory. Tests of executive function included: the Stroop Neuropsychological Screening Inventory Test (Stroop, (Stroop 1935)), a measure of focused attention and concentration in the face of interference; and the Wisconsin Card Sorting Task (WCST, (Heaton et al., 1991)), a measure of executive function requiring the ability to maintain appropriate problem-solving strategies across changing conditions. Scores from these tests were part of another study and thus only the test averages for each experimental group are presented in Table 1. Control subjects were excluded if they scored less than 26 on the MMSE, less than -1.5 SD below the mean in recall measures of the CVLT or less than 127/144 on the DRS. The average scores of these test scores for controls, aMCI, and AD patients can be found in Table 4.1. As expected by inclusion criteria, patients with aMCI scores were worse than in normal controls, and scores from mild AD patients were worse than in aMCI patients. Full analysis of these data is part of a separate study.
<table>
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<th>STROOP</th>
<th>CVLT</th>
<th>DRS</th>
<th>WISCONSIN (%)</th>
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</tr>
<tr>
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<td>58 ± 26</td>
<td>3 ± 4</td>
<td>125 ± 9</td>
<td>41 ± 11</td>
</tr>
</tbody>
</table>

**Table 4.1.** Psychometric test scores: Mean test results ± standard deviation for psychometric tests categorized by experimental group. N = number of subjects with valid scores on at least the MMSE; MMSE = Mini Mental Status Examination, total score out of 30; STROOP = Stroop task, number of errors, CVLT = California Verbal Learning Test, long-delay free recall; DRS = Dementia Rating Scale, total score out of 144; WISCONSIN = Wisconsin Card Sorting Task, percentage of errors.

### 4.3.3 Saccade paradigm

Subjects performed one block of the prosaccade task (120 trials; Fig. 4.1A), and two blocks of the antisaccade task (120 trials per block; Fig. 4.1B). Subjects were seated alone in a dark room 100 cm away from a translucent visual screen. Visual stimuli consisted of red light emitting diodes (LEDs; central fixation point = 2.0 cd/m²; peripheral stimuli = 5.0 cd/m²). The visual screen was diffusely illuminated (1.0 cd/m²) between trials to reduce dark adaptation. Each trial began when the background illumination was turned off and after 250 ms of complete darkness, the fixation point (FP) appeared. After 1000 ms, one of two possible events occurred depending on the trial condition. In the ‘overlap’ condition, the FP remained illuminated while a stimulus (S) appeared 20º left or right (Fig. 4.1C), while in the ‘gap’ condition, the FP disappeared for 200 ms before the peripheral stimulus appeared (Fig. 4.1D).
Introducing a gap period between disappearance of the fixation point and appearance of the stimulus serves to reduce reaction times (Dorris and Munoz 1995; Saslow 1967), and the difference between gap and overlap SRTs is used as a measure of automatic saccade control (known as the ‘gap-effect’). In both conditions the stimulus remained illuminated for 1000 ms, after which all LEDs were turned off and the background illumination came on for 500 ms.

Figure 4.1. The prosaccade paradigm (A) and the antisaccade paradigm (B) with time courses for both overlap (C) and gap conditions (D), including individual saccade traces in the gap condition for a representative 75 year old elderly control (E), a 76 year old aMCI patient (F), and a 75 year old AD patient (G). Solid lines = correct saccades, dotted lines = direction errors. S = stimulus, FP = fixation point.
 Stimulus location (left, right) and fixation condition (gap, overlap) were randomly interleaved within a block of trials. In the prosaccade task (Fig. 4.1A), subjects were instructed to look towards the peripheral stimulus as soon as it appeared. During the blocks of antisaccade trials (Fig. 4.1B), subjects were instructed to look in the opposite direction of the stimulus as soon as it appeared. The difference between pro and antisaccade reaction times (known as the ‘anti-effect’) provides a measure of the time it takes for additional antisaccade processes – the inhibition of a stimulus-driven saccade and the voluntary initiation of the correct saccade.

4.3.4 Recording and apparatus

Horizontal eye movements were measured using DC-electrooculography (EOG). The EOG signal was amplified (Grass P18 Amplifier) and low-pass filtered (50Hz). The experiment was controlled with REX, ver 5.4 (Hays et al., 1982) and horizontal eye position was digitized at a rate of 1 kHz, consistent with our previous database. Subjects wore two horizontal eye electrodes and a forehead ground electrode for ten minutes prior to beginning the experiment to minimize drift. Each subject was also asked to shift their eyes between peripheral and central stimulus locations in order to calibrate the EOG signal. We then set the EOG amplification to 1 Volt = 10°. Within this range (± 20°) the horizontal eye position signal remained linear (Goldring et al., 1996) and the noise remained < 2°. Digitized data were stored on a hard disk, and analyzed off-line.

4.3.5 Data analysis

The onset and termination of each saccade was determined when eye velocity exceeded 30°/s. Trials were scored as correct if the first saccade after stimulus appearance was in the
correct direction. For this study, multi-step saccades were not categorized separately. Trials were classified as direction errors if the first saccade after stimulus appearance was in the wrong direction. Saccadic reaction time (SRT) was measured from stimulus appearance to onset of the first saccade.

Saccades initiated occurring prior to when visual stimulus information was able to reach the oculomotor brain regions (Bell et al., 2006; Schmolesky et al., 1998) were categorized as anticipatory saccades, because they were equally likely to be correct or incorrect. In our data, saccades with SRTs < 100 ms were classified as anticipatory and analyzed separately (Fig. 4.2, lighter shaded bars). Short-latency stimulus-driven saccades within the first peak of a multimodal distribution of SRTs are traditionally identified as express saccades (Fischer and Boch 1983; Fischer et al., 1997). However, saccades with latencies within this range vary according to stimulus intensity (Bell et al., 2006), contrast (Carpenter 2004; White et al., 2006), and even age (Pelsch et al., 2009). Due to the task-specific variability of SRTs within this epoch, we refer to these saccades as short-latency saccades, using both time and bimodality to define them. We used antisaccade direction error latencies (erroneous stimulus-driven prosaccades) in combination with correct prosaccade latencies to help identify the short latency saccade epoch (see (Pelsch et al., 2009) for review). A binomial sign test determined the start and end of the short latency saccade epoch by measuring when the proportion of antisaccade error trials (in 10 ms bins) significantly exceeded that of correct antisaccade trials for each experimental group (Fig. 4.2, darker shaded bars) and averaged between gap and overlap conditions.
Figure 4.2. Distribution of SRTs for each experimental group in the prosaccade task (A – F), and the antisaccade task (G – L), showing both gap and overlap conditions. Correct responses are on the positive ordinate; incorrect responses are on the negative ordinate. Lighter grey shaded boxes represent calculated range of anticipatory saccades (0 – 100 ms); darker grey shaded boxes represent range of calculated short latency saccades (100 – 200 ms) based on the antisaccade error latencies.
For each subject, the following values were computed for pro and antisaccade trials with latencies from 100 to 1000 ms (for both gap and overlap conditions): mean SRT and coefficient of variation of SRT (SD/mean x 100) for all correct trials, percentage of anticipatory saccades, percentage of short latency saccades, and the percentage of direction errors (erroneous prosaccades to the stimulus in the antisaccade task). In controls, the anticipatory epoch ranged from 0 – 100 ms (Fig. 4.2; lighter grey shaded bars), and the short latency saccade epoch ranged from 100 – 200 ms (Fig. 4.2; darker grey shaded bars). These normative epochs were used to determine the percentage of short latency saccades in all experimental groups. However, we also computed group-specific short-latency saccade epochs using the binomial sign test as described above to assess changes in the time needed to process the visual transient and make the correct response in each experimental group.

4.3.6 Statistical analysis

For all tasks, the appropriate statistical corrections for heterogeneity (Levene’s) and sphericity of variance (Greenhouse-Geisser) were made as needed, and trials that differed by more than 3 standard deviations from the mean (for each measure in each experimental group) were removed. Repeated measures ANOVAs were used to evaluate the results from each group. The independent variable used to carry out the ANOVAs was group (aMCI, AD, control), and the repeated measures were experimental task (pro vs. antisaccades), and fixation condition (gap vs. overlap). One-way ANOVAs were used to evaluate differences between aMCI patients who converted to AD, and those who did not, with the same within subject measures as listed above. Values for right and left stimulus positions were not significantly different (paired t-test; p > .05), allowing the data to be collapsed across direction for each task.
4.4 Results

4.4.1 Saccadic reaction time

SRT characteristics were analyzed separately for each subject. Figure 4.1 (E-G) shows eye position traces recorded from three representative subjects (ages 75-76 years), in the gap condition for correct (solid traces) and incorrect (dotted traces) antisaccades. A qualitative look at the traces revealed that patient performance was more variable with more errors and longer correct reaction times, compared to control performance. Figure 4.2 shows the distribution of correct (positive values on ordinate) and direction error (negative values on ordinate) SRTs in the gap and overlap conditions for all subjects in each group for prosaccades (Fig. 4.2A-F) and antisaccades (Fig. 4.2G-L). Controls exhibited a small proportion of both anticipatory (0 -100 ms; light grey box, Fig. 4.2) and short latency (100 - 200 ms; dark grey box, Fig. 4.2) saccades in the prosaccade task. The SRT distributions of both aMCI and AD groups were markedly different from controls. Notably, the number of antisaccade errors was greater in the patient groups. Similar patterns were seen in gap and overlap conditions.

Analyzing cumulative SRT distributions with a non-parametric Kolmogorov-Smirnov test allowed us to determine if each curve comes from the same or different underlying distributions (e.g., how well cumulative distributions differed from one another). Removing outliers from each group helped to ensure that any differences seen were not driven by only a few subjects.

Antisaccade SRT distributions proved the most informative, especially for incorrect antisaccade latencies; in the gap condition, both AD (K = .20, p = .03) and aMCI (K = .90, p <
.01) curves differed significantly from the elderly controls, and from each other (K = .70, p < 0.01; Fig. 4.3C). Similarly, in the overlap condition (Fig. 3D), both AD (K = .38, p < .01) and MCI (K = .30, p < .01) error curves differed from controls, and also from each other (K = .21, p = .02). Only AD differed from controls in correct antisaccade latencies in the overlap condition (K = .23, p < .01). For prosaccades (Fig. 4.3A, B), gap, overlap, or correct and incorrect latencies showed minimal differences; only the AD group showed a distinct profile that was different from the aMCI and control curves.

