INVESTIGATING COGNITIVE IMPAIRMENTS IN AMYOTROPHIC LATERAL SCLEROSIS (ALS) USING EYE MOVEMENTS AND FUNCTIONAL MAGNETIC RESONANCE IMAGING (FMRI)

By

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Abstract

Patients with Amyotrophic lateral sclerosis (ALS) often experience cognitive impairment that accompanies degeneration of the motor system. A valuable tool for assessing cognitive control over behaviour is the antisaccade task which requires: 1) inhibition of the automatic response to look towards an eccentric visual stimulus (prosaccade) to instead 2) redirect gaze in the opposite direction of the stimulus (antisaccade). Psychometric tests were used to quantify the degree of impairment, while eye tracking, functional magnetic resonance imaging (fMRI) and structural MRI were combined to identify the neural correlates of cognitive impairment in ALS. We predict ALS patients will have executive dysfunction and grey matter loss in executive and oculomotor control areas that will affect antisaccade performance and will alter the corresponding brain activation. ALS patients and age-matched controls participated in a rapid-event-related fMRI design with interleaved pro- and antisaccade trials. Catch trials (no stimulus presented after instructional cue to prepare pro- or antisaccade) allowed us to discern the preparatory period from the execution period. ALS patients were biased towards automatic saccade responses, and had greater difficulty with antisaccades relative to controls in terms of correct and timely responses. We found that worsened antisaccade performance in ALS correlated with the degree of cognitive impairment. Generally, we found trends of increased brain activation during the preparatory period of antisaccades in ALS patients compared to controls in most oculomotor areas; meanwhile few differences were seen during execution. Structural analyses revealed ALS patients had decreased grey matter thickness in frontotemporal and oculomotor regions such as the frontal and supplementary eye fields (FEF, SEF) and the dorsolateral prefrontal cortex (DLPFC). These findings suggest that loss of structural integrity and executive dysfunction may
elicit compensation mechanisms to improve functional and behavioural performance. Despite this compensation, ALS patients still performed worse on antisaccades than controls. Further investigation to expand the current data set should improve our ability to assuredly identify the neural correlates of cognitive decline in ALS, and may provide a model system to use for critical evaluation of future therapies and interventions for ALS.
Co-Authorship

This research was carried out by Kelsey Witiuk under the supervision of Dr. Douglas P. Munoz and Dr. Michel Melanson. The idea for this research project was conceived by Dr. Douglas Munoz and Dr. Michel Melanson, and was originally started by a medical student, Dr. Ryan McKee. Dr. Michel Melanson recruited participants with ALS from his neuromuscular clinics at Saint Mary’s of the Lake and Kingston General Hospitals. Dr. Ryan McKee collected preliminary data from eight ALS patients, while Kelsey Witiuk collected the remaining ALS participants, and performed all of the data analyses and writing of this thesis.
Acknowledgements

I would first like to express my deep gratitude for the opportunity to work under the primary supervision of Dr. Douglas P. Munoz for my Master’s thesis. I will be forever appreciative of the guidance, kindness and mentorship that Doug has provided me during the course of my MSc. Doug’s passion for neuroscience is infectious, and is clearly reflected by the dedicated members of his lab, and the high-calibre research that is produced. It has been a privilege to be a part of the Munoz lab extended family over the last 2 years.

I would also like to extend great thanks to my secondary supervisor Dr. Michel Melanson for providing me with the unique opportunity to work directly with ALS patients, an experience that has provided me with invaluable clinical and life skills. I truly appreciate Michel’s hard work on patient recruitment, and for encouraging my interests in neurology that extend beyond the requirements of my project.

To my lab mates: Dr. Ian Cameron, Dr. Alicia Peltsch, Dr. Nadia Alahyane, Rebecca Schwerdtfeger and Stephen Lee, thank you for all of your academic help and continuing friendship. In particular, I must thank Nadia, Ian, Alicia and Rebecca for ‘showing me the ropes’ of the Munoz lab, and for being the clinical fMRI and Nemo-task ‘trail-blazers’. I would like to thank Dr. Brian Coe, my go-to ‘Mr. Fix-It’, for his endless hours of tech support, help with fMRI data acquisition, and many laughs. I would like to thank Don Brien for his programming support and help with the structural analyses. I am also grateful for the administrative help offered by Ann Lablans, Lucy Russo-Smith and Kelly Moore.

I must extend a warm thank you to the ALS patients who participated in this study, and to their families for bringing them to appointments, and for providing support along the way.
Without their combined generosity, cooperation and patience, this research would never have taken place. It was truly a pleasure working with them, and their courage and resilience has provided me with great inspiration to remain dedicated to this research, even when discouraging obstacles were thrown my way.

Additionally, I would like to acknowledge the ALS Society of Canada for their great work, not only helping families affected by ALS, but also for their fundraising efforts at events like the ALS Walk. I had the honour of presenting my research at one of their fundraising events, and I am very appreciative of their enthusiasm towards ALS research. During their annual ALS Society of Canada conference, which I had the privilege of attending the past two years, I have gained invaluable experiences both academically and personally.

Most importantly, I must graciously thank my own support team. To my parents Sid and Karole, my sister Sarah and my extended family, thank you all for your words of encouragement, endless love and support. To my girls back home, thank you for always being there, for believing in me, and for trying to understand my long rants about my research. I must also give special thanks to Steve Lund for his unconditional support and friendship throughout this project, without which I would have been hard-pressed to make it to the finish line.

My apologies if I have inadvertently omitted anyone to whom acknowledgement is due. For any errors or inadequacies that may remain in this work, of course, the responsibility is entirely my own. This research was supported by operating grants from the Canadian Institutes of Health Research (CIHR) and the Bernice Ramsay Discovery Grant from the ALS Society of Canada.
Dedication

This thesis is dedicated to my late grandmother, Kathleen “Louise” Margaret Witiuk.

“You can dig a ditch (for a living) if you want, but it’s going to be an educated ditch.”

- Grandma
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<tr>
<td>AC-PC</td>
<td>anterior commissure – posterior commissure</td>
</tr>
<tr>
<td>ALS</td>
<td>Amyotrophic lateral sclerosis</td>
</tr>
<tr>
<td>ALSFRS-R</td>
<td>Amyotrophic lateral sclerosis Functional Rating Scale Revised</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>
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<tr>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>calcium ion</td>
</tr>
<tr>
<td>CD</td>
<td>caudate nucleus</td>
</tr>
<tr>
<td>CIHR</td>
<td>Canadian Institutes of Health Research</td>
</tr>
<tr>
<td>CNS-LS</td>
<td>Centre for Neurologic Study Lability Scale</td>
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<tr>
<td>COWA</td>
<td>Controlled Oral Word Association</td>
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<tr>
<td>COGNISTAT</td>
<td>Neurobehavioural Cognitive Status Examination</td>
</tr>
<tr>
<td>CSF</td>
<td>cerebrospinal fluid</td>
</tr>
<tr>
<td>CT</td>
<td>computed tomography</td>
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<tr>
<td>CV&lt;sub&gt;SRT&lt;/sub&gt;</td>
<td>coefficient of variation of SRT</td>
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<tr>
<td>DLPFC</td>
<td>dorsolateral prefrontal cortex</td>
</tr>
<tr>
<td>DTI</td>
<td>diffusion tensor imaging</td>
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<tr>
<td>EPI</td>
<td>echo-planar imaging</td>
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<tr>
<td>FA</td>
<td>flip angle</td>
</tr>
<tr>
<td>FBI</td>
<td>Frontal Behavioural Inventory</td>
</tr>
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<td>FDA</td>
<td>Food and Drug Administration</td>
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<td>FEF</td>
<td>frontal eye fields</td>
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<td>fMRI</td>
<td>functional magnetic resonance imaging</td>
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<td>FOV</td>
<td>field of view</td>
</tr>
<tr>
<td>FP</td>
<td>fixation point</td>
</tr>
<tr>
<td>FTD</td>
<td>frontotemporal dementia</td>
</tr>
<tr>
<td>FTLD</td>
<td>frontotemporal lobar degeneration</td>
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<tr>
<td>FUS/TLS</td>
<td>fused in sarcoma/translated in liposarcoma</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>--------------</td>
<td>--------------------------------------------------</td>
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<tr>
<td>FVC %pred</td>
<td>forced vital capacity percent predicted</td>
</tr>
<tr>
<td>FWHM</td>
<td>full width at half maximum</td>
</tr>
<tr>
<td>GLM</td>
<td>general linear model</td>
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<tr>
<td>GM</td>
<td>grey matter</td>
</tr>
<tr>
<td>HADS</td>
<td>Hospital Anxiety and Depression Scale</td>
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<tr>
<td>LGN</td>
<td>lateral geniculate nucleus</td>
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<tr>
<td>LMN</td>
<td>lower motor neuron</td>
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<tr>
<td>LPF</td>
<td>lateral prefrontal</td>
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<tr>
<td>MMSE</td>
<td>Mini Mental State Examination</td>
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<td>MND</td>
<td>motor neuron disease</td>
</tr>
<tr>
<td>MNI</td>
<td>Montreal Neurological Institute</td>
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<tr>
<td>MoCA</td>
<td>Montreal Cognitive Assessment</td>
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<tr>
<td>MP-RAGE</td>
<td>Magnetization Prepared Rapid Gradient Echo</td>
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<td>MRI</td>
<td>magnetic resonance imaging</td>
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<tr>
<td>MVIC</td>
<td>maximal voluntary isometric contraction</td>
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<tr>
<td>PD</td>
<td>Parkinson’s disease</td>
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<td>PEF</td>
<td>parietal eye fields</td>
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<td>PET</td>
<td>positron emission tomography</td>
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<td>PL</td>
<td>parietal lobe</td>
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<tr>
<td>PPRF</td>
<td>paramedian pontine reticular formation</td>
</tr>
<tr>
<td>rCBF</td>
<td>regional cerebral blood flow</td>
</tr>
<tr>
<td>SC</td>
<td>superior colliculus</td>
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<tr>
<td>SCI</td>
<td>intermediate layers of superior colliculus</td>
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<tr>
<td>SCs</td>
<td>superficial layers of superior colliculus</td>
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<tr>
<td>SD</td>
<td>standard deviation</td>
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<tr>
<td>SE</td>
<td>standard error</td>
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<td>SEF</td>
<td>supplementary eye fields</td>
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<td>SOD-1</td>
<td>superoxide dismutase-1</td>
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<td>SPECT</td>
<td>single photon emission computed tomography</td>
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<td>SRT</td>
<td>saccadic reaction time</td>
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<tr>
<td>Term</td>
<td>Definition</td>
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<tr>
<td>T</td>
<td>target</td>
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<tr>
<td>TDP-43</td>
<td>TAR-DNA-binding protein 43</td>
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<tr>
<td>TE</td>
<td>echo time</td>
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<tr>
<td>TR</td>
<td>repetition time</td>
</tr>
<tr>
<td>TTL pulse</td>
<td>transistor-transistor logic pulse</td>
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<tr>
<td>UMN</td>
<td>upper motor neuron</td>
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<tr>
<td>VF</td>
<td>verbal fluency</td>
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<tr>
<td>WM</td>
<td>white matter</td>
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<td>†, ‡, *, **</td>
<td>to highlight statistical significance where described</td>
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Chapter 1

General Introduction

Amyotrophic lateral sclerosis (ALS), also known as motor neuron disease (MND), is a progressive neurodegenerative disease affecting motor neurons in the cerebral cortex, brainstem and spinal cord. The neuropathology of ALS is marked primarily by degeneration of upper motor neurons (UMN) in the brainstem and motor areas of the cortex, and of lower motor neurons (LMN) in the brainstem and spinal cord (Brooks, 1994; Brooks, Miller, Swash, & Munsat, 2000). This is accompanied by a loss of alpha motor neurons in the anterior horn of the spinal cord and gliosis of the corticospinal tracts, the former causing voluntary muscles in the body to become denervated (Abrahams, Goldstein, Kew, & Brooks, 1996; Hofmann, Ochs, Pelzl, & Warmuth-Metz, 1998; Mu, He, Anderson, Springer, & Trojanowski, 1996). Denervation generates physical symptoms of muscle weakness, atrophy and loss of tone (Mu et al., 1996; Murphy, Henry, & Lomen-Hoerth, 2007a), which progress until the production of voluntary movement becomes impossible. ALS is more common in men than women (1.6:1) (Mitchell & Borasio, 2007), and most often affects people in their sixties. Women tend to have a later onset (Haverkamp, Appel, & Appel, 1995) which is associated with a worse prognosis than those who are diagnosed at a younger age (A. D. Rosen, 1978). In the majority of cases, patients rapidly decline in function and succumb to respiratory failure within 2 to 5 years of symptom onset (Hillel & Miller, 1989; Kwiatkowski et al., 2009; Mitchell & Borasio, 2007).