We computed the mean proportion of anticipatory saccades (all saccades between 0 – 100 ms; see Methods) for each subject (Fig. 4.4A, B) for both pro and antisaccade blocks. A repeated
measures ANOVA revealed a main effect of experimental group (F (2, 170) = 5.26, p <.01); AD patients generated the most anticipatory saccades (Fig. 4.4A). A condition x disorder interaction also revealed that AD patients made the highest proportion of anticipations, especially in the overlap condition in both prosaccade and antisaccade tasks (F (2, 170) = 5.50, p <.01). Post-hoc analysis revealed that both controls (p = .01) and aMCI patients (p = .01) make fewer anticipatory saccades than AD patients. This can also be seen in the cumulative reaction time distributions (Fig. 4.3) where the AD curves typically begin earliest.

**Figure 4.4.** Prosaccade and antisaccade results in all three experimental groups, plotted by task (pro/anti) and condition (gap/overlap). Results include: Percent anticipations (A – B), and percent short latency saccades (C – D). * Significant between patients and controls, p < 0.05; ‡ significant between aMCI and AD, p < 0.05.
We also computed the mean proportion of correct short latency saccades (100 – 200 ms; see Methods) for each subject (Fig. 4.4C, D) in the pro and antisaccade tasks. In the prosaccade task, AD patients made more short latency correct responses than both aMCI and control participants in the gap condition (F (2, 170) = 11.91, p < .01); aMCI and control groups did not differ from each other (Fig. 4.4C). Short latency correct saccades were also less frequent in the overlap condition of the prosaccade task compared to the gap condition (Fig. 4.4D; F (2, 170) = 103.23, p < .01). In the antisaccade task, the proportion of short latency saccades was similarly attenuated in all three groups compared to the prosaccade task (Fig. 4.3D; F (2, 170) = 113.93, p < .01).

Finally, we calculated mean SRT for all correct responses for each subject. A 3-way interaction between tasks, condition, and disorder (F (2, 122) = 3.45, p = .04) revealed that both patient groups had differing SRTs depending on the task and condition. It appeared that aMCI patients had the longest mean SRTs in anti overlap trials and AD patients had longer SRT than controls in anti gap trials (Fig. 4.5B). Otherwise, group did not influence mean SRT (Fig. 4.5A, B). The ANOVA revealed significant main effects between saccade tasks (anti SRT > pro SRT) and conditions (overlap SRT > gap SRT) that were consistent with previous studies (e.g., (Abel et al., 2002; Chan et al., 2005; Munoz et al., 1998; Peitsch et al., 2009) (Fig. 4.5A, B)). Post-hoc (Tukey) analysis revealed a task x condition interaction where the longest latencies in all groups occurred in the anti overlap trials (F (2, 122) = 13.85, p < .01).
Figure 4.5. Prosaccade and antisaccade results in all three experimental groups, plotted by task (pro/anti) and condition (gap/overlap). Results include: mean SRT (A – B), mean coefficient of variation of SRT (standard deviation/mean x 100) (C – D), mean percent direction errors (erroneous prosaccades) (E – F). * Significant between patients and controls, p < 0.05; ‡ significant between aMCI and AD, p < 0.05.

4.4.2 Intra-subject variability in SRT

To contrast intra-subject variability in SRT, we computed the coefficient of variation in SRT (CV; see Methods). A three-way ANOVA revealed that intra-subject variability in SRT
increased ($F(2, 122) = 7.21 \ p < .01$) in aMCI and AD relative to controls (Fig. 4.5C, D). Post-hoc analysis showed that both aMCI ($p = .02$) and AD ($p < .01$) groups had more intra-subject variability than controls, and also differed from each other in the antisaccade task (Fig. 4.5D). For all groups, $CV$ was higher in the prosaccade task compared with the antisaccade task ($F(2, 122) = 19.18, \ p < .01$), likely due to shorter SRTs in the prosaccade task.

### 4.4.3 Direction Errors

The proportion of direction errors provides a robust measure of inhibitory control (Munoz and Everling 2004). We calculated the percentage of direction errors each subject made, and then computed a mean proportion of direction errors for each of the three groups. A three-way ANOVA revealed that disorder ($F(2, 122) = 6.08, \ p < .01$) and task significantly affected the proportion of direction errors. All groups elicited a higher proportion of errors in the antisaccade task compared to the prosaccade task (Fig. 4.5E, F); however, considerably more direction errors were seen in aMCI and AD groups in the antisaccade task (Fig. 4.5F). Post-hoc comparisons within the ANOVA showed that both AD ($p < .01$) and aMCI ($p < .01$) patients made substantially more direction errors than controls, but did not differ from each other. A two-way interaction between task (pro vs. anti) and fixation condition (gap vs. overlap) also revealed that subjects made more direction errors in the anti versus prosaccade task ($F(2, 122) = 37.06, \ p < .01$), and more errors in gap versus overlap conditions in the antisaccade task ($F(2, 122) = 19.69, \ p < .01$).

We also measured the proportion of errors occurring during the short-latency epoch (100 – 200 ms) to determine if the increased error rate in AD and aMCI was due to a selective increase
in errors with faster SRTs. A task x disorder interaction (F (2, 170) = 4.10, p = .02) revealed that only AD patients differed from controls (p = .01) in the proportion of short-latency errors made in the antisaccade task (Fig. 4.4F), while the difference between aMCI patients and controls approached significance (p = .06). Therefore, in the gap and overlap conditions of the antisaccade task, AD patients made more short-latency errors than aMCI patients, and aMCI patients made more short latency errors than controls. This is also illustrated in Fig. 4.3D (negative ordinate) where the aMCI and AD curves contain more errors than the control group. All three groups made minimal short latency errors in the prosaccade block (Fig. 4.4E).

4.4.4 Gap and anti effects

Contrasting the gap effect (overlap SRT – gap SRT) between groups is a measure of automatic saccade control, because shorter SRTs are typically produced by the disappearance of central fixation prior to stimulus onset (Dorris and Munoz, 1995). The gap condition produced shorter SRTs than the overlap condition in both pro and antisaccade tasks (Fig. 4.6A; F (1, 59) = 84.74, p < .01). A one-way ANOVA revealed that group did not directly influence the gap effect (F (2, 59) = 1.635, p = .20), however, AD subjects had a significantly shorter gap effect than both aMCI patients and controls in the antisaccade task only (F (2, 59) = 10.37, p < .01).

Conversely, contrasting the anti-effect (antisaccade SRT – prosaccade SRT) between groups is a measure of voluntary saccade control, indicative of good executive functioning (Munoz and Everling 2004). We found that correct prosaccade reaction times were faster than correct antisaccade reaction times in both gap and overlap conditions (Fig 4.6B; (1, 59) = 173.45, p < .01). A one-way ANOVA revealed that group influenced the anti effect (F (2, 59) = 3.651, p = .03), revealing that this voluntary effect differed with disease. Further analysis showed that both
aMCI (p = .04) and AD (p = .03) patients exhibited larger anti effects than controls, but did not differ from each other (Fig. 4.6B).

**Figure 4.6.** The mean gap-effect (overlap SRT – gap SRT) (A) and mean anti-effect (antisaccade SRT – prosaccade SRT) (B) for each experimental group in each task (pro/anti) and each condition (gap/overlap).

### 4.4.5 Age effects

Prosaccade and antisaccade performance (e.g., SRT, variability, error rates) worsens with aging (Munoz et al., 1998; Pelsch et al., 2009; Sharpe and Zackon 1987). To confirm that the performance differences we described here for aMCI and AD patients are related to disease pathology rather than aging, Pearson correlations were computed (for all three groups) across age for each task (pro and anti) and each fixation condition (gap and overlap) for SRT, variability in SRT, and direction errors. Notably, SRT and the proportion of direction errors in both pro and antisaccades worsened with age in elderly controls (Table 4.2). However, only pro gap SRT correlated with age in aMCI patients, whilst *no other saccade parameter* changed with age in the
two disease groups (Table 4.2). Interestingly, we found that the patient groups were already performing with the same executive dysfunction as the eldest of the controls.

<table>
<thead>
<tr>
<th>Task</th>
<th>Condition</th>
<th>Measure</th>
<th>CTRL (Pearson R/p)</th>
<th>MCI (Pearson R/p)</th>
<th>AD (Pearson R/p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRO</td>
<td>GAP</td>
<td>SRT</td>
<td>0.242*/0.035</td>
<td>0.507**/0.010</td>
<td>0.293/0.155</td>
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<tr>
<td></td>
<td></td>
<td>Error</td>
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<td>-0.241/0.246</td>
<td>0.038/0.857</td>
</tr>
<tr>
<td></td>
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<td>SRT</td>
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<td>0.334/0.103</td>
<td>0.298/0.148</td>
</tr>
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<td></td>
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<td>-0.209/0.316</td>
<td>0.094/0.654</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Error</td>
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<td>0.274/0.185</td>
</tr>
<tr>
<td>ANTI</td>
<td>GAP</td>
<td>SRT</td>
<td>0.357***/0.002</td>
<td>0.281/0.164</td>
<td>0.291/0.159</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CV</td>
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<td>0.035/0.863</td>
<td>-0.002/0.994</td>
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<td>0.328/0.110</td>
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<td>-0.002/0.993</td>
<td>-0.002/0.994</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Error</td>
<td>0.290*/0.011</td>
<td>0.122/0.551</td>
<td>0.331/0.106</td>
</tr>
</tbody>
</table>

**Bolded text:** * = p < .05; ** = p < .01

**Table 4.2.** Pearson Correlations: Saccade performance measures in the three experimental groups by AGE (years).

### 4.4.6 Conversion to AD

We had the opportunity to determine which aMCI patients converted to AD at 3 years follow-up. Comparing saccade performance between aMCI patients that converted to AD and those who did not yielded very interesting trends. Out of the 26 aMCI patients, follow-up diagnoses were available three years after initial testing for 19 patients. We found that 2 aMCI patients had clinical scores that returned to a normal range, 8 aMCI patients remained stable and
9 aMCI patients converted to AD. We then compared saccade performance (see Methods) between patients who converted to AD (mean age 79 years old) at 3 years follow-up and those who remained aMCI (mean age 73 years old). Automatic performance (e.g., prosaccade SRT, anticipations, short latency errors, direction errors, and the gap effect) did not differ between aMCI patients who converted to AD (aMCI→AD) versus aMCI patients who remained stable (data not shown). As expected, we did not see any SRT differences in the antisaccade task between the two groups (Fig. 4.7A). However, the percent of anticipatory saccades, an indication of saccade control, increased in aMCI→AD patients compared to aMCI patients (approaching significance, p = .09) in the antisaccade overlap condition (Fig 4.7B). Furthermore, aMCI→AD patients generated more direction errors than aMCI patients in the antisaccade task (especially in the gap condition), showing a reduced ability to inhibit erroneous saccades, although non-significant [Fig. 4.7C; F (1, 14) = 1.11, p = .31].
Figure 4.7. Antisaccade SRT (A), percentage of antisaccade anticipations (B), and the percentage of antisaccade direction errors (C) for aMCI patients only, to compare those who converted to AD at 3 years follow-up, to those who did not.

4.5 Discussion

The goals of this study were to determine if the antisaccade task could detect subtle executive function impairments in aMCI and AD patients, and to determine if aMCI patients exhibited deficits in executive functions (assessed via the antisaccade task) that corresponded to
an increased likelihood of conversion to AD. We found that although automatic prosaccade distributions differed between groups, individual saccade parameters could not distinguish them. For instance, aMCI and AD groups did not generate prosaccades any slower than controls, and showed similar proportions of short latency saccades, aside from AD patients making considerably more short latency saccades in the prosaccade gap condition. Conversely, voluntary or executive saccade parameters (saccade inhibition and voluntary saccade initiation, as measured in the antisaccade task) were significantly more impaired in both aMCI and AD relative to healthy aging. Importantly, aMCI and AD patients exhibited similar deficits, consistent with studies showing both patient groups have similar underlying pathology. We also found that executive saccade parameters had the potential to predict conversion from aMCI to AD.

Our data reveal that many aMCI patients had executive function deficits that were less significant on standard psychometric tests (see Table 4.1), but were more obvious and similar to AD patients in antisaccade performance. Our data support the hypothesis that aMCI patients have similar to, but subtle, executive function impairment as AD patients that can be assessed using the antisaccade task. Our results may therefore be indicative of a specific breakdown in controlled inhibitory functioning in both aMCI and AD patient groups, while automatic processing remains intact. These deficits may be analogous to the deficits seen in AD while performing the Stroop paradigm (Amieva et al., 2004). Therefore, specific antisaccade measures such as the proportion of direction errors could be used to indicate subtle executive dysfunction in aMCI patients, and potentially predict future conversion to AD.
4.5.1 Relation to previous studies

Previous studies have reported that AD patients did not differ from elderly controls in prosaccade measures, showing similar SRT and rates of anticipation (Crawford et al., 2005; Pratt et al., 2006). Similarly, we found prosaccade SRTs and anticipations in aMCI and AD patient groups differed minimally from elderly controls. The increase in intra-subject variability of SRT in AD patients only may be analogous to the frequent saccadic intrusions seen in fixation control and horizontal saccade studies in previous studies of AD (Fletcher and Sharpe 1986; Jones et al., 1983; Schewe et al., 1999). Overall, prosaccade measures were neither robust nor sensitive enough to predict abnormal aging patterns.

Alternatively, the enhanced executive impairment seen in voluntary antisaccade parameters in both patient groups is more promising. Previous studies have found increased antisaccade latencies and direction errors (Abel et al., 2002; Bylsma et al., 1995; Garbutt et al., 2008; Shafiq-Antonacci et al., 2003) in AD patients relative to controls. Similarly, we observed similar trends, wherein the AD group made substantially more direction errors, more anticipatory saccades, and more short latency errors. We observed these same trends in our aMCI patients, contrary to (Versino et al., 1996), who reported that none of their “non-demented memory-impaired” patients showed abnormal latencies or error rates in the antisaccade task. This could be explained by the prior lack of diagnostic criteria for aMCI patients compared to the current criteria (Petersen et al., 2009), or due to the relatively young mean age (67 years old) of their memory-impaired cohort.

We additionally observed an increased anti-effect (Fig. 4.6) in both of our patient groups, supporting the notion that executive dysfunction exists in not only AD patients, but in aMCI
patients too. Age strongly correlated with voluntary saccade parameters in controls, but patients already performed at the same impaired level as our eldest controls (75+ yrs old). This suggests that AD/aMCI disease pathology influenced behavior more than simply age alone.

4.5.2 Implications for aMCI and its link to AD

Between 4 and 15% of aMCI patients annually progress to AD (Ravaglia et al., 2008; Solfrizzi et al., 2004; Tschanz et al., 2006). Therefore, identifying measures that can be used to predict which aMCI patients are most similar to AD is crucial. Our data revealed that aMCI patients had antisaccade deficits that were similar to mild AD patients. aMCI patients who later converted to AD generated more anticipatory saccades (both correct and incorrect) and direction errors in the antisaccade task than those who did not. Therefore, we speculate that similar brain changes as those occurring in AD may be influencing saccade behavior in aMCI patients, providing the potential for simple antisaccade measures to predict a future AD diagnosis. However, we only assessed our patients at 3 years follow-up, and there was a small age difference between the two groups, so a longer longitudinal study with a larger patient sample is needed before it can be convincingly demonstrated that the same brain changes influence behavior in aMCI and AD patients.

Patients with mild stage AD and aMCI have been reported to have similar memory impairments, whereas AD patients are impaired in other cognitive domains, such as executive functioning (Petersen and Bennett 2005). We restricted our sample to mild-stage AD to determine if AD patients were indeed more impaired on frontal oculomotor parameters, or if these differences were instead due to the advanced pathology seen in moderate-to-severe stage AD.
patients. Using later stage AD patients could potentially exaggerate the differences between patient groups. Our data revealed that antisaccade measures may be sensitive enough to pick up executive impairments in aMCI, and that aMCI patients who later converted to AD had somewhat increased executive impairments, suggesting that although they do not always show clinical impairment in these non-memory cognitive domains, antisaccade performance may be indicative of future progress to AD. However, our aMCI patients varied in age, resulting in an older average in patients who converted to AD. This may be contributing to some of the trends seen in Fig. 4.7; therefore, this needs to be verified with larger-scale studies. We also found, similar to results published by (Shafiq-Antonacci et al., 2003), that using our data to differentiate between patient groups and controls only has high reliability at the group level, presumably due to the high variability in performance seen in all elderly subjects. Further experimentation to increase the sensitivity of the paradigm is needed.

4.5.3 Linking eye movement performance to brain structures

aMCI and AD patients exhibited impairments in the antisaccade task such as reduced ability to initiate voluntary movements and to suppress unwanted reactive saccades. Reduced performance on the antisaccade task is suggestive of attenuated frontal function (Munoz and Everling 2004) and delayed frontal processing. Because the circuitry underlying saccadic eye movements is well understood (Hikosaka et al., 2006; Leigh and Zee 2006; Moschovakis et al., 1996; Munoz and Everling 2004; Scudder et al., 2002; Sparks 2002; Wurtz and Goldberg 1989), the behavioral observations seen in aMCI and AD patients relative to controls can infer involvement of specific structures or regions of the brain. Lesion studies have shown that frontal regions like the frontal eye fields (FEF), supplementary eye fields (SEF), and the dorsolateral
prefrontal cortex (DLPFC) are important for voluntary saccade initiation and suppression of automatic saccades (Guitton et al., 1985; Pierrot-Deseilligny et al., 1991; Rivaud et al., 1994). Electrophysiological recordings of single neurons in monkeys have confirmed that FEF (Everling and Munoz 2000), SEF (Amador et al., 2004; Schlag-Rey et al., 1997), and DLPFC (Johnston and Everling 2006) neurons are selectively recruited for the antisaccade task. Transcranial magnetic stimulation has revealed that the FEF is involved in antisaccade preparation (Juan et al., 2008). Human neuroimaging studies have also confirmed that the DLPFC, FEF, and SEF are involved in saccade inhibition and voluntary saccade initiation, processes required for generating antisaccades (Connolly et al., 2002; Connolly et al., 2005; Ettinger et al., 2005; Ford et al., 2005; O'Driscoll et al., 1995; Sweeney et al., 1996). Furthermore, the DLPFC elicits higher fMRI response for the preparation of an antisaccade than the execution of the antisaccade (DeSouza et al., 2003). Therefore, we expect that alterations in any of these frontal regions could alter the input to other oculomotor regions involved in the initiation and execution of antisaccades, leading to increases in SRT and error rates.

In AD, antisaccade SRT and error rate increases likely indicate frontal cortex dysfunction. The fact that our AD patients generated most antisaccade direction errors during the short-latency epoch (e.g., unable to inhibit visually-triggered responses) further suggests executive function impairment, and thus, frontal lobe involvement, particularly of the DLPFC. It has been suggested that the DLPFC may be a good tracking structure to predict aMCI conversion to AD (Kaufman et al., 2010). However, the hallmark AD pathology of tangles of the microtubules targets the frontal cortex last, and amyloid plaque deposits in AD have been suggested non-significant for the differentiation of pathological stages (Braak and Braak 1991). The first structures influenced by AD pathology are in the medial temporal lobe (MTL), including
the hippocampus (Braak and Braak 1991). Nevertheless, disruptions in hippocampal connectivity have been noted in mild AD patients (Wang et al., 2006); reduced connectivity was found between the hippocampus and the prefrontal cortex. We speculate that aberrant projections from hippocampus and/or MTL could influence frontal cortex function prior to AD-related anatomical changes. Pathology in mild AD is thought to be limited to the parietal-temporal junction (Rabinovici et al., 2007), so a lack of impairment in prosaccade generation is not surprising. It has been shown previously that volumetric changes in the SEF correlate to antisaccade latency, but that DLPFC and FEF volume were not correlated with antisaccade performance (Boxer et al., 2006), supporting the notion that functional changes could precede anatomical changes in the frontal cortex of AD patients. However, Boxer et al. (2006) did not separate frontal temporal dementia (FTD) patients from AD patients, and the increased variability by combining the two groups may have masked some relationships between structure and behavior (Kaufman et al., 2010). However, other fMRI studies have shown that FEF, SEF, and DLPFC activity does indeed correspond to saccade performance changes in healthy adults (Connolly et al., 2005; Ford et al., 2005). Therefore, tracking functional changes in frontal structures may be useful to determine upcoming anatomical changes.