Historically, motor impairments have highlighted the progressive disability seen in ALS, with relative sparing of the sensory system (Brooks, 1994). Traditionally, ALS was thought to affect
the motor system but leave all cognitive functions intact; however within the last few decades there has been consistent evidence that cognitive impairments can be detected within a large proportion of patients (for review see: (Neary, Snowden, & Mann, 2000; Phukan, Pender, & Hardiman, 2007; Woolley & Katz, 2008)). Despite this growing body of evidence, the diagnostic criteria for ALS continue to omit the requirement of any cognitive symptoms for a positive diagnosis; diagnosis relies solely on confirmation of the physical symptoms associated with motor neuron loss.

With this increasing recognition of cognitively impaired individuals within the ALS population, it is an opportune time to explore this facet of the disease and gain new insights into how ALS is viewed and diagnosed. Because a decline in cognitive function can sometimes precede the onset of physical symptoms in ALS (Guedj et al., 2007; Mackenzie et al., 2005; Murphy et al., 2007a), it is likely that testing a patient’s cognitive status can serve as a valuable new screening tool, particularly for individuals with a family history of ALS. This thesis intends to document cognitive deficits identified through a combination of neuropsychological tests and other reliable tools, such as saccadic eye movement tasks designed probe executive function, or neuroimaging techniques such as functional and structural magnetic resonance imaging (MRI). In order for this multi-disciplinary method of studying cognitive impairment to be useful for disease diagnosis, we must first understand and document the fundamental cognitive, behavioural, functional and structural changes that can be expected over time when someone is afflicted with ALS. This thesis aims to investigate and record these changes in as many ALS patients as possible within the limited timeframe of a Master’s program.
1.1 Clinical and Diagnostic Features

The most widely-accepted diagnostic criteria for ALS are the Revised El Escorial Criteria (see Appendix A), set by The World Federation of Neurology (Brooks et al., 2000) which were adapted slightly from the original criteria (Brooks, 1994) to increase their overall sensitivity.

Location of symptom onset can vary from bulbar (brainstem) to cervical, thoracic or lumbosacral regions of the spinal cord, however a progressive spread of impairment from one region to another is necessary for diagnosis (Brooks et al., 2000). There is a lack of reliable biomarkers for ALS. Hence, the El Escorial criteria have established several classifications that quantify the certainty of diagnosis as determined by neurological exams, standard electromyographic needles and nerve conduction studies (Brooks et al., 2000). To receive a diagnosis of clinically ‘definite’ ALS, the patient must display both UMN (spastic muscle tone, exaggerated reflexes and clonus) and LMN (muscle weakness, atrophy, fasciculations) signs in the bulbar region and in at least two other spinal regions, or, alternatively, the presence of UMN and LMN symptoms in three spinal regions (Brooks et al., 2000). Clinically ‘probable’ ALS is diagnosed when UMN and LMN symptoms are present in at least two regions, with some UMN symptoms presenting rostrally to the LMN symptoms (Brooks et al., 2000). Diagnosis can only be confirmed in the absence of neuroimaging or electrophysiological findings supporting other disease processes that would explain the impairments (i.e. oesophageal carcinoma or myasthenia gravis) (Brooks et al., 2000; Mitchell & Borasio, 2007).

Patients can be categorized into sub-clinical groups based on the onset location of their symptoms. An initial loss of UMN, LMN, or both in the brainstem is associated with bulbar-onset ALS and is usually marked by a loss of cranial nerve functions (Hillel & Miller, 1989). Bulbar-
onset patients display signs of speech (dysarthria) and swallowing (dysphagia) difficulties with excessive saliva production (sialorrhoea) that is often accompanied by shoulder and neck weakness and noticeable emotional changes such as labile affect (Hillel & Miller, 1989; Mitchell & Borasio, 2007). Spinal-onset patients display initial LMN loss in various regions of the spinal cord and present with proximal or distal weakness of the limbs, which usually manifests as difficulty climbing stairs, weakened grasp, or foot drop (Hillel & Miller, 1989; Mitchell & Borasio, 2007). In comparison to spinal-onset, bulbar-onset is associated with a worse prognosis (A. D. Rosen, 1978) and more severe cognitive dysfunction (see Section 1.3).

1.2 Etiology

The etiology of ALS is uncertain, although hypotheses of viral infections (Mitchell & Borasio, 2007), toxic heavy-metals (Haverkamp et al., 1995; Mitchell, 1987), lifestyle-related risk factors (such as poor diet or smoking) (Armon, 2003), and oxidative stress leading to excitotoxic motor neuron death (for review see: (Carri, Ferri, Cozzolino, Calabrese, & Rotilio, 2003)) have previously been investigated. ALS is a rare disease with an incidence between 1.5 and 2.0 per 100,000 people per year, and a prevalence of 6 per 100,000 people (Mitchell & Borasio, 2007). Of these cases, it is estimated that 10% are genetically inherited (‘familial ALS’), while the remaining 90% of cases are ‘sporadic’ (Kwiatkowski et al., 2009; Mitchell & Borasio, 2007; Phukan et al., 2007). In familial ALS, the Mendelian pattern of inheritance is most often autosomal-dominant (Mitchell & Borasio, 2007), although instances of autosomal-recessive and x-linked inheritance have also been noted (Figlewicz & Orrell, 2003).

Pathological reviews suggest that ALS is often associated with aggregations of neurofilaments and misfolded proteins which induce a cascade of oxidative stress and
excitotoxic motor neuron death (Brown Jr, 1995; Carì et al., 2003). Of the identified causative genes in ALS, the superoxide dismutase 1 (SOD-1) locus on chromosome 21 accounts for approximately 20% of familial ALS cases (D. R. Rosen et al., 1993). SOD-1 is a potent antioxidant that is expressed in virtually every cell type, but in mutant forms increases neurotoxicity through mechanisms that are not yet understood (Banci et al., 2008; Brown Jr, 1995; Carì et al., 2003).

Another suspected cause of ALS is overproduction of the nervous system’s primary excitatory neurotransmitter: glutamate. Glutamate receptors are responsible for allowing large amounts of calcium (Ca$^{2+}$) to enter the cell, which is affiliated with excitotoxic cell death (Choi, 1992). Neurotoxic effects related to activation of glutamate receptors were documented in rat motor neurons that had been cultured in cerebrospinal fluid (CSF) recovered from ALS patients (Couratier et al., 1993). This hypothesis was further supported by findings that chronic increases in glutamate via blocked glutamate transport produced motor neuron toxicity in spinal cord slices (Rothstein, Jin, Dykes-Hoberg, & Kuncl, 1993). Riluzole™ is a glutamate-release inhibitor that has been clinically proven to slow the progression of ALS; it remains the only Food and Drug Administration (FDA) approved and efficacious therapy for ALS (Miller, Mitchell, Lyon, & Moore, 2007).

The aggregation of misfolded protein inclusions is a hallmark of neurodegenerative pathology (Forman, Trojanowski, & Lee, 2004). Ubiquitin-positive and tau-negative inclusions have been found in the cytoplasm of LMNs (Arai et al., 2006; Mackenzie, A H, & Feldman, 2005; Yoshida, 2004) and in cortical and hippocampal brain regions of patients with familial or sporadic ALS (Bergmann, Kuchelmeister, Schmid, Kretzschmar, & Schröder, 1996) and frontotemporal lobar degeneration (FTLD) (Jackson, Lennox, & Lowe, 1996). FTLD is a clinical
syndrome affecting the frontal and temporal lobes and is characterized by progressive dementia producing cognitive, language and behavioural changes. FTLD encompasses three sub-clinical syndromes (see Neary Criteria: (Neary et al., 1998; Neary et al., 2000)), most notably frontotemporal dementia (FTD), and is widely believed to represent a spectrum of disease sharing a common pathological entity with ALS (Arai et al., 2006; Bergmann et al., 1996; Jackson et al., 1996; Mackenzie et al., 2005; Neary et al., 1998; Strong et al., 1999; Wilson, Grace, Munoz, He, & Strong, 2001; Yang, Sopper, LeystraLantz, & Strong, 2003; Yoshida, 2004). This hypothesis of an ALS – FTLD continuum was further confirmed upon discovery of TAR-DNA-binding protein 43 (TDP-43), a component of ubiquitin-positive tau-negative inclusions, as the major disease protein in both ALS and FTLD (Neumann et al., 2006). TDP-43 is widely expressed in heart, lung, muscle, and brain tissue (Buratti et al., 2001), and is normally restricted to the nucleus but builds up in the cytoplasm during pathogenesis (Neumann et al., 2006).

Another mutation that is commonly linked to familial ALS is the fused in sarcoma/translated in liposarcoma (FUS/TLS) gene on chromosome 16 (Kwiatkowski et al., 2009). FUS/TLS proteins are responsible for diverse functions including RNA binding, and follow a similar pathological mechanism as TDP-43 (Kwiatkowski et al., 2009). The numerous documented protein alterations involved with ALS pathology (some of which were not discussed here in the interest of brevity), and the support of a common biological substrate between ALS and FTLD suggests that the cause of this disease is likely dependent on complex interactions between genetic predisposition and environmental factors.
1.3 Cognitive Impairment in ALS

Previously, changes in cognitive function had only been detected in a subgroup of 3% to 5% of ALS patients (Kew & Leigh, 1992; Neary & Snowden, 1996); however, more recent studies have identified some degree of cognitive impairment and/or behavioural disturbance in 30% to 50% of patients through neuropsychological assessments (Lomen-Hoerth et al., 2003; Massman et al., 1996; Ringholz et al., 2005). The cognitive impairments found in ALS patients are usually attributed to changes in frontal lobe, or ‘executive’ functions that include, but are not limited to, mental flexibility (Strong et al., 1999), abstract reasoning and problem solving (Flaherty-Craig, Eslinger, Stephens, & Simmons, 2006; Kew et al., 1993), attention and concentration (Frank, Haas, Heinze, Stark, & Münte, 1997; Massman et al., 1996; Ringholz et al., 2005), and internally guided responses such as verbal fluency (Abrahams et al., 2000; Abrahams et al., 2005; Abrahams, Leigh, & Goldstein, 2005; Evdokimidis et al., 2002; Frank et al., 1997; Ludolph et al., 1992; Massman et al., 1996; Murphy et al., 2007b). In addition to frontal lobe dysfunction, subtle deterioration of working memory (Abrahams et al., 1996; Abrahams et al., 1997; Abrahams et al., 2000; David & Gillham, 1986; Kew et al., 1993a; Neary et al., 1990; Ringholz et al., 2005; Strong et al., 1999), and occasional reports of language deficits (Neary et al., 1990) which worsen over time (Abrahams et al., 2005) have also been documented. The behavioural changes noted most often in ALS include apathy, irritability and aggression, loss of insight, social disinhibition, impulsivity, poor judgement, emotional lability (dramatic mood fluctuations), compulsiveness and rigidity of thinking (Merrilees, Klapper, Murphy, Lomen-Hoerth, & Miller, 2010; Phukan et al., 2007; Woolley & Katz, 2008).
1.3.1 Evidence of an ALS-FTLD continuum

FTD, the most common behavioural subtype of FTLD, is characterized by the Neary Criteria as a gradual decline in social and personal conduct accompanied by emotional blunting, change in character, and loss of insight (Neary et al., 1990; Neary & Snowden, 1996). These symptoms overlap substantially with cognitive and behavioural impairments seen in ALS, which further supports the hypothesis of a biological disease continuum. It is estimated that 15-25% of ALS patients have definite FTLD, although it is believed that up to 50% of patients display FTD-like deficits without meeting all criteria for official diagnosis (Lomen-Hoerth et al., 2003; Ringholz et al., 2005). In addition to the pathological (TDP-43) and behavioural evidence of an ALS and FTLD continuum, neuroimaging techniques have shown that the degree and pattern of degeneration in extramotor brain areas in ALS draws close similarities to that in FTD (Abrahams et al., 1996; Kew et al., 1993b). Furthermore, the degeneration of extramotor brain areas has been shown to increase with the degree of cognitive impairment (Ringholz et al., 2005; Wilson et al., 2001; Yang et al., 2003). This suggests that neuropsychological tests are useful for identifying cognitive and behavioural changes that likely result from frontotemporal pathogenesis, and may be extremely valuable in providing insight to the extramotor neurological changes that occur in ALS.