In this study, the mild AD patients showed similar impairments in the antisaccade task as the oldest of the controls (over age 75 yrs), suggesting a form of “accelerated aging with disease,” where pathology may be accelerating the natural age-related attenuation of performance. However, recent theory suggests that age-related deficits result primarily from frontal-striatal changes, whereas AD deficits arise primarily from changes to the hippocampal circuit (Buckner 2004; Head et al., 2005), implying that AD is not simply an accelerated form of aging. This is more likely, based on what is now known about AD pathology, but the resulting behavior may be
similar to that seen in advanced aging, due to the attenuated hippocampal-to-frontal projections and the consequential dysfunction in the frontal cortex. Therefore, although hippocampal/MTL dysfunction could be influencing the frontal cortex and thus executive function, it has been suggested that tracking frontal regions such as the DLPFC may then be useful for assessing the progress of AD pathology (Kaufman et al., 2010). In this case, reduced function, assessed with imaging or behavioral executive function impairments, may predict upcoming pathological changes, providing a good indication for early therapeutic intervention. Future mixed-method paradigms, such as combining behavioral tasks with fMRI, may provide insightful information on how functional brain changes relate to behavioral changes, such as how DLPFC function changes in aMCI/AD patients compared to controls. Determining the functional connectivity between frontal and hippocampal areas using MRI or EEG will also be useful. Finally, and most importantly, longitudinal studies that track aMCI patients to determine precise rates of conversion are also imperative.

4.6 Conclusions

These data provide a detailed description of saccade performance changes in aMCI and AD patients who underwent rigorous neuropsychological assessment. Although the current diagnostic criteria for aMCI is memory-based (listed as “cognitive complaint not normal for age, not demented, memory decline, essentially normal functional activities;” (Petersen et al., 2009)) and not sensitive to frontal changes, we now know that our aMCI patients exhibited similar frontal impairments, as revealed by antisaccade performance, to AD patients, and that aMCI patients with a higher proportion of antisaccade errors and anticipatory saccades were more
likely to convert to AD. This indicates the importance of quantifying the similarities between aMCI and AD due to the higher prevalence of conversion to dementia.

Performance in the antisaccade task may indicate subtle executive function deficits and identify patients at risk for conversion to dementia. Although performance on clinical tests (e.g., Stroop) often corresponds with antisaccade errors (Hodgson et al., 2009), our results suggest that the antisaccade task is more sensitive and objective at detecting the subtle deficits seen in aMCI. Combining these results with future longitudinal studies that track which aMCI patients develop AD, and with neuroimaging parameters, will have strong potential for clinical application.

4.7 Acknowledgements

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4.8 Funding

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Chapter 5
Preserved response inhibition corresponds to increased frontal recruitment in elderly

5.1 Abstract
Frontal executive functions are compromised in aging. The comparison of prosaccades (automatically look toward an eccentric stimulus) to antisaccades (inhibit the automatic saccade to the eccentric stimulus to instead initiate a voluntary saccade away from the stimulus) is often used to investigate executive functions. Here, we combine functional magnetic resonance imaging (MRI) with eye tracking to investigate the relationship between aging brain changes and behaviour. It is expected that differing activation between older and younger subject groups will be related to the deteriorating saccade performance seen in aging (e.g., longer latencies, increased direction errors). Furthermore, we expect to see differences in activation between high-performing older adults (< 10% direction errors) and poor-performing older adults (> 10% direction errors). Healthy elderly adults (age 60-85 yrs) performed a rapid event-related (interleaved) pro- and anti-saccade task while blood oxygen level dependent (BOLD) signal changes were recorded using MRI. Preliminary analysis revealed that poor-performing older subjects had reduced BOLD response in both the frontal eye fields and dorsolateral prefrontal cortex compared to high-performing older. In conclusion, healthy older adults show decreased frontal recruitment during the saccade task when performing at lower-than-optimal levels (> 10% error rate), and high-performing subjects show over-recruitment or compensation of frontal cortical areas. This may be related to cognitive reserve often seen in the elderly. These results
provide insight into how functional changes in the aging brain may alter behaviour, specifically behaviour subserved by the frontal cortex.

5.2 Introduction

It is well known that the brain changes physiologically across the human lifespan. Some changes have been associated with decreases in overall cognitive functioning in the elderly, such as cell loss, synapse loss, overall volume loss, and white matter abnormalities (Wang and Snyder, 1998). The processes underlying executive functions – a set of higher order cognitive functions including planning, inhibition, and goal-directed or voluntary behaviours – are thought to be subserved by the frontal cortex and are also attenuated in the elderly (Kaup et al., 2011). Executive functions can be assessed using the antisaccade task (Hallett, 1978; Guitton et al., 1985). To complete an antisaccade, the subject must first inhibit an automatic reaction to the presentation of a suddenly appearing peripheral stimulus, followed by initiation of a voluntary saccade in the opposite direction. Successful performance on the antisaccade is reflective of good frontal functioning (Munoz and Everling, 2004). More specifically, a network of cortical and subcortical structures must ‘come online’ prior to the appearance of the visual stimulus so the motor system can then generate the appropriate action. These include the dorsolateral prefrontal cortex (DLPFC) (Guitton et al., 1985; Pierrot-Deseilligny et al., 1991), the frontal, supplementary and parietal eye fields (FEF, SEF, PEF) (Connolly et al., 2005; Curtis and D'Esposito, 2003; DeSouza et al., 2003; Ford et al., 2005; Brown et al., 2007), and the basal ganglia (Ford and Everling, 2009; Watanabe and Munoz, 2009). This phenomenon is referred to as ‘task set’ or ‘preparatory set.’ Recent evidence has shown that hyper-activation measured with fMRI occurs throughout the frontoparietal network during saccade performance in the elderly (Nelles et al., 2009), particularly antisaccade trials (Raemackers et al., 2006). However, prior evidence has also
shown that if the elderly are broken down into those who perform similar to young adults, versus those with impaired performance (e.g., impaired ability to inhibit automatic saccades to stimulus), the elderly subjects who could maintain performance at higher levels on tasks of antisaccade trials (Raemaekers et al., 2006), memory of previously studied words (Cabeza et al., 2002), and verbal memory encoding (Logan et al., 2002) showed increased or hyper-activation in frontal brain regions compared to young adults.

In the present study, we provide evidence that elderly subjects who perform with a higher rate of accuracy (< 10% errors, see Methods) show hyper-activation, or a shift in activity from the parietal to the frontal oculomotor areas during the preparatory stage, rather than during the execution of the actual saccade response. We speculate that this corresponds to a similar ‘cognitive reserve’ (Tucker and Stern, 2011), or compensation reported in other modalities, where extra recruitment of blood oxygen (and theoretically neural activity) is necessary to compensate for what would otherwise be a decrement in performance.

Previous studies comparing prosaccades and antisaccades in young adults have found an increased level of activation in the FEF, SEF, and the PEF for antisaccades compared with prosaccades (Brown et al., 2006, Ford and Everling, 2006). In the elderly, higher activation was found in PEF, FEF, SEF, and the CN for antisaccades versus prosaccades, and the percentage of signal change is higher in the elderly than young adults (Nelles et al., 2009; Raemaekers et al., 2006). Therefore, we are focused on the following regions of interest (ROIs) in the oculomotor circuit: DLPFC, FEF, SEF, PEF, and CN. We interleaved prosaccades and antisaccades in a rapid-event fashion in order to add ‘preparatory’ trials, where an instruction for which type of saccade (pro or anti) to generate is presented, but no stimulus appears, so subjects must ‘prepare’
for the appropriate saccade without later generating one. This method also allows us to separately investigate the ‘execution’ of saccades.

We (Peltsch et al., 2009; Peltsch et al., ; Munoz et al., 1998) and others (Abel and Douglas, 2007; Abrams et al., 1998; Yang and Kapoula, 2006) have shown that in elderly subjects compared to young adults, performance on voluntary/executive measures (e.g., the antisaccade task) was considerably more impaired than performance on the prosaccade task (automatic saccade to peripheral stimulus). Because the processes underlying the antisaccade were far more sensitive to aging, we expect the underlying neurological substrates of antisaccades to also be sensitive to changes occurring in the aging brain. We speculate that the frontal oculomotor regions such as DLPFC, FEF, and SEF should prove most different between young adults, high-performing older adults, and low-performing older adults since these frontal regions appear to be over-recruited when compensating for performance.

5.3 Methods

5.3.1 Participants

The experiment was approved by the Research and Ethics Board of Queen’s University, and adhered to the principles of the Tri Council Policy. Twenty-two healthy young volunteers (aged 18 – 25 years, 11 female) and 21 healthy elderly (aged 61 – 83 years, 14 female) participated in one two-hour experiment at the Queen’s University magnetic resonance imaging facility. All participants had normal or corrected-to-normal vision, or verified that they could distinguish the stimuli without their glasses. Participants reported no neurological or psychiatric illnesses. All participants gave informed written consent prior to engaging in the experiment.
5.3.2 Paradigm

A rapid event-related design was used, allowing for presentation of 64 trials in one run (approximately 6 minutes; 277.5 s); each no longer than 4.5 s. Each run consisted of 16 ‘prosaccade’ trials, 16 ‘antisaccade’ trials, 8 prosaccade ‘preparation’ trials, 8 antisaccade ‘preparation’ trials, and 16 ‘fixation trials,’ all pseudorandomly interleaved within the run (Fig. 5.1). Each participant performed 6 runs. Prosaccade and antisaccade trials began with 1000 ms of fixation on a neutral fixation stimulus (hollow gold coin) at center screen, subtending approximately 2 degrees of visual angle. The pro or anti instruction was then presented for 1300 ms, and was either a central green stimulus (turtle), or a central red stimulus (lobster), both of similar sizes and luminance (these stimuli were chosen based on the conduction of this identical experiment across other age groups including children). Participants were instructed to make a prosaccade to the stimulus (green turtle) or an antisaccade away from the stimulus (red lobster) depending on the instruction. If participants executed an incorrect response (direction error), they were instructed to correct themselves. A gap of 200 ms in the central fixation stimulus then occurred prior to the presentation of the peripheral ‘target’ stimulus (another gold coin) for 100 ms at 6 or 7 degrees to the right or left of fixation. The gap was employed to increase automatic responding ((Dorris and Munoz, 1995), thus increasing the occurrence of short latency automatic prosaccades in both the pro and antisaccade task. A period of 1400 ms of darkness occurred after presentation of the target, in which participants held their gaze at the location of the target stimulus on a prosaccade trial, or at its mirror location on an antisaccade trial. A period of 500 ms of neutral fixation then followed to return participants fixation to center.
Figure 5.1. Experimental paradigm; representation of stimuli and timing of events in the 4 trial types. Trials were pseudorandomly presented and intermixed with periods of fixation on Neutral Fixation stimulus that were 1.5, 3 and 4.5 s in length.

‘Preparation’ trials were identical to pro and antisaccade trials, but did not include the presentation of the target stimulus, instead containing a 1700 ms period of darkness (200 + 100 + 1400) following the prosaccade or antisaccade instruction, where subjects were required to maintain fixation (Fig. 5.1). Therefore, both saccade and ‘preparation’ trials were 4500 ms in length (or three TR’s (repetition time = 1500 ms); described below). Trials containing only the neutral fixation point were also included, varying in lengths from one TR (4 trials), two TRs (4 trials), and three TRs (8 trials). The inclusion of preparation trials and fixation trials of varied lengths was necessary for the deconvolution analysis of the rapid event-related design, explained in fMRI parameters (Ollinger et al., 2001; Dale, 1999). All runs began with an additional period of fixation for 3 s to allow the BOLD signal to reach steady-state longitudinal magnetization, and ended with a period of fixation for 16.5 s to allow for return of the hemodynamic response signal to baseline.
5.3.3 Eye tracking and visual display

Visual stimuli were generated using e-prime software (Psychology Software Tools Inc., Pittsburgh, PA, USA) running on a PC, and an NEC LT265 DLP video projector (Toyko, Japan) was used to back-project the image onto a custom-built screen. Eye tracking was conducted using an ISCAN ETL-400 camera (Burlington, MA, USA) running dqw software v1.10X and sampling eye position at 120 Hz. The camera was positioned next to the screen, approximately 50 cm from the bore of the magnet to view the right eye of the subject in the mirror. An infrared fiber-optic illuminator was fixed to the head coil prior to the participant entering the bore of the scanner. This illuminated the participant’s right eye from an angle of approximately 45° below the eye. Prior to the first functional scan, calibration of the eye tracker was conducted using a nine-point calibration routine, with the nine points covering the maximum available visual field on the screen (approximately 16 inches in width).

5.3.4 fMRI parameters

All MRI scans were conducted with a Siemens 3T Magnetom Trio system (Erlangen, Germany) with a 12-channel receive-only head coil. Participants lay supine in the scanner and viewed visual stimuli back-projected onto a screen located at the head-end of the scanner. A mirror attached to the head coil and angled at approximately 45° allowed subjects to view the screen. Subjects were presented with a text screen informing them to prepare for the onset of each experimental run. Functional images were acquired with 24 horizontal slices (3.3 mm thick) covering the brain from the top and including the frontal/prefrontal, parietal, visual areas, and BG to the level of the ventral striatum. Each functional volume consisted of a T2*-weighted echo-planar image (EPI) sensitive to BOLD contrast (Ogawa et al., 1990; Kwong et al., 1992) acquired
in an interleaved fashion (repetition time, TR = 1500 ms; echo time, TE = 30 ms; flip angle, FA = 72 deg; field-of-view, FOV = 211 x 211 mm, matrix size 64 x 64, 3.3 mm isovoxel resolution, 185 volumes). High-resolution MP-rage 3D T1-weighted scans were acquired for anatomical localization (TE = 2.2 ms, TR = 1760 ms, FA = 9 deg, 176 slices, 1 mm thick).

5.3.5 Statistical analysis

Behavioral data were analyzed using custom programs in Matlab 7.4 (The Mathworks Inc., Natick, MA, USA) and imaging data was analyzed using the Brain Voyager QX v2.1 software package (Brain Innovation, Maastrict, The Netherlands). Saccade reaction time (SRT) was defined as the first saccade away from fixation after stimulus onset, when the velocity exceeded the mean + 3 deg the SD of the background velocity. Errors in which participants failed to fixate the instruction, failed to maintain fixation (measured by saccades in any direction during the fixation period), failed to initiate a saccade, or executed multiple saccades during the response period were also removed from behavioral analysis but modeled separately as ‘null trials’ in the fMRI analysis (see section 5.3.7). Trials in which participants failed to correct direction errors were also placed in the null category. The percentage of direction errors was calculated by dividing the errors by the total number of valid trials. After the initial analysis, the elderly were broken down into two categories. Poor-performing older adults (N = 10; aged 62-83 yrs) were categorized as those who generated greater than 10% direction errors, a proportion analogous to what younger adults generate in the antisaccade task (based on the scatter plot in Fig. 5). High-performing older adults (N = 11; aged 61-78 yrs) therefore made less than 10% errors. Finally, entire runs were excluded if the participant had more than 25% of their trials removed for any of the above reasons or if successful eye tracking was not possible. In total, all participants provided
between 4 and 6 functional runs, and no subject had more than 15% of trials excluded from further analysis for any given run that was included.

5.3.6 Behavioural analysis

Paired t-tests (non-directional) were conducted to compare mean SRTs, mean percentage of direction errors, and mean CV across subjects between anti and pro trials. One-way ANOVA’s were used to compare behaviour across the three experimental groups. Left and right target responses were collapsed to increase statistical power. P values were corrected for multiple comparisons (Bonferroni, p < .05).

5.3.7 Functional analysis

Analysis of the functional brain data was conducted using Brain Voyager 1.9 (Maastricht, the Netherlands). Functional images were first pre-processed to remove motion artifacts and linear drift (high pass filtered at 3 cycle/time-course, motion corrected by aligning the images of the time series to the first volume in the series, and corrected for slice timing differences by means of a sinc interpolation). The first two volumes were removed from analysis in order to include only data obtained with a steady-state longitudinal magnetization. Each participant’s high-resolution anatomical scans was transformed into Talairach coordinates (Talairach and Tournoux, 1988) by first using cubic spline interpolation to align the anatomical images into the anterior commissure – posterior commissure (AC – PC) plane and then using trilinear interpolation to transform the anatomical images into Talairach coordinates. Functional volumes were overlaid onto one representative brain from each experimental group (Figs. 5.4-5.6). The events of interest were modeled with boxcar predictors with a width of the 3 s ‘trial’ period (Fig.
convolved with Brain Voyager’s canonical (2 gamma) hemodynamic response function to map the BOLD response time locked to the onset of the visual stimulus, and spanning into the 12 s period (‘neutral gold coin’) following the response period. A total of seven predictors for the trial period were created based on: instruction (pro or anti), response (pro or anti), and performance (correct direction or erroneous direction that was subsequently corrected). In addition, the initial ‘instruction’ period was also modeled with separate predictors (pro, green; anti, red). Finally, all ‘null trials’ plus trials in which tracking was lost, trials in which the participant made multiple eye movements, failed to correct an error, or ‘uncorrected’ a correct response were modeled with a separate ‘null predictor’ in the trial period. This was done so that trials that could not be classified as a correct trial or corrected error were still modeled so as not to effect the calculation of the BOLD signal change from baseline. Group analysis was conducted using a fixed-effects general linear model (GLM) with separate subject predictors, Bonferroni corrected for multiple comparisons at p < .05 and cluster-size corrected at p < .05 (yielding a cluster threshold of nine contiguous voxels, as estimated using Brain Voyager’s Cluster-level Statistical Threshold Estimator at 1000 iterations). One-way ANOVA’s were conducted using SPSS 19 on the beta weight values (GLM parameter estimates) for comparisons described in the Results and figure legends. BOLD signal time courses were aligned to the onset of the peripheral stimulus, and the baseline was averaged from the mean three time-points (including the time-point at stimulus onset) of the ‘instruction’ period.
5.4 Results

5.4.1 Behaviour

Figure 5.2A shows a sample eye trace overlaying three saccades for one representative subject. Overall, we found that older subjects generated a higher percentage of direction errors than younger subjects (F(2, 45) = 6.12, p = .004) in the antisaccade trials only (Fig. 5.2B). SRT differences were not noted between the two age groups (Fig. 5.2C). CV in SRT however, was significantly increased in both the prosaccade (F(2, 45) = 8.79, p = .001) and antisaccade (F(2, 45) = 3.67, p = .033) trials in the older subject group relative to the younger subject group (Fig 2D). These results are consistent with ours (Peltsch et al., 2009; Munoz et al., 1998) and other (Abel and Douglas, 2007; Versino et al., 1996; Sharpe and Zackon, 1987) previous behavioural studies. We also calculated the difference in direction errors and SRT for anti trials minus pro trials, which is analogous to our fMRI contrast. The results were similar; the direction error difference (anti – pro) was greater for the older subjects than the younger subjects (Fig. 5.2E). SRT did not differ between groups (Fig. 5.2F).
Figure 5.2. Experimental behaviour. (A) Sample eye traces comparing correct antisaccade and erroneous antisaccade trial (direction error) that was subsequently corrected. (B) Mean percentage direction errors (initial saccade away from T on prosaccade trials, toward T on antisaccade trial). (C) Mean saccadic reaction time (SRT) on correct trials. (D) Mean intrasubject coefficient of variation in SRT (CV). Error bars correspond to standard error of the mean. (E,F) Mean subtractions between antisaccade and prosaccade trials for (E) direction errors and (F) SRT, with individual data points.
Previous research shows that older subjects who perform similar to younger subjects may indeed be ‘compensating’ with cognitive reserves to maintain adequate performance in various modalities (Cabeza et al., 2002; Tucker and Stern, 2011; Grady and Craik, 2000; Buckner, 2004). Thus, we categorized the older subjects into two performance-related groups before continuing the behavioural subtractions and the imaging analysis (see Statistical methods). Interestingly, a one-way ANOVA revealed a main effect of the direction error subtraction (anti – pro) (F (2, 44) = 21.55. p < .001), where post-hoc analysis (Tukey) revealed that the poor-performing older group generated more direction errors than both the high-performing older group (p < .001) and the young group (p < .001; Fig. 5.3A). Again, no SRT differences were observed (Fig. 5.3B).