Cognitive impairments also exist in a number of other neurodegenerative diseases (Alzheimer’s, Parkinson’s, and progressive supranuclear palsy), so it is important to study the nature of impairment specific to ALS, in order to distinguish how ALS affects brain function in relation to other neurodegenerative diseases. The pattern of extramotor degeneration and resulting dementia seen in ALS with or without memory loss is pathologically and psychometrically distinct from the dementia associated with Alzheimer’s disease (Neary et al.,
1990; Takeda, Uchihara, Arai, Mizutani, & Iwata, 2009). However, it is not uncommon for patients who display dementia to be initially diagnosed with Alzheimer’s, and then switched to a diagnosis of ALS/FTD when they start to display motor symptoms, which can often appear after dementia begins (Heidler-Gary & Hillis, 2007).

The correct identification of FTD-like impairments in ALS patients through neuropsychological evaluation may be additionally beneficial since recent evidence found ALS/FTD comorbidity was associated with an adverse effect on survival in bulbar patients, more so than a diagnosis of ALS or FTD alone (Neary et al., 2000; Olney et al., 2005). It should be noted that while this adverse effect was independent from the patient’s age at bulbar onset, it was not independent from the type of onset (bulbar or spinal) (Olney et al., 2005). These findings must therefore be interpreted with caution, since bulbar-onset is associated with a worse prognosis (Neary et al., 2000; A. D. Rosen, 1978) and more profound executive dysfunction (Abrahams et al., 1997; David & Gillham, 1986; Lomen-Hoerth et al., 2003; Massman et al., 1996). Further caution must be applied considering that bulbar-onset occurs more frequently in patients with ALS/FTD (Neary et al., 2000; Olney et al., 2005). Meanwhile an absence of bulbar involvement does not imply an absence of cognitive impairment (Strong et al., 1999). In contrast, others have found no difference between bulbar- and spinal-onset ALS patients on tests of executive function at the initial test date (Frank et al., 1997; Ringholz et al., 2005; Strong et al., 1999), but found progressive frontal dysfunction in bulbar patients when tests were re-administered during follow-up evaluations (Strong et al., 1999).

Despite their usefulness in identifying FTD-like symptoms, the executive dysfunction and behavioural deficits seen in ALS are not exclusive to patients with bulbar-onset or ALS/FTD
comorbidity; deficits have also been reported, to a degree, in ‘non-demented’ ALS patients (Abrahams et al., 1997; Abrahams et al., 2005; David & Gillham, 1986; Talbot et al., 1995). Neuropsychological evaluations can therefore be employed not only to identify impairments in non-demented ALS patients, but can also play a key role in the early identification of dementia in ALS (Strong et al., 1999). However, some warn that mild and/or early dementia can sometimes present without any cognitive impairments; it has been recommended that tests sensitive to behavioural deficits be employed as well, in order to better detect more subtle forms of dementia that affect behaviour before cognition (Neary et al., 2000). Additionally, others advise that detection of dementia should rely on neuropsychological assessments in conjunction with pathological or neuroimaging evidence for the best predictive outcomes (Barson, Kinsella, Ong, & Mathers, 2000).

1.3.2 The importance of neuropsychological testing

Neuropsychological evaluations may also provide important insight into the degree of pathological involvement and the ensuing physical impairment in ALS; research has shown that the degree of cognitive impairment is correlated with greater motor deficits (Massman et al., 1996), the presence of dysarthria (although some non-dysarthric patients were also impaired) (Massman et al., 1996), and pseudobulbar palsy (a combination of dysarthria, dysphagia, and emotional lability signs) (Abrahams et al., 1997; David & Gillham, 1986; Lomen-Hoerth et al., 2003; Massman et al., 1996).

One of the most strikingly consistent reports of executive dysfunction in ALS has been shown through measures of verbal fluency. Verbal fluency reliably reflects executive function because it requires the generation of internally-guided responses and preserved working
memory, but is independent of primary linguistic abilities (Abrahams et al., 2000). ALS patients invariably demonstrate verbal fluency deficits in comparison to normative data (Abrahams et al., 2000; Frank et al., 1997; Kew et al., 1993a; Strong et al., 1999), with deficits even more marked in bulbar-onset patients (Strong et al., 1999). Additionally, verbal fluency is sensitive to subtle reductions in cognition that manifest early on in the disease, and these deficits remain consistent over time even in non-demented ALS (Abrahams et al., 2005). Furthermore, ALS patients who demonstrate verbal fluency impairment display decreased regional cerebral blood flow (rCBF) in the prefrontal cortices compared those who were unimpaired (Kew et al., 1993a).

Despite the increased use of neuropsychological tests to evaluate cognitive impairment in ALS, the degree and pattern of progression is still not well understood. A high level of attrition due to the rapidly progressive nature of ALS makes studying cognitive and behavioural changes over time within a cohort of patients extremely challenging. However, early administration of such tests may be vital for the early diagnosis of FTD in ALS which may precede, follow, or coincide with the onset of motor symptoms (Mackenzie et al., 2005) and is linked to adverse effects on survival (Olney et al., 2005). A recent study found that in 58% of ALS/FTD patients, the onset of FTD symptoms occurred approximately 3 years before the onset of ALS symptoms, while 38% had ALS/FTD concurrent onset (Guedj et al., 2007). A prospective study found that ALS patients who met the FTD criteria showed cognitive and behavioural decline, on average, more than 7 years before the onset of motor symptoms (Murphy et al., 2007b). Thus, it is useful to develop robust tools for identifying the FTD-like cognitive deficits in ALS, as FTD symptoms may provide an earlier marker for ALS diagnosis.
Considering the validity of neuropsychological tests in predicting the onset or severity of cognitive decline in ALS, the current study evaluated the degree of behavioural and cognitive impairment in ALS using measures of verbal fluency, working memory, attention, abstract reasoning, signs of FTD comorbidity, and emotional lability (see Methods for details). The current study also included evaluations of functional and physical abilities to assess the degree of progression on an individual basis. Anxiety and depression measures were taken to eliminate the possibility of the patient’s emotional state influencing their cognitive profile (see Methods).

1.4 Saccadic Eye Movement System

Saccades are rapid eye movements used to direct the fovea to an object or location of interest. Saccades offer a popular model for studying cognitive function and flexible control of behaviour because: 1) they can be triggered automatically towards novel stimuli as part of the ‘visual grasp’ reflex (Hess, Burgi, & Bucher, 1946), or suppressed when inappropriate to instead be elicited voluntarily towards a desired location; 2) they are easily implemented in clinical- or laboratory-based settings; 3) their properties and underlying brain areas are well understood; and 4) saccadic control is often impaired in a variety of clinical groups.

In the prosaccade task, subjects are instructed to look towards a peripheral target (T) immediately after it is presented, which requires an automatic saccade response that is often referred to as ‘visually-guided’ (Fig. 1.1A). In the prosaccade task, the target location and the saccade goal are compatible and therefore require a direct sensorimotor transformation (Munoz & Everling, 2004). In the antisaccade task (Hallett, 1978; Munoz & Everling, 2004), subjects are instructed to look away from T to its mirrored position. In order to do this, the subject must suppress the automatic response to instead make a voluntary saccade in the opposite direction.
of T (Fig. 1.1A). In this task, the target location and the saccade goal are incompatible, which requires a more difficult sensorimotor transformation of vector inversion (Munoz & Everling, 2004). The prosaccade and antisaccade instructions are conveyed to the subject by the colour of the fixation point (FP) (see Methods for details). Sometimes subjects make direction errors (i.e. erroneous prosaccade on an antisaccade trial), which they are encouraged to correct immediately in order to convey that they understood the task, but just had difficulty suppressing the automatic response (Fig. 1.1B).

**Figure 1.1: Schematic of prosaccade and antisaccade tasks.** (A) Subject is instructed to look from the fixation point (FP) either towards (prosaccade) or away from (antisaccade) the target (T) based on the colour of FP. Failure to suppress the automatic response results in a direction error (dashed arrow). (B) Example of eye position traces for the antisaccade task showing correct responses (solid line) and corrected direction errors (dashed line). FP disappears 200 ms before T appears, creating a gap. Latency of response is shown as saccadic reaction time (SRT). Figures adapted with permission from Munoz et al. (2007).
1.4.1 The oculomotor circuit

The oculomotor circuit is highly complex; several brain regions and multiple neuronal populations contribute to the control and production of saccades (Fig. 1.2). Brain regions including areas in the frontal and parietal cortices, the superior colliculus (SC), basal ganglia (BG), thalamus, paramedian pontine reticular formation (PPRF), and cerebellum have been identified as neural correlates of saccadic control through anatomical, radiological, physiological and clinical studies (Hikosaka, Takikawa, & Kawagoe, 2000; Hikosaka, Nakamura, & Nakahara, 2006; Leigh & Kennard, 2004; McDowell, Dyckman, Austin, & Clementz, 2008; Moschovakis, Scudder, & Highstein, 1996; Munoz & Everling, 2004; Pierrot-Deseilligny, Ploner, Muri, Gaymard, & Rivaud-Pechoux, 2002; Scudder, Kaneko, & Fuchs, 2002; Wurtz, Sommer, Paré, & Ferraina, 2001).
Visual information enters the oculomotor circuit through the retina to the lateral geniculate nucleus (LGN) of the thalamus where it is relayed to the visual cortex via the retino-geniculo-cortical pathway. Projections from the visual cortex extend directly to the superficial and intermediate layers of the SC (SCs/Sci) and carry information required for producing automatic saccades (C. E. Collins, Lyon, & Kaas, 2005; Schiller, Stryker, Cynader, & Berman, 1974).

Information from the visual cortex can also be carried, via the dorsal stream, to motor areas within the parietal lobes, such as the parietal eye fields (PEF) (Greenlee, 1999). The parietal
cortex transforms sensory coordinates into motor coordinates (Colby & Goldberg, 1999) and projects to the SCi (Paré & Wurtz, 2001), which is organized retinotopically to produce saccades to a specific location in space. The parietal cortex appears to be important for initiating automatic saccades, since lesions to the PEF significantly increases prosaccade latencies (Pierrot-Deseilligny, Rivaud, Gaymard, & Agid, 1991a). The PEF also act as an interface with oculomotor areas in the frontal cortex including the frontal eye fields (FEF), supplementary eye fields (SEF) and the dorsolateral prefrontal cortex (DLPFC) (Connolly, Goodale, Menon, & Munoz, 2002; DeSouza, Menon, & Everling, 2003; Pierrot-Deseilligny et al., 2002).

Lesions of the DLPFC result in higher percentage of direction errors made on the antisaccade task (Pierrot-Deseilligny et al., 1991a; Pierrot-Deseilligny et al., 2003), which suggests that DLPFC is responsible for the suppression of unwanted automatic saccades. In contrast, lesions to FEF cause a normal percentage of errors but result in longer antisaccade saccadic reaction times (SRTs) (Pierrot-Deseilligny et al., 2003; Rivaud, Müri, Gaymard, Vermersch, & Pierrot-Deseilligny, 1994), suggesting that FEF is more involved in producing correct antisaccades. SRT is a by-product of the time required to process visual information, choose a target and program a motor response, and varies depending on contrast, luminance, or the cognitive difficulty of the task (Leigh & Kennard, 2004). Lesion and neural recording studies reveal that SEF is involved with sequencing multiple saccades and the associated intrinsic decision-making process (Coe, Tomihara, Matsuzawa, & Hikosaka, 2002; Gaymard, Rivaud, & Pierrot-Deseilligny, 1993).