**Figure 5.3.** The same subtractions as Figure 5.2 E, F, but comparing all three experimental groups, with individual data points.
5.4.2 fMRI

From the behaviour, it is evident that several neural correlates in the brain should be activated related to the antisaccade impairments seen in aging. In fMRI volume maps (e.g., Figs. 5.4-5.6), ‘hot spots,’ or orange-coloured areas are thought to reflect a greater ‘recruitment’ of neural processes when two conditions are compared. Therefore, we based our analysis on directly comparing anti trials to pro trials (correct saccades only). We hypothesized that activation, or recruitment should be greater in areas more critically involved in the voluntary processes of antisaccade generation opposed to the automatic processes underlying prosaccade generation.

Firstly, we use this method to reveal fMRI brain areas of antisaccade deficits in aging (and between high-performing elderly and poor-performing elderly) when these groups were contrasted across groups (within-group). Using the deconvolution method allowed us to use three time points that corresponded to the region of peak activation, and not by convolution with a canonical hemodynamic response function (see 5.3.7 Methods). These time points occurred at approximately 7.7, and 9.2 seconds and corresponded to the 5th and 6th time points from trial onset (Fig. 5.4B). The ROIs determined from our anti-pro contrast were then used to determine the magnitudes of BOLD activation (via the general linear model) in these areas in two sub-processes of saccade generation; preparation of the saccade, and execution of the saccade. Including instruction-only ‘preparation’ trials allowed us this ability (peak activation then corresponded to the 4th and 5th time points). These contrasts are described below. Secondly, we then ran a second random effects analysis of the peak locations described above with the five ROI’s for pro preparation trials, anti preparation trials, pro execution trials, and anti execution trials (for execution-only, preparation trials were subtracted from saccade trials for both pro and antisaccade trials and then compared to each other). The results are displayed in Fig. 5.7. One-way ANOVA’s revealed group differences, also described below.
Figure 5.4. Whole saccade trials contrast map. (A) Contrast of pro saccade trials subtracted from anti saccade trials, cluster size corrected at $P < 0.05$ (9 contiguous voxels). Significantly greater BOLD activation is shown in 5 oculomotor regions of interest for antisaccades (‘hot’ colors) as labeled. Contrast maps are overlaid on one representative T1 anatomical scan from each age group. Coordinate values of planes in Talairach space are indicated. (B) Representation of mean BOLD signal time course for pro and antisaccade trials from right FEF region in A. Shaded area corresponds to a region of peak activation from trial onset. The three mean time points (5,6,7) under the shaded area were used at whole brain level to derive the voxels showing significantly greater activation on antisaccade trials in A. Error bars correspond to standard error of the mean (SE).

5.4.3 Antisaccades versus prosaccades

The volume map for subtracting prosaccade trials from antisaccade trials, for only the young and overall older group, is shown in Figure 5.4. This allowed us to confirm that the appropriate oculomotor ROIs were activated during the task. The regions in orange are those that
exhibited a higher BOLD signal change for antisaccade trials versus prosaccade trials. Our ROIs are labeled and it can be observed that the spread of the response differs between groups, with the older group often showing increased spread of BOLD signal. To determine the magnitude of the response between groups, we then calculate the mean ‘beta weight’ (parameter estimate of how much each predictor contributed to the variability in the model) for each ROI using the general linear model approach, and then do a simple t-test (Bonferroni corrected) between the peak values (Fig. 5.4B).

Figure 5.5 shows the volume map for the same contrast but between all three experimental groups: young adults, high-performing older adults, and poor-performing older adults. Regions in orange are those that exhibited a higher BOLD signal change for antisaccade trials versus prosaccade trials. We also noted that the high-performing older group shows the highest spread of BOLD signal change in the DLPFC (p < 0.05, Bonferroni corrected). Behavioural differences are listed above. The following results will more precisely examine preparation versus execution of the saccade.
Figure 5.5. Whole saccade trials contrast map as in Figure 5.4., but with all three experimental groups shown. Contrast of pro saccade trials subtracted from anti saccade trials, cluster size corrected at $P < 0.01$ (8 contiguous voxels).

5.4.4 Saccade preparation

A volume map for antisaccade preparation minus prosaccade preparation trials is shown in Figure 5.6A. This allowed us to measure the BOLD signal change during the preparatory period only. The high-performing older group showed the largest extent of BOLD signal change in the DLPFC, the FEF, and the SEF ($p < 0.05$, Bonferroni corrected). We than calculated the mean beta weights for all five ROIs. A one-way ANOVA to compare BOLD signal change between experimental groups revealed a main effect for the FEF only: in the antisaccade preparation-only trials, the FEF exhibited an increased signal change ($F(2, 40) = 4.929, p = .012$) where high-performing older subjects had the highest signal change compared to the young subject group ($p = .015$) and although non-significant, the poor-performing older group (Fig. 5.7A) ($p = 0.673$, ...
n.s.). In the prosaccade preparation-only trials, a main effect was seen in several oculomotor areas, such as the DLPFC (F(2, 40) = 3.696, p = 0.034), the FEF (F(2, 40) = 5.355, p = 0.009), and the CN (F(2, 41) = 3.280, p = 0.048).

**Figure 5.6.** Saccade preparation and saccade execution contrast maps. (A) Contrast of pro prep trials subtracted from anti prep trials, cluster size corrected at $P < 0.05$ (9 contiguous voxels). The 5th and 6th time points relative to trial onset were used in subtraction. (B) Contrast of pro saccade execution processes subtracted from anti execution processes. The 6th and 7th time points from trial onset were used in subtractions.
Figure 5.7. Region of interest (ROI) analysis for saccade preparation and saccade execution (A). Mean beta weight values for time points 5 and 6 from second-level random effects analysis of prosaccade and anti-prep trials for 125 cubic voxels surrounding peak activations in the regions showing greater activation on antisaccade trials compared to prosaccade trials. Unless specified, left and right hemispheres were averaged. Error bars correspond to standard error of the mean (SE). (B) Mean beta weight values for time points 6 and 7 for saccade execution period (saccade trials – prep trials).

Post-hoc analysis confirmed that for the DLPFC, the high-performing older group showed the highest signal change compared to the poor-performing older group (p = 0.032), and showed higher signal change compared to the young group (p = 0.111, n.s.), although not significant. In
the FEF, results were similar; the high-performing older group showed the highest signal change compared to both the poor-performing older group (p = 0.199, n.s.) and the young group (p = 0.006). Similarly, in the CN, the high-performing older group exhibited the highest signal change compared to the poor-performing older group (p = 0.037) and the young group (p = 0.271, n.s.). Significance was usually attained between high-performing and poor-performing groups, whereas good-performing older subjects and young subjects showed more closely related values. No other significant effects were found (Fig. 5.7A). Overall, the DLPFC, FEF, and CN exhibited the highest signal change in the high-performing older adults in both prosaccades and antisaccades. In prosaccades only, the PEF also followed this same trend. The SEF did not show this pattern.

5.4.5 Saccade execution

Figure 5.6B shows a volume map for antisaccade execution minus prosaccade execution. This allowed us to measure the BOLD signal change during the execution of the saccade only. BOLD signal changes were difficult to interpret in this volume map as the difference between antisaccade and prosaccade execution was not that different. However, the mean beta weights to determine group differences were more insightful (Fig. 5.7B). A one-way ANOVA to compare BOLD signal change between experimental groups revealed several frontal oculomotor ROI’s showing main effects: in the antisaccade execution-only contrast, the DLPFC (F(2, 41) = 18.020, p < 0.001), the FEF (F(2, 41) = 19.382, p < 0.001), and the SEF (F(2, 41) = 19.782, p < 0.001). Post-hoc analysis revealed that DLPFC activation differed between the young group and high-performing older group (p < 0.001), and the young group and poor-performing older group (p = 0.005), but not between poor-performing and high-performing older groups (p = 0.139, n.s.). FEF signal change showed similar results, only differing between young and older groups (both high-
performers (p < 0.001) and poor-performers (p < 0.001)), but not between the two older groups (p = 0.850, n.s.). The SEF showed differing levels of BOLD response in the same fashion; young subjects differed from high-performing older subjects (p < 0.001) and poor-performing older subjects (p < 0.001), but the older groups did not differ from each other (p = 0.937, n.s.). The CN and PEF did not differ between the experimental groups in the antisaccade execution contrast.

In the prosaccade execution-only contrast, only the FEF (F(2, 41) = 3.508, p = 0.039) and SEF (F(2, 41) = 8.107, p = 0.001) showed main effects for signal change differing between experimental groups. Post-hoc analysis confirmed that in the FEF, only young and the poor-performing older group differed from each other in the level of signal change (p = 0.034). The same pattern was seen in the SEF (p = 0.001). Overall, only the frontal ROIs (DLPFC, FEF, and SEF) showed higher signal change in both elderly groups compared to the young adults. No significant differences were seen in the PEF or CN.

5.5 Discussion

The purpose of this study was two-fold: to a) examine the neural correlates underlying saccade impairment in aging to see if altered activation in structures related to voluntary saccade processes correspond to changes in performance, and to b) determine if increased activation in frontal oculomotor regions corresponded to higher performance in the elderly when compared to lower performance in the elderly. We instead found that most oculomotor structures were in some way related to performance in the elderly. Firstly, we observed increased preparatory activity in three out of five ROIs (DLPFC, FEF, and CN) that was highest for the high-performing elderly. Secondly, we observed that the frontal oculomotor structures (DLPFC, FEF, and SEF) also showed the highest execution activity in both elderly groups compared to young adults, but did
not differ from each other. From these findings, we propose two things: the more efficient an elderly individual is at building up preparatory activity, the better they perform (e.g., generate fewer errors on antisaccade trials); and that elderly individuals appear to increase recruitment (and thus theoretically neural activity) to compensate for task demands. This second proposition has two facets: that ALL elderly, regardless of performance or task, tend to over-recruit neural areas related to saccade generation, but those that perform at a higher level recruit even more neural activity during the preparation stage of saccade generation. Interestingly, the motor, premotor (FEF and SEF) and prefrontal (DLPFC) areas are all influenced by basal ganglia output, which may explain why preparatory activity in the CN also was shown highest in high-performing elderly (suggesting that the CN may boost frontal signals to allow for better performance). Our data lead us to support two of the aging controversies to describe what could be happening in an elderly brain.