The FEF, SEF and DLPFC all project to the SCi to mediate voluntary saccade control, either through direct projections or indirectly through the primary input area of the BG, the caudate nucleus (CD) (Everling & Munoz, 2000; Huerta, Krubitzer, & Kaas, 1986; Huerta & Kaas, 1990).
Neural activity in CD has been correlated with automatic and voluntary saccade behaviour (Hikosaka et al., 2000), which can be modulated by reward (Hikosaka et al., 2006). In particular, the CD is presumably critically involved with online control of initiating antisaccades (Watanabe & Munoz, 2009). The BG has a number of pathways, the direct, indirect, and hyperdirect pathways (Fig 1.2), which are separately involved in triggering, suppressing or rapidly halting automatic responses through the nature of their connections with SC, respectively (Hikosaka et al., 2000). Inputs from these BG pathways and direct inputs from frontal and parietal cortices must be integrated by SC to produce eye movements (Hikosaka et al., 2000; Hikosaka et al., 2006).

Upon integrating the various inputs of descending projections from visual, parietal and frontal cortices and the BG, the SCi acts as a critical interface by sending motor commands to the ‘saccade generator’ in the brainstem: the PPRF. The PPRF is involved with sending motor commands to the six extraocular muscles, which ultimately produce the programmed saccade. PPRF is known to incorporate inputs from the SCi and cerebellum to monitor the accuracy of the eye movement using online visual feedback acquired during the execution of a saccade.

1.4.2 Express saccades

Automatic, visually-guided saccades are often so rapid that they occur before any cognitive processing has taken place; therefore their timeliness is determined by the amount of pre-target (before presentation of peripheral T) neural activity in the SC (Everling, Dorris, & Munoz, 1998; Leigh & Kennard, 2004). High levels of pre-target activity within the SC combine with the visual response to produce the most rapid automatic saccade: the ‘express’ saccade (Fischer & Boch, 1983; Fischer & Weber, 1993; Paré & Munoz, 1996). Typical express saccade latencies span 90 –
140 ms (Dorris, Pare, & Munoz, 1997; Fischer & Boch, 1983; Fischer & Weber, 1993; Munoz, Broughton, Goldring, & Armstrong, 1998) and are close to the minimum conduction time for a simple afferent and efferent command (Carpenter, 1981); in other words, the minimum time for a visual response to be converted into a motor command. Express saccades represent the visual response taking the most direct route from the visual cortex, through SCi, to the brainstem saccade generator where the motor command is executed (Dorris et al., 1997; Schiller, Sandell, & Maunsell, 1987).

Express saccades can occur when subjects have been instructed to execute a prosaccade, and there has been enough pre-target neural activity in SC to surpass the saccade threshold upon the occurrence of a visual response. The likelihood of an express saccade being generated is increased when the FP is removed before the presentation of the peripheral T to create a ‘gap’ condition (usually = 200 ms) where the subject sees a black screen and receives no visual input (Fig. 1.1B) (Dorris & Munoz, 1995). The gap condition facilitates express saccades by disengaging fixation neurons to reduce the inhibition of the retinotopic saccade map in SC, thereby allowing pre-target activity to build up so that when the visual response occurs, the threshold is readily surpassed to evoke an express saccade (Dorris & Munoz, 1995; Dorris et al., 1997; Fischer & Ramsperger, 1984; Fischer & Weber, 1997; Paré & Munoz, 1996). The SRTs for the gap condition tend to resemble a bimodal distribution, where the first peak of reaction times fall around 100 ms, making them ‘express’ saccades, while the second peak falls around 150 ms, making them ‘regular’ latency automatic saccades (Fischer & Boch, 1983; Schiller et al., 1987). Lesions to SC cause attenuation of express saccade production, suggesting express
saccades require an intact SC (Pierrot-Deseilligny, Rosa, Masmoudi, Rivaud, & Gaymard, 1991b; Schiller et al., 1987).

1.4.3 Preparatory set

The successful production of antisaccades depends upon executive control and the ability to suppress the visual grasp reflex, a phenomenon where visual input is directly transformed into a motor command within the SC to produce an orienting saccade (Munoz & Everling, 2004). In particular, the success of forthcoming saccades is often dictated by the amount of pre-target neural activity in the SC (Everling et al., 1998; Everling & Munoz, 2000) where lower levels of activity are associated with correct performance of the antisaccade task (Everling, Dorris, Klein, & Munoz, 1999). The amount of pre-target activity is also modulated by the likelihood of the target being presented in a particular location in the visual field (Dorris & Munoz, 1998; Paré & Munoz, 1996), with greater neural activity building up in areas that are predicted to display the visual response. This phenomenon is known as ‘preparatory set’ and refers to the task-appropriate amount of build-up neural activity in the SC, and is important for preparing the desired motor response (for example, lower levels of activity would be more desirable for producing correct antisaccades, while higher levels would be more desirable for producing fast express saccades).

Preparatory set in the SC can be influenced by other brain areas that project to it, particularly those involved with executive function; the frontal lobes are largely responsible for producing adequate inhibition of the saccade map to reduce pre-target activity in the SC and prevent erroneous prosaccades on an antisaccade task (Everling et al., 1998; Everling et al., 1999; Everling & Munoz, 2000). Inhibitory signals evoked in the frontal lobes and BG might
explain the higher levels of blood oxygen-level dependent (BOLD) signal that have been consistently reported in FEF, SEF, PEF (Brown, Vilis, & Everling, 2007; Connolly, Goodale, Goltz, & Munoz, 2005; Connolly, Goodale, Cant, & Munoz, 2007; Curtis & D'Esposito, 2003b; Everling & Munoz, 2000; Schlag-Rey & Amador, 1997), DLPFC (Guitton, Buchtel, & Douglas, 1985; Pierrot-Deseilligny et al., 1991), and CD (Watanabe & Munoz, 2009) when preparing for an antisaccade compared to a prosaccade. Pre-target activity in FEF is particularly important in predicting the resulting motor response, as it can influence the rate of antisaccade errors, percentage of express saccades, and can influence SRT (Connolly et al., 2002; Everling & Munoz, 2000). Therefore, the current investigation will focus on FEF, SEF, PEF, DLPFC, and CD as regions of interest (ROIs) (Fig. 1.2) for analysis of the functional magnetic resonance imaging (fMRI) data collected due to their projections to SC, their involvement with antisaccade preparatory set, and their ability to influence response times.

1.4.4 Eye movements in ALS

Saccades may function as a diagnostic tool for identifying neurological disorders (Leigh & Kennard, 2004), since eye movement deficits have been well documented in a number of clinical populations (Cameron, Watanabe, Pari, & Munoz, 2010; Chan, Armstrong, Pari, Riopelle, & Munoz, 2005; Green, Munoz, Nikkel, & Reynolds, 2007; Meienberg, Muri, & Rabineau, 1986; Munoz, Armstrong, Hampton, & Moore, 2003; Munoz, Armstrong, & Coe, 2007; Peltsch, Hoffman, Armstrong, Pari, & Munoz, 2008; Perneczky et al., 2011; Reuter & Kathmann, 2004; Serra, Derwenskus, Downey, & Leigh, 2003). Recent findings have shown promise for using saccades to distinguish ALS from its mimic disorders (Donaghy, Thurtell, Pioro, & Leigh, 2010b; Donaghy, Thurtell, Pioro, Gibson, & Leigh, 2011) because saccade properties will often deviate
when lesions overlap with oculomotor brain regions (Pierrot-Deseilligny, et al., 1991b; Pierrot-Deseilligny et al., 2003; Rivaud et al., 1994). Atrophy of the frontal lobes has been noted in ALS patients with FTD-like cognitive changes, where more widespread atrophy was associated with more substantial executive dysfunction and behavioural deficits (Abrahams et al., 1996; Abrahams et al., 2000; Guedj et al., 2007; Murphy et al., 2007b; Neary et al., 2000; Yoshida, 2004). In addition to being responsible for executive processes such as inhibition, the frontal lobes house oculomotor control areas such as the FEF and DLPFC, which are required to correctly perform the antisaccade task (Pierrot-Deseilligny et al., 2003; Rivaud et al., 1994). Arguably, if a proportion of ALS patients demonstrated widespread atrophy of the frontal lobes as a result of an ALS/FTD-related pathology, this would be expected to have implications on their ability to inhibit unwanted prosaccades in order to correctly perform an antisaccade.

Saccades also provide a unique opportunity to study one of the last functioning voluntary motor circuits in ALS (Leveille, Kiernan, Goodwin, & Antel, 1982; Martí-Fàbregas & Roig, 1993). The machinery required for producing an eye movement remains intact even in the late stages of ALS; motor neurons from the trochlear and oculomotor nuclei control most of the extraocular muscles and maintain their integrity according to pathological findings (Okamoto et al., 1993). Only when patients had been on end-of-life respiratory support did they sometimes develop ophthalmoplegia, a weakness of the extraocular muscles caused by damage to their nuclei, and therefore displayed saccadic abnormalities in terms of saccade latency and range of motion (Averbuch-Heller, Helmchen, Horn, Leigh, & Büttner-Ennever, 1998; Kato, Hayashi, & Yagishita, 1993). Therefore, it is reasonable to assume that if eye movement abnormalities are detected in ALS patients near the beginning of their prognosis, it is likely a result of upstream damage within
oculomotor cortical control areas, rather than a result of downstream deterioration of the trochlear and oculomotor nuclei causing ophthalmoplegia.

While there have only been a handful of studies evaluating eye movements in ALS, they have consistently demonstrated saccade deficits (Averbuch-Heller et al., 1998; Donaghy et al., 2010a; Donaghy et al., 2011; Evdokimidis et al., 2002; Leveille et al., 1982; Marti-Fàbregas & Roig, 1993; Ohki et al., 1994; Shaunak et al., 1995). Specifically, ALS patients generated more direction errors and had longer latencies for antisaccades than healthy controls (Donaghy et al., 2010a; Donaghy et al., 2010b; Shaunak et al., 1995); meanwhile, no abnormalities were documented for prosaccades (Shaunak et al., 1995). These impairments point to FEF and DLPFC inadequately suppressing the visual grasp reflex, and might be an indication of frontal lobe pathology in ALS.

There has been some evidence that different locations of symptom onset in ALS may influence SRTs; in particular, bulbar-onset patients tended to have longer prosaccade latencies than spinal-onset and controls (Donaghy et al., 2010a). Longer latencies were also noted during delayed and remembered saccade tasks and were partly attributed to a difficulty suppressing unwanted saccades, despite saccade accuracy being unaffected (Evdokimidis et al., 2002). In the same study, the percentage of direction errors on the antisaccade task correlated with the degree of frontal lobe impairment and occurred more often in patients displaying bulbar symptoms such as dysarthria (Evdokimidis et al., 2002). Pseudobulbar palsy (which includes dysarthria) in ALS has previously been associated with executive dysfunction (Abrahams et al., 1997), and likely explains the greater difficulty for dysarthric patients to inhibit unwanted saccades. The greater saccade impairments seen in bulbar ALS patients might be attributed to a
more advanced deterioration of cortical areas involved with saccade control, or perhaps is a result of more extensive pathological involvement of the brainstem.

Saccadic intrusions are involuntary conjugated eye movements that occur naturally during fixation in healthy adults, and are usually characterized by an initial fast saccade away from the FP that is followed by a slightly delayed second saccade or drift back to the fixation location (Abadi & Gowen, 2004). Saccadic intrusions have also been reported in ALS patients (Donaghy et al., 2009; Marti-Fàbregas & Roig, 1993); however, the conditions under which these intrusions occur are conflicting. Intrusions happened significantly more in ALS patients than controls when fixating on a central FP (Shaunak et al., 1995); while others only found this when no FP was present (Donaghy et al., 2009). Additionally, some have reported an association between the frequency of intrusions and bulbar symptoms (Marti-Fàbregas & Roig, 1993), while others found this association to be stronger in spinal-onset patients (Donaghy et al., 2009). Despite this controversy, it is generally accepted that intrusions during fixation occur more often in ALS patients with impaired frontal lobe functions (Donaghy et al., 2009; Shaunak et al., 1995) and might be attributed to attentional shifts during fixation (Abadi & Gowen, 2004).