5.5.1 Aging controversies

There have been several proposed hypotheses of frontal changes in the elderly that help maintain or attenuate performance on a variety of cognitive tasks. These can be broken down into three major categories: compensation/adaptation, inhibition hypothesis, and dysfunction in deactivating the default mode network, described in that order. Firstly, it has been suggested that an over-recruited prefrontal cortex (PFC) may influence behaviour. The idea of compensatory over-recruitment or ‘cognitive reserve’ has been discussed in great detail (Cabeza et al., 2002; Tucker and Stern, 2011; Grady and Craik, 2000; Buckner, 2004), wherein older adults over-recruit frontal structures to maintain performance levels. Velanova et al (2007) found delayed PFC activity in older adults; however we have not yet assessed all time course curves. Dennis et al (2007) found less activity in medial temporal areas but more PFC activity suggesting that older
people had to engage more regions positively related to the task, perhaps explaining why they are slower. However, our elderly did not initiate significantly slower saccades than our young adults, so the shift in signal from visual areas to frontal areas may not be underlying the performance differences we have observed. Park and Reuter-Lorenz (2009) have suggested the elderly brain is adaptive, and engages a compensatory ‘scaffolding’ to protect cognitive function in the aging brain. All of these ideas revolve around the hypothesis that there is some sort of compensation occurring in an aging brain where some elderly can maintain a higher level of cognitive functioning. Our data support this hypothesis.

Secondly, an ‘inhibition hypothesis’ has been proposed, in which older adults cannot filter out irrelevant information (Gazzaley et al., 2005; Stevens et al., 2008). Our behavioural data do indeed support this hypothesis, in that elderly appear impaired at inhibiting unwanted saccades, rather than impaired at initiating voluntary saccades. We have also noted in unpublished data that the ability to filter out extraneous information measured with neuropsychology tests such as the Stroop paradigm (Stroop, 1935) correlates with the ability to ignore the peripheral stimulus when attempting a correct antisaccade (Supplementary Fig 5.1).
Supplementary Figure 5.1. Correlations (with Pearson r values) between saccade measures and Stroop score. Higher Stroop score indicates higher performance. (A, B) Prosaccade and antisaccade direction errors correlate with Stroop score. (C, D) Prosaccade and antisaccade CV correlate with Stroop score. * = p < 0.05

Thirdly, Stevens et al (2008) have also suggested that older adults may have a reduced ability to suppress default mode processing due to increased distractions hindering this ability, perhaps contributing to encoding failures. Our behavioural data do not show slowed responses in the elderly, implying that the older adults studied here may not have difficulties deactivating their default modes. Our functional data also implies changes in executive control mechanisms in the frontal cortex (Miller and Cohen, 2001). Therefore, overall our data primarily support both the compensation and inhibition hypothesis, where it looks like throughout the oculomotor network, high-performing older adults showed the highest level of preparatory activity, suggesting they
were able to use the instruction information to prepare their response. This could be further clarified by looking at time course curves to see if the peak of the BOLD signal in frontal regions is delayed in the elderly.

Furthermore, antisaccade trials have consistently been shown to elicit greater activation than prosaccade trials (Sweeney et al., 1996, Luna et al., 1998, Curtis and D’Esposito 2003, DeSouza et al., 2003, Connolly et al., 2005, Ford & Everling 2006, Brown et al., 2006, Curtis and Connolly 2008), which is suggested to reflect additional neural processes needed to generate an antisaccade (Munoz and Everling 2004). Therefore, an increase in activation in frontal voluntary areas should correspond to improved performance, or vise versa, a decrease in frontal activation should correspond to impaired performance. Our results reflect this phenomenon, including during saccade preparation, where the DLPFC, FEF, SEF, and PEF showed larger signal change for anti preparation versus pro preparation.

5.5.2 Neural circuitry

Logothetis and colleagues (Logothetis and Wandell, 2004) have suggested that the BOLD signal correlates with neural activity. Specifically, it seems to correlate with synaptic input more than spiking output. Therefore, reduced BOLD signal changes in the frontal oculomotor regions in the poor-performing older group could reflect reduced input signals from other areas, particularly those responsible for developing preparatory activity. For instance, no differences between pro and antisaccade trials were seen in the poor-performing older group in the oculomotor ROIs. We could therefore speculate that reduced input from the DLPFC or the CN (both known to influence the FEF) could cause reduced activation in the FEF, and so on. Whether the reduced input from DLPFC is caused by aging brain atrophy (e.g., tissue loss) or other factors
is yet to be determined in this subject pool. Lesions to the DLPFC in humans have been reported
to elicit more direction errors on antisaccade trials (Guitton et al., 1985), showing a reduced
ability to suppress automatic saccades to the visual stimulus. Our current and previous (Peltsch et
al., 2009) behavioural measures show that inhibiting the unwanted response is considerably
impaired in elderly, while initiating the voluntary response (measured by antisaccade SRT) is not.
Combined with reduced activation in frontal structures such as the DLPFC in poor-performing
elderly, this suggests deficits in executive control mechanisms caused by prefrontal dysfunction.
Thus, it is expected that in future studies, we should also find relationships between frontal
structures and behaviour. Previous studies have shown a relationship between brain volume and
saccade behaviour (Ettinger et al., 2005; Boxer et al., 2006). We have collected some preliminary
data that does indeed show that volume loss does increase with age, but is not different in the
high-performing versus poor-performing elderly (supplementary Fig. 5.2A). However, when all
aged-subjects are considered, both white and gray matter volume correlates to the percentage of
direction errors made by all subjects (supplementary Fig. 5.2B, C). Further studies will include
developing software than enables us to look directly at the volume of specific oculomotor
structures, like the DLPFC or FEF.
5.5.3 Limitations and future directions

Although we observed interesting differences between the two elderly groups as a function of performance, it is important to note that the age groups of the two groups differed. The poor-performing older group was aged 62 – 83 years, whereas the high-performing group was aged 61 – 78 years. This difference is not large, but we have previously shown how saccade characteristics can be altered with age (Chapter 2; Peltsch et al., 2009). In order to be sure the
differences are due to performance and not simply age alone, it is imperative in the future to include more subjects to age-match both groups more accurately.

We now have evidence that both a reduced ability to inhibit irrelevant information and that functional compensatory strategies may exist in the elderly. This data could provide insight into neurological disease, such as Mild Cognitive Impairment, Alzheimer’s disease and Huntington’s disease. A longitudinal study determining whether the elderly who can compensate for aging declines end up being more resilient to the onslaught of AD, or whether those who do not compensate end up being at-risk or later developing AD could provide informative. Firstly however, a cohort study is needed to determine if the oculomotor neural correlates are altered in the same way as in normal aging, or whether or not different structures come into play. For example, in HD the lack of input from the BG may influence DLPFC activation to a different extent than we’ve noted here, or in AD hippocampal changes may influence the frontal structures differently. Furthermore, determining if aMCI patients have the same functional changes measured with fMRI could be useful in further determining which patients end up being most at-risk for progression to AD.

5.6 References


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Chapter 6

General Discussion

These studies were conducted to explore the feasibility of using the antisaccade task as an objective tool for characterizing and assessing the behavioural and brain changes resulting from normal and abnormal aging. Thus, the aim was three-fold: 1) to demonstrate characteristics of oculomotor control across the elderly lifespan, 2) to measure executive dysfunction via the antisaccade task in age-related patient populations such as HD, aMCI, and AD and, 3) to examine the underlying neural correlates of saccade control and how these are functionally altered with normal aging. We provide evidence that the antisaccade task, a measure of voluntary behavioural control, can be successfully used to assess executive dysfunction in normal versus abnormal aging. We have found that reductions in executive abilities are increased across the elderly lifespan (age 60 years and up), and that the executive deficits observed in neurodegenerative diseases such as HD and AD are more severe than those seen in normal aging, and potentially discernable using saccadic eye movement tasks. The general hypotheses and major findings from each paper are summarized below.

Beginning with Chapter 2, I examined the precise rates of decline in prosaccade and antisaccade performance in individuals aged 60 years and older. We elucidated the patterns of eye movement deficits in aging to help determine both the feasibility of eye movement testing to evaluate the aging process and the aspects of the saccade system that are most resilient to the aging process. We proposed in this chapter that voluntary saccade control would be more influenced by aging than automatic saccade control. We also proposed that the performance decrements we observed should correspond with the natural cognitive slowing and cerebral
atrophy that occur in the aging brain (Aizenstein et al., 2004; Creasey and Rapoport, 1985; Kramer et al., 2007). We found that the generation of prosaccades, a simple sensory-motor process, was minimally influenced by age. In contrast, processes required for voluntary saccade initiation and voluntary saccade suppression were more sensitive to aging and the deficits correlated with age. For example, error rates increased dramatically – by approximately 11% from ages 60 to 85 years (Fig. 2.4F). We speculated that because different processes underlie the mechanisms driving antisaccades versus prosaccades, they likely degrade at different rates in the elderly.

We also explored the difference between prosaccades and antisaccades in Chapter 2. The increased processing required to complete antisaccades despite the same visual stimuli as prosaccades results in greater antisaccade error rates overall, demonstrating that there is a bias toward more automatic behaviour (Figures 2.2, 2.4). In aging, the ability to suppress that bias toward automatic responses was reduced. Inputs from both the basal ganglia (BG) and the frontal eye fields (FEF) are known to affect the prefrontal cortex and motor regions (Mink, 1996; Alexander et al., 1986), which we suggest may alter behaviour and reduce voluntary control.