In summary, the saccadic behaviour previously documented in ALS patients such as increased antisaccade latencies, greater antisaccade direction errors, and more saccadic intrusions, all appear to occur more frequently in those patients with impaired executive control and likely points toward ALS/FTD pathology. Therefore, measures of saccadic performance can provide important insight into the degree of cognitive impairment in ALS patients.
1.5 Neuroimaging in ALS

While eye movements and neuropsychological tests have previously been employed simultaneously in ALS research, it is believed that no other study has combined these techniques with neuroimaging to provide a more holistic approach to studying the neural correlates of cognitive impairment in ALS. There have been a number of neuroimaging techniques employed by others to explore the structural and functional changes that occur in the ALS brain, ranging from magnetic resonance imaging (MRI), to single-photon emission computed tomography (SPECT), to computed tomography (CT), to positron emission tomography (PET), each with their own advantages and disadvantages. The current investigation used fMRI to study the degree of brain activation (in terms of BOLD signal) in combination with detailed structural images acquired using an MRI to evaluate changes in cortical grey matter.

There has been substantial evidence pointing to abnormalities in brain regions that lie outside of the motor cortex in ALS (Abrahams et al., 1996; Abrahams et al., 2000; Abrahams et al., 2005; Chang et al., 2005; Guedj et al., 2007; Neary et al., 2000). ALS patients tend to have reduced perfusion (in terms of rCBF) in frontal, temporal, and parietal regions (Abrahams et al., 1996; Guedj et al., 2007; Kew et al., 1993a; Kew et al., 1993b; Talbot et al., 1995) in addition to lower overall cortical metabolism (in terms of nutrient uptake measured by SPECT), which tends to be more marked in patients displaying cognitive impairment (Abe et al., 1997; Ludolph et al., 1992; Neary et al., 1990; Talbot et al., 1995).

Functional differences have been reported previously in ALS patients using BOLD fMRI, where reduced brain activation was found in premotor, supplementary motor and parietal cortical areas (Tessitore et al., 2006). The structural changes that are most commonly noted in
ALS, with or without an abnormal cognitive profile, include neuronal loss in the motor cortex of bulbar-onset patients, which also becomes evident later on in spinal-onset patients (Strong et al., 1999; Strong, Kesavapany, & Pant, 2005). Additionally, enlarged ventricles (Frank et al., 1997), and reductions in white (Abrahams et al., 2005) and grey matter (Chang et al., 2005; Murphy et al., 2007b) within motor, premotor and frontotemporal regions were previously described in ALS patients, although reductions were more substantial in those patients with cognitive impairment. BOLD signal is predominantly measured from areas of grey matter; therefore, if a reduction in grey matter within frontotemporal regions is characteristic of the cortical degeneration in ALS, it may translate to changes in the level of brain activation in these same areas.

White matter changes within the corticospinal tract are a classic pathological finding in ALS (Hofmann et al., 1998; Neary et al., 2000; Thivard et al., 2007; Toosy, Werring, Orrell, & Howard, 2003). Studies using diffusion tensor imaging (DTI) to investigate the integrity of white matter tracts in ALS have found reduced diffusion in the corticospinal tracts in multiple spinal segments and in subcortical and cortical areas (Nair et al., 2010; Thivard et al., 2007; Toosy et al., 2003). A change in the integrity of white matter tracts has clinical relevance and may underlie the functional and cognitive impairment seen in ALS due to a lack of ‘connectedness’ between subcortical areas. Interestingly, extramotor structural abnormalities have been noted even in non-demented ALS patients, suggesting that structural changes may precede or perhaps be responsible for the onset of cognitive abnormalities (Abrahams et al., 2003). This may have valuable implications for using structural MRI as a biomarker for the early detection of the
pattern of cerebral involvement that is associated with the ALS/FTLD continuum (Murphy et al., 2007b; Talbot et al., 1995).

1.6 Thesis Objectives

The main objective of this thesis is to gain new insights into the cognitive impairments seen in ALS as the first study to combine eye movement recordings and neuropsychological assessments with structural and functional MRI. I intend to capitalize on the overlap between the oculomotor circuit and the brain regions that are pathologically altered in ALS to highlight the neurological changes that occur in this disease. I hypothesize that a significant proportion of ALS patients will have cognitive disturbances that will alter their flexible control over behaviour and will be revealed in eye movement tasks designed to probe this flexibility. Behaviourally, I expect to see task-related differences across prosaccades and antisaccades in measures of SRT, coefficient of variation in SRT ($CV_{\text{SRT}}$), and direction errors. More specifically, I predict slower antisaccade SRTs and more antisaccade direction errors across ALS and control groups due to the added cognitive processing required to perform the antisaccade task (prosaccade suppression, attention redirection, saccade vector inversion) (DeSouza et al., 2003; Munoz & Everling, 2004). This same task difference should apply to the functional data; I hypothesize that antisaccades should elicit greater brain activation during saccade preparation than prosaccades in terms of BOLD signal in the brain areas that are involved with executive function and oculomotor control such as DLPFC and FEF (Pierrot-Deseilligny et al., 1991a; Pierrot-Deseilligny et al., 2003) and in other oculomotor regions that modulate SC activity such as SEF, PEF, and CD. I expect reductions in the amount of grey matter in ALS patients compared to controls, both globally and specifically in frontotemporal regions and I predict this reduction in grey matter will
correspond to functional overcompensation during the saccade tasks. Ultimately, my aim in combining these techniques is to identify the neural substrate for some of the cognitive impairments in executive function that are present in ALS.
Chapter 2

Investigating cognitive impairments in amyotrophic lateral sclerosis using saccadic eye movements and functional magnetic resonance imaging

2.1 Introduction

Amyotrophic lateral sclerosis (ALS) is a terminal neurodegenerative disease affecting the upper and lower motor neurons that causes progressive weakness and eventual respiratory failure. While ALS has traditionally been thought of as a motor disease, recent findings provide evidence that up to 30-50% of ALS patients display some degree of cognitive or behavioural impairment through neuropsychological examination (for review see: (Neary et al., 2000; Phukan et al., 2007; Woolley & Katz, 2008)). Cognitive abilities associated with ‘executive functions’ are most vulnerable to decline in ALS because the pattern of extramotor pathological degeneration typically follows that of frontotemporal lobar degeneration (FTLD) and is thought to share a common biological disease continuum (Abrahams et al., 1996; Kew et al., 1993; Lomen-Hoerth et al., 2003; Ringholz et al., 2005). In addition to executive processing capabilities, the frontal lobes play a key role in the initiation of voluntary saccades and the suppression of saccades to irrelevant visual stimuli (via their projections to the superior colliculus (SC) (Everling et al., 1999; Munoz & Everling, 2004; Segraves & Goldberg, 1987) and basal ganglia (BG) (Hikosaka et al., 2000; Hikosaka et al., 2006).

Saccades can help provide measures for identifying neurological disorders (Leigh & Kennard, 2004; Munoz & Everling, 2004; Munoz et al., 2007; Ramat, Leigh, Zee, & Optican, 2007), as
different saccade deficits have been documented in a number of clinical populations through the use of various oculomotor tasks (Cameron et al., 2010; Chan et al., 2005; Green et al., 2007; Meienberg et al., 1986; Munoz et al., 2003; Munoz et al., 2007; Peltsch et al., 2008; Perneczky et al., 2011; Reuter & Kathmann, 2004; Serra et al., 2003). More specific to ALS, saccades provide a valuable tool for probing the variable cognitive deficits that have been consistently reported in the literature that closely resemble deficits of frontotemporal dementia (FTD), a sub-type of FTLD (for review see: (Sharma et al., 2011)). Finally, the oculomotor network provides the unique opportunity to study one of the last functioning voluntary motor circuits in ALS, due to the progressive loss of motor control (Leveille et al., 1982; Marti-Fàbregas & Roig, 1993).

The ‘prosaccade’ task (Fig. 1.1A) requires subjects to look towards a peripheral visual target when it appears, and provides a measure of their ability to generate automatic, visually-triggered saccades. Visual input travels from the retina to the visual cortex, where it can extend directly to the superior colliculus (SC) (C. E. Collins et al., 2005; Schiller et al., 1974) or to parietal areas via the dorsal stream to guide an automatic saccade (Greenlee, 1999). ‘Express’ saccades are elicited on prosaccade trials when pre-target neural activity in SC is sufficient to surpass the saccade threshold upon the occurrence of a visual response. In an express saccade, the visual response takes a direct route from the visual cortex, through the SC, to the brainstem saccade generator (Dorris et al., 1997; Schiller et al., 1987) where the motor command is executed.

The ‘antisaccade’ task (Fig. 1.1A) requires subjects to suppress the automatic saccade to instead generate a saccade in the opposite direction of the target, providing a measure of voluntary control (Hallett, 1978; Munoz & Everling, 2004). A properly executed antisaccade requires intact frontal lobes, in particular the dorsolateral prefrontal cortex (DLPFC) and frontal
eye fields (FEF) which are responsible for suppression of unwanted saccades and initiation of voluntary saccades (Guitton et al., 1985; Pierrot-Deseilligny et al., 1991a; Pierrot-Deseilligny et al., 1991b; Pierrot-Deseilligny et al., 2003; Rivaud et al., 1994). Antisaccade deficits, including increased occurrence of direction errors (erroneous prosaccades) and longer latencies, have been reported in ALS (Donaghy et al., 2010a; Donaghy et al., 2010b; Shaunak et al., 1995), with a relative absence of prosaccade deficits (Shaunak et al., 1995). These oculomotor impairments may arise from the widespread pathological involvement of the frontal lobes in ALS, which is often accompanied by executive dysfunction and behavioural deficits (Abrahams et al., 1996; Abrahams et al., 2000; Guedj et al., 2007; Murphy et al., 2007b; Neary et al., 2000; Yoshida, 2004). ‘Catch trials’ and ‘fixation trials’ were necessary for isolating preparation processes from execution processes due to the nature of fMRI rapid event-related designs (Dale, 1999; Ollinger, Shulman, & Corbetta, 2001) (see Section 2.2.7). Catch trials were identical to prosaccade and antisaccade trials, however no peripheral target appeared.

To investigate the neural substrate of cognitive impairment and voluntary saccade control in ALS, we combined neuropsychological evaluations, oculomotor tasks such as the prosaccade and antisaccade, functional magnetic resonance imaging (fMRI) and structural MRI in group of patients compared to age-matched controls to gain new insights into cognitive decline in ALS. To our knowledge, this study marks the first to combine all of these techniques.

We hypothesize that a significant proportion of ALS patients will display cognitive impairments that will alter their ability to exert flexible voluntary control over eye movement behaviour. More specifically, we predict changes in the amount of brain activation (in terms of blood oxygen-level dependent (BOLD) signal) in areas involved with executive function and
oculomotor control such as DLPFC and FEF, and in oculomotor regions that modulate SC activity such as supplementary and parietal eye fields (SEF/PEF) and the caudate nucleus (CD). We further hypothesize that structural analyses of cortical thickness will reveal decreased grey matter in ALS patients compared to controls, both globally and specifically in frontotemporal and oculomotor control regions, which will likely be more marked in patients displaying cognitive impairment typical of FTD.