In Chapter 3, we explored saccade behaviour in Huntington’s disease, a neurodegenerative disease where the primary brain atrophy begins in the striatum ((Purdon et al., 1994; Sharp and Ross, 1996). We confirmed deficits in executive function, and inferred that the pathological changes in the BG could be influencing the inputs to the FEF and PFC and thus inhibiting the ability to control behaviour. Because HD patients also showed deficits in automatic performance but to a lesser extent, and executive dysfunction increased as a function of disease
severity, we also speculated that changes in indirect pathway through the BG in HD patients influences frontal structures prior alterations in the direct pathway.

In Chapter 4, we investigated saccade characteristics in normal aging, aMCI, and mild AD. We proposed that AD patients would exhibit impaired voluntary saccade control via the antisaccade task, because mild AD pathology affects frontal regions (Buckner, 2004; Rabinovici et al., 2007), and frontal executive functions are impaired in mild AD (Balota and Faust, 2001; Takeda et al., 2010). We also expected that the similar brain and behavioral changes in aMCI (Morris et al., 2001) would elicit similar alterations in antisaccade performance, despite maintaining good executive function in some standard clinical tests. We determined whether the antisaccade task could measure executive dysfunction in aMCI patients that may aid in the future tracking of executive impairments and conversion to AD. We found similar phenomena in aMCI and AD patients to what we saw in HD, where although early pathology is reported to begin in the hippocampus (e.g., not necessarily in the frontal cortex), executive dysfunction was observed in both patient groups. The reduction in hippocampal inputs to frontal cortex could influence executive control.

Finally, in Chapter 5, we explored the neurological substrates of antisaccades compared to prosaccades in younger versus older subjects, and in high-performing elderly compared to poor-performing elderly. Knowing that the processes underlying antisaccades were far more sensitive to aging than prosaccades, we proposed that the underlying neurological substrates of antisaccades would be more affected by aging brain changes. We speculated that the frontal oculomotor regions such as DLPFC, FEF, and SEF should show the highest BOLD signal changes between young adults, high-performing older adults, and low-performing older adults
since these frontal regions appear to be over-recruited when compensating for poor performance (e.g., maintaining higher levels of performance). We found that high-performing older adults appeared to over-recruit areas in the frontal cortex critical for antisaccade control compared to poor-performing older adults, supporting the idea of cognitive reserve in the elderly.

6.1 Clinical relevance

The need for standardized, quick, easy objective tools to assist in the assessment of individuals with neurodegenerative disorders is necessary. In HD, where diagnostic tools such as genetic testing and neurological exams are already established, the antisaccade task could be used as a screening tool for HD gene carriers, as we found that error rates and intra-subject variability correlated with disease severity in HD. Similarly, Blekher and colleagues (Blekher et al., 2006) and Smith and colleagues (Smith et al., 2000) observed that fully diagnosed HD patients were more severely impaired than pre-symptomatic HD gene carriers, who were more impaired than controls in measures of movement control (oculomotor and reaching, respectively). Since we found this same trend, it appears that eye movement performance could be used to track disease severity or progression. Detection of a simple behavioural measure that correlates with functional disease progression is important to track clinical changes in HD patients and could also be used as a tool in controlled clinical trials to assess improvements in motor and functional abilities during therapeutic interventions.

The usefulness of saccade tasks for dementia would be somewhat different, although assessing changes in therapeutic interventions would be useful. Our data reveal that the antisaccade task shows potential for value if used in conjunction with other standardized clinical tests. It is now well appreciated that individuals who receive an early diagnosis and thus earlier
therapeutic intervention demonstrate significantly better outcomes, including societal costs (Teipel et al., 2007). First, the antisaccade task shows promise for being a predictive measure of disease progression in aMCI patients or mild stage AD patients (Fig 4.7). Second, the antisaccade task is a hands-free and language-free measure of executive control (Kaufman et al., 2010), which is ideal for a patient group like AD where language deficits are often profound, even early on in the disease (Benzi and Moretti, 1998). Furthermore, many of the standardized techniques in Canada that are available for clinical assessment are not ideal for culturally diverse populations (e.g., if English is not the first language), so the antisaccade task could provide another avenue for testing those non-proficient in English. We have preliminary data showing that antisaccade error rates correlate with the total Stroop score, a standardized test of selective attention (the ability to filter out irrelevant stimuli to focus on the task at hand), indicating that for someone with a language deficit (or lack of English proficiency), perhaps the antisaccade task could be an ideal substitute to measure this selective attention. Thus, the potential for saccadic eye movement tasks and tests of executive function have significant applicability for use as potential screening tools to assist in assessing the progression of aMCI.

6.2 Future directions

We examined a normal aging population in Chapter 2 and provided evidence that the antisaccade task is an ideal tool for tracking age-related brain and behavioural changes. Further similar normative studies are needed with larger populations to develop a set of normative standards. Then, patient groups can always be age matched, as we now know how much age can alter executive control (Chapter 2). These future data sets could be compared to the data generated from our study to further assess the validity of the antisaccade task for assessment purposes and potentially clinical use.
Based on performance on oculomotor tasks and functional neuroimaging, it appeared that adults over 60 years old have impairments in executive control and functional alterations in frontal lobe structures, in particular the DLPFC, FEF, and SEF. In particular, older individuals appear to over-recruit these frontal structures to successfully perform the antisaccade task, which may be a compensatory way to maintain good performance (e.g., SRT and errors similar to younger adults). To further support or refute this claim, a larger number of elderly subjects are needed to ensure age matching between both elderly groups. Furthermore, the relationship between structural and functional changes in the aging brain needs to be addressed. Do functional changes precede structural ones? Or does a loss of tissue lead to functional changes? Furthermore, “normal” aging needs to be re-defined. Brain alterations such as white matter hyperintensities have become so common (Polvikoski et al., 2010) that they are becoming “normal.” It may be more effective to differentiate successful versus unsuccessful aging (based on behaviour and performance).

In all chapters of this thesis, a common theme was that processes underlying voluntary control (assessed with the antisaccade task or memory-guided saccade task) are considerably less resilient to both healthy aging changes and disease states than processes underlying automatic control. Therefore, these measures proved more informative regarding executive dysfunction changes, or comparisons between experimental groups. On that note, future studies in this field could focus less on prosaccade or automatic control, and more on voluntary control.
6.2.1 Future directions for HD

Both our study and the study done by Blekher and colleagues (Blekher et al., 2006) found very comparable results. However, the memory-guided saccade task proved very difficult for HD patients in the more advanced stages. Further studies are necessary with more subjects at differing disease stages to determine the usefulness of the memory-guided saccade task in assessing HD. Furthermore, both these studies were cohort studies, but longitudinal studies of oculomotor control in HD patients may shed light on the progression of indirect and direct pathway influences on the cortical function. This would include pre-symptomatic carriers, early stage HD patients, and advanced-stage HD patients, as current therapeutics target the pre-diagnosis period (Blekher et al., 2006). For antisaccades or memory-guided saccades to be used in a clinical setting to determine disease progression and to begin therapeutic intervention, more laboratory testing of oculomotor control in HD is needed.

6.2.2 Future directions for dementia

Hippocampal cell loss has been reported in the earliest stages of dementia (Braak and Braak, 1995). Hippocampal function can also be probed using oculomotor tasks, such as the memory-guided saccade task (Muri et al., 2000). The memory-guided saccade task proved very difficult for HD patients, but would be a suitable way to assess working memory in individuals with aMCI and AD. Although memory impairments are typically assessed easily with clinical tests, oculomotor tasks would be an ideal replacement in patients with language difficulties, as mentioned previously. To re-iterate, one version of this task involves subjects being seated in front of a computer screen and after a brief delay, three target lights appear at random locations one after another. After another brief delay, subjects must recall the location and order of the target lights by moving their eyes in the same sequence. This task could also easily be adapted for
functional neuroimaging.

One hypothesis we proposed regarding frontal changes in aMCI and AD was that pathology in the hippocampus or medial temporal lobe, evident many years prior to symptom onset (Seahill et al., 2002; Smith, 2002) may influence frontal function due to loss of input from the medial temporal areas. Studies confirming hippocampal influences directly on the DLPFC are necessary to further support this idea. Technology such as Diffusion Tensor Imaging (DTI) would be useful in identifying specific fiber tracts between these structures. Complementary methodologies with good temporal resolution such as EEG may be useful in confirming the performance-related shift in activation seen in fMRI studies from parietal regions to frontal regions (Park and Reuter-Lorenz, 2009).

Similarly, the Jagust lab at the University of California Berkeley has found amyloid deposition in the striatum of elderly subjects that corresponds to behavioural changes (e.g., more amyloid deposition in striatum leads to poorer performance on cognitive tasks; unpublished). The BG are not commonly implicated in AD, but the striatum, part of the direct and indirect BG pathways, may boost or suppress function of the frontal cortex in oculomotor tasks (Cameron, 2010). Because saccade response inhibition rather than response initiation was primarily altered in AD, the DLPFC is the most likely candidate for influencing this behaviour. However, the indirect pathway of the BG is known to affect the FEF, which in turn affects DLPFC function. It would be interesting to confirm these projections with DTI, and for future studies to further examine the relationship between striatum amyloid and oculomotor function. One study has found that amyloid deposition begins in the striatum of familial AD patients before any symptom onset (Klunk et al., 2007). We unfortunately do not have genetic information for the patient
volunteers in this thesis, but some of our younger patients (60-65 yrs old) could have indeed been genetic mutation carriers. Therefore, future studies (post-mortem or amyloid imaging) to confirm patient brain pathology and genetic mutations would be insightful.

6.3 Conclusions

Overall, this thesis has provided evidence that reduced executive function abilities worsen considerably across the elderly lifespan, and may be distinguishable between different neurodegenerative diseases using the antisaccade task. Furthermore, specific frontal lobe regions are involved in executive control as measured by the antisaccade task, and these regions are altered in normal aging. Regions such as the DLPFC, FEF, and SEF have previously been shown to be more critical for antisaccade than prosaccade generation, but we now specifically understand the role they play in normal versus abnormal aging. This thesis focuses on executive dysfunction. But remember, many cognitive functions are preserved in the elderly, such as musical memory (Vanstone and Cuddy, 2010) and memory involving highly practiced skills and familiar information (Burke and Mackay, 1997). It isn’t all downhill after 30.

6.4 References