2.2 Methods

2.2.1 Subjects

All experimental procedures were approved by the Queen’s University Research and Ethics Board, and complied with the principles of the Canadian Tri-council Policy Statement on Ethical Conduct for Research Involving Humans and the principles of the Declaration of Helsinki (1964). All subjects were recruited through the Queen’s University community or through the ALS clinics at Kingston General Hospital and Saint Mary’s on the Lake Hospital, and gave their written and informed consent. Twenty-one ALS patients (ages 44 – 89 years, 3 females, mean age = 63.3, SD ± 11.1) diagnosed by a neurologist (co-author M.M.) as having ‘definite’ or ‘probable’ ALS (See Appendix A) (Brooks, 1994) were recruited for this study. Some ALS patients were unable to complete all parts of this research protocol for various reasons; some patients were excluded from the analysis based on their inability to complete the MRI session due to respiratory compromise (N=2) (see Section 2.2.2), claustrophobia (N=1), MRI safety screening problems (N=1), magnet trigger failure (N=1), complications with eye tracking (N=3), or head motion exceeding 3 mm in any direction during scans (N=1) (Table 1).
Table 1: Clinical information for all recruited ALS patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (yrs)</th>
<th>Edu. Mo. (yrs)</th>
<th>Clinical Evaluation</th>
<th>Neuropsychological Evaluation</th>
<th>completion</th>
</tr>
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<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td>El Escor</td>
<td>FVC %pred</td>
<td>ALS FR-S-R</td>
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<tr>
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<td>M</td>
<td>71</td>
<td>14 60</td>
<td>prob</td>
<td>78</td>
<td>38</td>
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<td>2</td>
<td>M</td>
<td>69</td>
<td>16 20</td>
<td>def</td>
<td>90</td>
<td>41</td>
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<tr>
<td>3</td>
<td>M</td>
<td>44</td>
<td>12 84</td>
<td>def</td>
<td>92</td>
<td>29</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>68</td>
<td>18 12</td>
<td>def</td>
<td>101</td>
<td>41</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>54</td>
<td>14 168</td>
<td>def</td>
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<tr>
<td>6</td>
<td>M</td>
<td>53</td>
<td>13 13</td>
<td>def</td>
<td>100</td>
<td>31</td>
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<tr>
<td>7</td>
<td>F</td>
<td>53</td>
<td>15 13</td>
<td>def</td>
<td>62</td>
<td>28</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
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<td>def</td>
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</tr>
<tr>
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<td>M</td>
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<td>16 16</td>
<td>def</td>
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<td>M</td>
<td>66</td>
<td>16 10</td>
<td>def</td>
<td>77</td>
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<tr>
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<td>62</td>
<td>13 11</td>
<td>def</td>
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<td>75</td>
<td>19 18</td>
<td>def</td>
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</tr>
<tr>
<td>14 (resp)</td>
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<td>def</td>
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<td>10 12</td>
<td>def</td>
<td>84</td>
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<td>54</td>
<td>16 18</td>
<td>def</td>
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<td>25</td>
</tr>
<tr>
<td>17 (fs)</td>
<td>M</td>
<td>67</td>
<td>20 60</td>
<td>def</td>
<td>55</td>
<td>21</td>
</tr>
<tr>
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<td>45</td>
<td>13 12</td>
<td>def</td>
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</tr>
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<td>89</td>
<td>9 60</td>
<td>prob</td>
<td>91</td>
<td>33</td>
</tr>
<tr>
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<td>M</td>
<td>70</td>
<td>8 12</td>
<td>prob</td>
<td>73</td>
<td>29</td>
</tr>
<tr>
<td>21 (mo)</td>
<td>M</td>
<td>71</td>
<td>9 12</td>
<td>prob</td>
<td>95</td>
<td>36</td>
</tr>
</tbody>
</table>

| Mean (n=21) | 18M | 63.3 | 14 32.6 | 79.81 | 34.4 | 19.9 | 57.2 | 57 | 23.24 | 6.14 | 5.05 | 8.32 | 11 12.42 | 2.85 | 5.16 | 21 | 19 | 13 | 12 | 16 |
| Mean (n=12)  | 10M | 61.6 | 14.8 37.3 | 83.08 | 36.3 | 19.3 | 58.9 | 57 | 24.83 | 6.75 | 5.33 | 6.11 | 11     | 11 | 3     | 4  |   |   |   |    |

| Controls (n=12) | 9M | 62.6 |

| 32 |

| 32 |
ALSFRS-R, ALS Functional Rating Scale Revised version; behav, saccadic behaviour; CNS-LS, Centre for Neurologic Studies Lability Scale, Cognistat R/J, Verbal Reasoning and Judgment questions from Neurobehavioural Cognitive Status Examination; COWA, Controlled Oral Word Association test; def, definite diagnosis; Edu., education; El Esc, El Escorial Criteria; FBI, Frontal Behavioural Inventory; fMRI, functional magnetic resonance imaging; FVC %pred, forced vital capacity percent predicted when sitting; HADS D/A, Depression and Anxiety measures of Hospital Anxiety and Depression Scale; Hand, Modified Edinburgh Handedness Inventory; kg, kilograms; LLT, lower limb total of Manual Muscle test; Med, Medications; Mo. since diag., months since diagnosis; MoCA, Montreal Cognitive Assessment; MVIC, maximum voluntary isometric contraction; n/a, not applicable; prob, probable diagnosis; Struct, structural anatomical scan; ULT, upper limb total of Manual Muscle test; VFI verbal fluency index; Yrs, years; ‘(blank)’ not assessed; ‘?’ no information

Exclusions:
resp, respiratory compromise
tr, failed eye tracking
ax, HADS anxiety score considered abnormal
f, could not perform task
nc, neurological confound (stroke causing significant atrophy)
mo, motion > 3 mm in any given direction
mt, magnet trigger failed
fs, failed safety screening
clau, claustrophobia

ALS

<table>
<thead>
<tr>
<th>Medications</th>
<th>(n=21)</th>
</tr>
</thead>
<tbody>
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</tr>
<tr>
<td>Antianxiety</td>
<td>3</td>
</tr>
<tr>
<td>Antidepressant</td>
<td>5</td>
</tr>
<tr>
<td>Antiepileptic</td>
<td>1</td>
</tr>
<tr>
<td>Antihypertensive</td>
<td>7</td>
</tr>
<tr>
<td>Atypical antipsychotic</td>
<td>1</td>
</tr>
<tr>
<td>Baclofen</td>
<td>6</td>
</tr>
<tr>
<td>Riluzole</td>
<td>6</td>
</tr>
<tr>
<td>Other†</td>
<td>9</td>
</tr>
</tbody>
</table>

†Antibiotic, Antihistamine, Antiarthritis, Anticholesterol, Anticoagulant, Aspirin, Antidiabetic, Antiinflammatory, COPD treatments
Only 12 ALS patients (ages 44 – 76, 2 females, mean age = 61.6, SD ± 9.6) completed all experimental components and were used in the final functional analysis. Behavioural data were successfully acquired from 13 ALS patients, while structural images were available from 16 patients. Twelve controls were age-matched within 3 years of ALS patients (ages 41 – 76 years, 3 females, mean age = 62.6, SD = 10.8) and were included for comparison in the functional analysis. Thirteen age-matched controls were used for comparison in the behavioural analysis, while 16 controls were used for structural analysis.

No subjects reported a history of diagnosis with other neurological or psychiatric conditions, with the exception of one ALS patient who experienced menopausal-related anxiety over 20 years previous. No subjects had any visual disturbances and all subjects had normal or corrected-to-normal vision, and two ALS patients verified that they could distinguish the stimuli without their glasses. All participants included in the final analyses (behaviour, function, and structure) had no history of stroke or traumatic brain injury, with the exception of two ALS patients who each experienced one concussion over 30 years ago. Because of the difficulty associated with collecting fMRI data from ALS patients due to the physical restrictions of the disease (such as compromised breathing), we felt the benefits of including the patients with histories of concussion and menopausal anxiety to have a larger data set would outweigh the risks associated with including them, and therefore these patients were not excluded.

Subjects participated in a two-session experiment at the Kingston General Hospital and at the Queen’s University Magnetic Resonance Imaging facility (http://www.queensu.ca/neuroscience/MRI-facility.html), respectively, with each session lasting 1-2 hours in length. In the first session, controls were screened for mild cognitive impairment
using the Montreal Cognitive Assessment (MoCA) (Nasreddine et al., 2005) or the Mini Mental Status Examination (MMSE) (Folstein, Folstein, & McHugh, 1975) and were excluded if they scored below a cognitively normal range (<26/30). ALS patients underwent a more rigorous battery of neuropsychological evaluations, in addition to having a clinical evaluation in the first session to evaluate the severity of their functional decline (see Section 2.2.2). Both controls and patients completed the second session on a separate day, which involved partaking in the MRI study and performing a saccadic eye movement paradigm (see Section 2.2.3). Control subjects always completed the first and second sessions within three months of each other, however due to the rapid functional decline associated with ALS, patients completed the first and second sessions within 5-30 days to avoid substantial degeneration between the clinical evaluation and the behavioural and imaging evaluations.

**2.2.2 Clinical Evaluation**

Clinical evaluation of ALS patients was modelled after a previously described rapid screening battery used in ALS patients to measure physical function and frontal lobe impairments (Flaherty-Craig et al., 2006). These measures included pulmonary function tests of forced vital capacity in terms of percent predicted (FVC %pred) when sitting upright. Patients who had a FVC < 60%pred were considered to have insufficient pulmonary function to lie supine in the MRI, and therefore were excluded from completing the second session (N=2) (Table 1). The self-administered ALS Functional Rating Scale Revised version (ALSFRS-R) was included to evaluate the degree of physical impairment in ALS (Cedarbaum et al., 1999).

Neuropsychological evaluation using the rapid screening battery included the Neurobehavioural Cognitive Status Examination (COGNISTAT) (Kiernan, Mueller J., & Langston J.
W., 1995) to assess verbal reasoning and judgment. The Controlled Oral Word Association test (COWA) (Benton, 1969) was used to assess verbal fluency (VF), which is highly sensitive to executive dysfunction in ALS (Abrahams et al., 2000; Frank et al., 1997; Kew et al., 1993a; Strong et al., 1999). In the COWA, patients were asked to generate as many words as possible starting with the letters C, A, and S (excluding proper nouns and repeated words) within an allotted 3 minute time period. These letters were modified from the original COWA which included F, A, and S in order to avoid repetition from the MoCA (Nasreddine et al., 2005) where subjects were also asked to generate words starting with the letter F. The letter C was chosen as a replacement because it represented a consonant of equal difficulty level in word generation tasks (Borkowski, Benton, & Spreen, 1967). VF represents the average time taken to think of each word, as calculated in equation (1) (Abrahams et al., 2000), and is designed to control for individual variations in motor speed. Previously described normative data were used as a benchmark for age-matched control performance (Tombaugh, Kozak, & Rees, 1999).

\[
VF = \frac{\text{Time allotted} - \text{Time taken to generate words}}{\text{Total number of correct words generated}} \quad (1)
\]

**Equation 1:** Verbal fluency calculation (Abrahams et al., 2000).

Additional clinical evaluations included manual muscle testing based on the Medical Research Council (1943) scale, manual strength tests of maximum voluntary isometric contraction (MVIC) of the dominant hand, a detailed patient history, age and symptoms at disease onset, disease duration and current medications being taken by the patient. Further neuropsychological testing included the highly sensitive MoCA to detect mild cognitive...
impairments in executive functions, working memory, attention and concentration, abstract reasoning, visuospatial abilities, language and orientation (Nasreddine et al., 2005). The Frontal Behavioural Inventory (FBI) (Kertesz, Davidson, & Fox, 1997), which is widely approved in FTD literature and has been employed in ALS research (Heidler-Gary & Hillis, 2007), was completed by a family member or caregiver to rate changes in behaviour and/or personality of the patient, where a score of 27 or more would be sufficient for FTD diagnosis.

Supplementary tests were administered to measure mood dysfunction and pseudobulbar affect using the Centre for Neurologic Study Lability Scale (CNS-LS) (Moore, Gresham, Bromberg, Kasarkis, & Smith, 1997) and the Hospital Anxiety and Depression Scale (HADS) (Bjelland, Dahl, Haug, & Neckelmann, 2002; Zigmond & Snaith, 1983). The HADS was modified to exclude one question which falsely exaggerated the measure of depression due to the physical disabilities experienced by ALS patients, as previously modified for an ALS population (Abrahams et al., 2000). One subject (subject 16, Table 1) would have been excluded due to an abnormally high anxiety score on the HADS; however this patient never completed the MRI session due to respiratory compromise. Of the 12 patients included in the final analyses, most were right-handed with the exception of four who were functionally left-handed, as determined by the Modified Edinburgh Handedness Inventory (Oldfield, 1971).

2.2.3 Saccade paradigm

Subjects lay supine in the scanner and visual stimuli were back-projected onto a display screen placed at the head of the scanner. A mirror angled at approximately 45° was attached to the head coil to allow subjects to see the display screen. A text display instructed the subjects to prepare for the onset of the experimental run which consisted of 64 trials in total. Each
experimental run consisted of 16 ‘prosaccade’ trials, 16 ‘antisaccade’ trials, 8 ‘pro-catch’ trials, 8 ‘anti-catch’ trials, and 16 fixation trials and lasted 277.5 s in total. In this paradigm, each saccade and catch trial lasted 4500 ms; equivalent to the length of 3 repetition times (TRs), each 1.5 s long (see Section 2.2.7). Each subject successfully completed anywhere from 3-7 runs, as time and subject comfort permitted.

Each experimental run began with a 3 s (2 TRs) fixation period to allow the longitudinal magnetization of the fMRI signal to reach a steady state before commencing data acquisition. Saccade trials within a run started with a 1000 ms display of a central fixation point (a hollow ‘gold coin’, visual angle approximately 1°) that did not convey any instruction other than to fixate at centre (Fig. 2.1). The fixation point was then replaced by one of two instructional stimuli presented for 1300 ms. Instructional stimuli were either a green turtle instructing a prosaccade (to look towards the target) or a red crab instructing an antisaccade (to look away from the target). These stimuli were chosen to match a paradigm designed for across-population comparisons to the other clinical groups studied in our lab that includes young children. Next, all visual stimuli disappeared to reveal a black screen for a 200 ms ‘gap’ period before a peripheral target (hollow ‘gold coin’, at 4.5 or 8° of visual angle to allow participants to move only their eyes and not their head) pseudorandomly appeared to the right or left of the central fixation for 100 ms to prevent prediction of target location. The gap period was used to elicit the most automatic response possible by allowing for disengagement of active visual fixation prior to target appearance (Dorris & Munoz, 1995; Saslow, 1967). After the disappearance of the peripheral target, a black screen reappeared for 1400 ms and subjects were instructed to hold their gaze at the target location for a prosaccade trial, or in the opposite
location of the target for an antisaccade trial. Subjects were asked to: 1) return their gaze to the central fixation point, which reappeared at the centre of the screen, and 2) to maintain fixation for 500 ms until the trial ended. Before beginning the experiment, subjects were instructed to correct themselves if they made an incorrect saccade on a trial (a direction error, such as an erroneous prosaccade on an antisaccade trial), in order to convey that they understood the task but made an error of which they were aware.

**Figure 2.1: Experimental paradigm.** An illustration of the stimuli and duration of events for the four trial types. Figure adapted with permission from Cameron et al. (2011) in press.

On catch trials, subjects were instructed to continue fixating at centre for a period of 1700 ms of darkness until the central fixation point reappeared to end the trial. Trials containing only fixation varied in length such that out of the 16 fixation trials, eight of them were 1 TR, four of were 2 TRs, and four were 3 TRs in length. All runs ended with a fixation period of 16.5 s to allow
for the hemodynamic response to return back to the baseline signal before the commencement of the next run.

2.2.4 Eye tracking and visual display

Eye position was recorded using an ISCAN ETL-400 camera (Burlington, MA, USA) at a sampling frequency of 120 Hz. The camera was positioned approximately 50 cm from the bore hole at the head end of the magnet, next to a custom built screen where visual images were back-projected using a NEC LT265 DLP video projector (Tokyo, Japan) with a refresh rate of 60 Hz and a resolution of 1024 X 768. An infrared fibre-optic illuminator was attached to the head coil and angled at approximately 45° to illuminate the subject’s right eye. Visual images were created using E-PRIME software (Psychology Software Tools Inc., Pittsburgh, PA, USA) on a PC. Eye position was calibrated before the first functional scan using a nine point calibration presentation. Display software was triggered with a TTL pulse from the MRI to ensure the eye position recordings and MRI scans were synchronized.

Three ALS patients were excluded from analysis due to eye tracking difficulties (Table 1). Due to ineffective calibration, the pupil trace was often lost and substantial portions of eye position data were therefore unavailable. One patient was excluded from functional analysis because the TTL pulse did not fire correctly during fMRI acquisition; therefore only the eye tracking data were used for analysis. Most patients who had successful eye tracking completed five to seven runs, although two patients only had time to complete three or four runs due to extra time spent calibrating and adjusting the ISCAN camera.
2.2.5 Functional magnetic resonance imaging parameters

All scans were obtained using a Siemens 3 Tesla Magnetom Trio system (Erlangen, Germany) with a receiving-only 12-channel head coil. An initial localizer scan was taken to identify where the head was located in the magnet in order to position the slices for all subsequent scans. High-resolution anatomical scans were obtained using T1-weighting with an MP-RAGE 3D sequence (repetition time, TR = 1760 ms; echo time, TE = 2.2 ms; flip angle, FA = 9°; field-of-view, FOV = 256 x 256 mm; matrix size 256 x 256; 1 mm iso-voxel resolution; 176 volumes). Experimental runs were presented in a rapid event-related fMRI design, and images were obtained with T2*-weighted echo-planar imaging (EPI) sensitive to changes in BOLD signal contrasts (Kwong et al., 1992; Ogawa, Lee, Kay, & Tank, 1990). Slices were taken in the transverse plane and were angled slightly to avoid the eyes, with a phase-encoding direction of anterior-posterior. Twenty-four slices (3.3 mm thick) covered the frontal, parietal and occipital areas of the brain, and extended down to the ventral striatum to include the basal ganglia. Functional volumes were collected in an interleaved manner (TR = 1500 ms; TE = 30 ms; FA = 72°; FOV = 211 x 211 mm; matrix size 64 x 64; 3.3 mm iso-voxel resolution; 185 volumes).

2.2.6 Behavioural analysis

Analysis of behavioural data was performed using custom-made scripts in Matlab v7.90 (The MathWorks Inc., Natick, MA, USA). No differences were seen across rightward and leftward saccade trials; therefore all trials were pooled together. Correct trials were separated from incorrect trials which consisted of: direction errors (even if they were subsequently corrected), failure to respond, anticipatory errors (saccadic reaction time; SRT < 90 ms), delayed saccades (SRT > 1000 ms), multiple saccades following a correct response, and saccades during catch or
fixation trials. No subjects (ALS or control) exceeded plus or minus three standard deviations (SD) from their group mean on measures of SRT, coefficient of variation of SRT ($CV_{SRT}$), express saccades, or direction errors for either prosaccade or antisaccade tasks.

Using SPSS Statistics v19.0 (IBM, Chicago, IL, USA), 2 x 2 repeated measures ANOVA’s were performed on measures of saccade performance (mean SRT, mean $CV_{SRT}$, and percentage of direction errors) and saccade metrics (mean amplitude, mean velocity). The variables were Group with two levels (ALS, controls) and Task with two levels (pro, anti). Main effects of task were expected to be seen across the above measures of saccade performance (such as SRT and errors), due to the extensive documentation of such differences between pro- and antisaccades (Donaghy et al., 2010a; Munoz et al., 1998; Munoz & Everling, 2004; Shaunak et al., 1995). Significant differences across Groups (ALS and controls) will be reported in the Results section but will not be explicitly highlighted in the Figures for the sake of figure clarity. Significant differences across Task and Group-Task interactions will be explicitly highlighted in the Results section and Figures.

Express saccades were defined in this study as saccades with latencies between 90 – 160 ms of target onset. An independent t-test was used for analysis of express saccades on prosaccade trials only, since generation of an anti-express saccade is physiologically impossible due to the involvement of multiple cortical areas in generating antisaccades (Everling et al., 1998; Everling et al., 1999; Everling & Munoz, 2000). Two-tailed Pearson correlations were performed between neuropsychological test scores and the proportion of antisaccade errors to determine whether any clinical variables were related to oculomotor performance.
2.2.7 Functional analysis

Imaging data were analyzed using Brain Voyager QX v1.10 (Brain Innovation, Maastricht, The Netherlands). The first two functional volumes acquired were discarded from analysis (‘dummy scans’) to ensure the longitudinal magnetization had reached steady-state. Of the remaining volumes, functional imaging data were pre-processed by applying: 3D motion correction with trilinear interpolation to align images to the first volume within each run, slice scan-time correction with a cubic-spline interpolation, high-pass temporal filtering (cut off of 2 cycles/run and linear trend removed), and spatial smoothing using a 4 mm FWHM Gaussian kernel. Coregistration was performed to align each functional scan to the structural image. Normalization was performed by aligning anatomical images with the anterior commissure - posterior commissure (AC-PC) plane to convert images into Talairach space (Talairach & Tournoux, 1988) using trilinear interpolation.

In the rapid event-related fMRI design, the rapid succession of trials causes the time-locked hemodynamic response of each event to overlap significantly. To correct for this, a deconvolution-based general linear model (GLM) was used in Brain Voyager to predict the hemodynamic response associated with each trial type of interest (Dale & Buckner, 1997). A number of ‘stick predictors’ were created based on trial types of interest and included: ‘correct prosaccades’, ‘correct antisaccades’, ‘correct prosaccade preparation’, ‘correct antisaccade preparation’ trials, erroneous pro- and anti-‘direction errors’ that were corrected and ‘null trials’ of no interest. Stick predictors were modeled over a 13 point time scale to cover the temporal length of a typical hemodynamic response of 20 s with a resulting temporal resolution of around 1 TR ($20 \text{ s}/13 \approx 1.5 \text{ s}$). Correct fixation trials were not modelled since fixation was the control
state (or baseline) in this experiment, where trial types of interest were subtracted from fixation to produce the imaging contrasts (Ollinger et al., 2001). Remaining incorrect trials (including uncorrected errors, multiple eye movements per trial, correct trials that were subsequently ‘uncorrected’) and trials where tracking was lost were binned with the ‘null trials’ predictor to prevent them from interfering with the predicted BOLD response for the modeled trial types (Brown et al., 2007).

**Main contrast analysis**

ALS and control groups were analyzed separately using random-effects GLMs with separate subject predictors and Z-normalization to highlight which oculomotor areas were recruited for prosaccades and antisaccades. The 5th to 7th time points from the onset of a saccade trial (7.7, 9.3, 10.8 s) were used for the main contrast as these time points corresponded to the peak hemodynamic response upon examination of the signal a priori. Statistical maps were generated for each group with a threshold set to $p < 0.001$ ($t$ value $= 4.44$, $df = 11$; uncorrected) and were cluster-corrected across the population of voxels with $p < 0.05$ (10 contiguous voxels, as estimated by Brain Voyager’s Cluster-level Statistical Threshold Estimator with 1000 iterations). Group-level statistical maps were overlaid onto averaged structural images containing the anatomical scans (in Talairach space) of all twelve subjects in each group. These statistical maps were used in subsequent region of interest (ROI) analyses involving preparation and execution.

**ROI analysis**

Random-effects GLMs were used to extract beta weights (GLM parameter estimates) from five ROIs, where contrasts of interest were prosaccade preparation and execution, and antisaccade preparation and execution. ROIs were selected as 125 contiguous voxels (5 x 5 x 5),
in the form of a cubic cluster, surrounding the peak activation within DLPFC, FEF, SEF, PEF, and CD. These oculomotor areas were established based on anatomical landmarks and known locations within Talairach space. To isolate pro- and antisaccade preparation (pro and anti prep), BOLD activations were taken from the 5\textsuperscript{th} and 6\textsuperscript{th} time points of the pro-catch and anti-catch trials minus fixation trials. To isolate pro- and antisaccade execution (pro and anti exec), BOLD activations were taken from the 6\textsuperscript{th} and 7\textsuperscript{th} time points of prosaccade and antisaccade trials minus pro-catch and anti-catch trials. Beta weight estimates were then extracted from these BOLD contrasts of interest using the 5\textsuperscript{th} and 6\textsuperscript{th} time points for preparation, meanwhile the 6\textsuperscript{th} and 7\textsuperscript{th} time points were used for execution because the onset of the peripheral target was delayed by 1TR (1.5 s) from the instructional cue (Brown et al., 2007). Beta weights from each ROI were averaged across the right and left hemispheres to increase statistical power for all contrasts. Two-way repeated measures ANOVA’s were conducted on the ROI beta weight values for preparation and execution contrasts, where the variables were: 1) Task with two levels (pro, anti), and 2) Group with two levels (ALS, control). Pearson correlations (two-tailed) were performed to determine if any of the ROI mean peak beta weights predicted various measures of saccade performance, or whether they predicted neuropsychological scores as described in the Results and Figure captions.

Main effects of task were expected to be seen across the selected ROIs when comparing BOLD activation due to the extensive documentation of such differences between pro- and antisaccades in neuroimaging literature (Brown et al., 2007; Connolly et al., 2002; Connolly et al., 2005; Curtis & D'Esposito, 2003a; Curtis & D'Esposito, 2003b; Curtis & Connolly, 2008; DeSouza et al., 2003; Ford, Goltz, Brown, & Everling, 2005). For this reason, statistical
differences in the fMRI data across tasks will be reported in the Results section but will not be explicitly highlighted in the Figures for the sake of figure clarity. Meanwhile, significant differences across Groups (ALS and control) will be explicitly highlighted in the Results and Figures.

### 2.2.8 Structural analysis

Structural analysis was performed using CIVET pipeline software developed by Alan Evans and colleagues at the Montreal Neurological Institute (Ad-Dab'bagh, 2005; Ad-Dab'bagh et al., 2006; Boucher, Whitesides, & Evans, 2009; Chung et al., 2003; Chung & Taylor, 2004; D. L. Collins, Neelin, Peters, & Evans, 1994; Grabner et al., 2006; Kim et al., 2005; Lerch & Evans, 2005; Lyttelton, Boucher, Robbins, & Evans, 2007; MacDonald, Kabani, Avis, & Evans, 2000; Robbins, 2004; Sled, Zijdenbos, & Evans, 1998; Smith, 2002; Tohka et al., 2004; Tohka, Zijdenbos, & Evans, 2004; Zijdenbos, Forghani, & Evans, 1998) using T1-weighted MRI images. Outputs of this pipeline included transformations from native to linear to MNI space, partial volume estimates, segmented volumes, surface maps and cortical thickness maps. Cortical thickness was investigated for significant differences between ALS and control groups across the whole brain at the 95% significance level and was corrected for multiple comparisons using false discovery rate. Analyses were performed using a GLM accounting for age, gender, and group. Segmentations of white matter (WM), grey matter (GM) and cerebrospinal fluid (CSF) volumes were used to investigate significant differences between ALS and controls using independent t-tests. Additionally, partial volume estimations of GM in the four lobes and cerebellum, in addition to more specified areas such as medial frontal lobes, were tested for significant differences between ALS and control subjects using independent t-tests.
2.3 Results

2.3.1 Clinical and Neuropsychological Evaluation

In total, all of the 21 patients recruited for this study completed the clinical evaluation and 19 completed the neuropsychological evaluation. Scores from these evaluations are reported in Table 1. All patients met the El Escorial criteria for ‘definite’ diagnosis, with the exception of four patients who met the criteria of ‘probable’ diagnosis. Mean disease duration at the time of the clinical evaluation was 32.6 months (range, 10-168), and the mean vital capacity was 80% predicted (FVC range, 38-101). The evaluation of physical disability using the ALSFRS-R revealed a mean score of 34.4 ± 6.2 (SD) out of 48, where a score of 48 is considered completely independent and functional. The mean score from this ALS group was functionally worse than a previously reported average score of 38 from a sample demographic of 384 ALS patients with a mean disease duration of 25 months (Cedarbaum et al., 1999).

Patients and controls were evaluated for mild cognitive impairment using either the MMSE or MoCA. Specific scores for the controls were not tabulated, but a score below 26 would merit exclusion of a control subject; therefore we can assume that, on average, controls scored greater than or equal to 26. The mean MoCA score of ALS patients was 23.2 ± 4.3; therefore it is reasonable to assume that, on average, the ALS group displayed mild cognitive impairment that fell below normal standards of performance (26 or higher) (Nasreddine et al., 2005).

In depth neuropsychological evaluation revealed mean COGNISTAT reasoning scores of 6.1 ± 1.8 and judgment scores of 5.1 ± .9, both which fell within the average ranges reported for healthy adults (Kiernan et al., 1995). Verbal fluency calculations showed a mean score of 8.3 ±
5.2 (range 2.9 - 19.3) which is more impaired than previously noted in ALS populations without pseudobulbar palsy (Abrahams et al., 1997; Abrahams et al., 2000; Abrahams et al., 2005; Frank et al., 1997; Massman et al., 1996). Overall, ALS patients did not display frontal lobe dementia, as the average FBI score for ALS participants was 11.0 ± 10.0 and a score of 27 or higher is required for a diagnosis of frontal lobe dementia (Kertesz et al., 1997). The FBI score was extremely variable due to a handful of patients who came close to the cut off for frontal lobe dementia and one patient who met the criteria with a score of 42. Emotional lability, or dramatic mood fluctuations, was measured using the CNS-LS, where a score of 13 has been demonstrated as a reliable cut off for accurately predicting a neurologist’s diagnosis of emotional lability in 82% of ALS participants (Moore et al., 1997). Based on this cut off, the ALS group can be considered borderline labile with a mean score of 12.4 ± 4.1. By these standards, out of the 19 ALS participants who completed the CNS-LS, 9 of them were considered emotionally labile. The HADS evaluation revealed that as a group, the ALS participants did not display signs of depression or anxiety, except for one patient who fell within the abnormal range for anxiety (patient 16, Table 1); however this patient also met other exclusion criteria and therefore did not complete the experimental paradigm due to a low FVC causing respiratory compromise. Of the remaining patients, the average depression score of 2.85 ± 2.80 and anxiety score of 5.16 ± 2.93 fell within the normal range of 0-7 points (Zigmond & Snaith, 1983). Based on the large variability on the mean scores for each neuropsychological test, we can conclude that the ALS subjects in this study all had varying degrees of impairment perhaps because patients were at different stages in their disease progression, and they do not represent a homogenous sampling. However, in general, the ALS patients in this study demonstrated mild
cognitive impairment and executive dysfunction based on their performance on neuropsychological evaluations, despite the fact that most patients did not meet the criteria for frontal lobe dementia.

2.3.2 Behaviour

Saccadic eye movement tasks, such as the antisaccade, can be employed to investigate executive function. In a disease where executive dysfunction is present, ALS patients were hypothesized to display difficulties with inhibiting automatic saccades. Illustrated in Fig. 2.2 is the cumulative distribution of SRTs for the prosaccade and antisaccade tasks, displayed as a proportion of the total number of trials, where the latencies of correct and incorrect saccades were categorized into SRT bins of 10 ms increments. The greatest proportion of correct, short-latency saccades were executed by ALS patients on prosaccade trials, while direction errors were virtually negligible. The latencies of correct prosaccades made by control subjects were considerably more delayed than those of the ALS patients, and slightly more direction errors were made by the controls on prosaccade trials.

We analyzed cumulative SRT distributions using the non-parametric Kolmogorov-Smirnov test to determine whether the distributions of SRTs were significantly different across groups for correct and error trials (i.e., how much the distributions differed from each other). We found significant differences in SRT distributions between ALS and control subjects for correct antisaccades (K= 1.435, p< .033), incorrect antisaccade trials (K= 4.489, p< .001), and incorrect prosaccade trials (K= 2.490, p< .001). Distributions of correct prosaccade SRTs between ALS and controls were not significantly different when all SRT bins were included (K= 1.272, p< .079),
however SRT distributions in the express saccade epoch only (90 – 160 ms) were significantly different between groups (K= 1.500, p< .022).

Control subjects demonstrated faster latencies on correct antisaccade trials than the ALS subjects, who lagged closely behind. The most direction errors were made during antisaccade trials by ALS subjects. These antisaccade errors occurred mostly between 100 and 180 ms after the target appearance, and were likely in response to inadequate suppression of the automatic prosaccade, whereas antisaccade errors made by the controls were fewer and more delayed.
Shown in Fig. 2.3 are the behavioural results of prosaccade and antisaccade trials in ALS and control subjects. Analysis of variance for SRT (Fig 2.3A) showed a significant main effect of Task ($F(1,12)= 41.961, p< .001$), with significantly prolonged antisaccade latencies, which has been previously shown in the ALS literature (Donaghy et al., 2010a; Donaghy et al., 2010b; Shaunak et al., 1995). Contrary to our predictions, there was no main effect of Group ($F(1,12)= .271, p= .612$), while Group by Task interactions failed to reach significance ($F(1,12)= 4.145, p= .064$).

Intrasubject variability of SRT was expressed as a coefficient of variation ($CV_{SRT}$). A main effect of Task was found for $CV_{SRT}$ ($F(1,12)= 22.832, p< .001$) (Fig. 2.3B) such that prosaccades were significantly more variable than antisaccades. A noticeable trend of a Group main effect was seen ($F(1,12)= 3.752, p= .077$), where ALS patients had more variability for $CV_{SRT}$ than controls when prosaccade and antisaccade trials were pooled together. Increased $CV_{SRT}$ is common in many patient groups (Cameron et al., 2011; Munoz et al., 2003; Peltsch, 2011), generally as a result of the variability of the disease across patients. The interaction between Group and Task reached significance ($F(1,12)= 7.083, p= .021$), and further analysis showed prosaccade $CV_{SRT}$ was significantly greater than that of antisaccades within both ALS ($p= 0.003$) and control groups ($p= 0.035$). Further analysis of the Group-Task interactions also revealed ALS patients displayed significantly greater prosaccade $CV_{SRT}$ than controls ($p= .033$).
Figure 2.3: Behaviour. (A) Mean saccadic reaction time (SRT) on correct trials. (B) Mean intra-subject coefficient of variation (CV) for SRT. (C) Mean percentage of express saccades (90 – 160 ms). (D) Mean percentage of direction errors. (E) Correlation of Montreal Cognitive Assessment (MoCA) score (/30) and percentage of antisaccade direction errors. Error bars represent standard error of the mean (SE).

The express saccade epoch was defined as 90 –160 ms (Fig. 2.2). An independent t-test revealed that the ALS group made a significantly greater proportion of express saccades on prosaccade trials compared to controls ($t(24)= 3.364$, $p = .003$) (Fig. 2.3C). Analysis of variance of direction errors revealed no main effect of Group ($F(1,12)= 1.232$, $p = .289$) (Fig. 2.3D). However, there was a significant main effect of Task ($F(1,12)= 11.100$, $p = .006$) and significant interaction between Group and Task ($F(1,12)= 4.749$, $p = .050$). Follow-up analysis showed the proportion of direction errors was significantly greater on antisaccades than prosaccades for both the ALS ($p = .012$) and control groups ($p = .034$). There was only a trend between groups for direction errors, where ALS patients seemed to make more antisaccade direction errors ($p = .125$) while controls
seemed to make more prosaccade direction errors ($p = .109$). A two-tailed Pearson’s correlation revealed a significant negative correlation between the ALS group’s performance on the MoCA and the percentage of antisaccade errors made ($r(12) = -.686, p < .010$) (Fig. 2.3E), where antisaccade error rates increased as MoCA scores worsened.

Figures 2.4A and B illustrate the metrics of pro- and antisaccades in terms of saccade amplitude and velocity, respectively, across ALS and control subjects. Analysis of variance revealed no significant main effect of Group on saccade amplitude ($F(1,12) = .504, p = .491$) or saccade velocity ($F(1,12) = .749, p = .404$), suggesting ALS patients did not have significantly altered saccade metrics. A main effect of Task was observed for saccade amplitude ($F(1,12) = 6.012, p = .030$), where antisaccades had significantly greater amplitudes than prosaccades despite the target locations remaining constant at either 4.5 or 8° of visual angle. These results differed from previous findings in elderly controls, where accuracy of amplitude worsened with age on the prosaccade task, but antisaccade accuracy was preserved (Guitton et al., 1985; Olincy, Ross, Youngd, & Freedman, 1997). A significant interaction effect between Group and Task on saccade amplitude ($F(1,12) = 15.296, p = 0.002$) was followed by pairwise comparisons which revealed antisaccades had significantly greater amplitudes than prosaccades within the ALS group only ($p=0.003$). In general, both ALS and control subjects appeared to generate slightly hypometric saccades, where the subjects undershot the target location. Saccade velocity showed no main effect of Task ($F(1,12) = 2.708, p = .126$), and displayed a strong trend of an interaction effect between Group and Task, although this failed to reach significance ($F(1,12) = 4.424, p = .057$).
Figure 2.4: Saccade metrics. (A) Mean saccade amplitude. (B) Mean saccade velocity. Error bars represent standard error of the mean (SE).

Our focus was to evaluate group differences in saccadic behaviour across ALS and control subjects. In summary, ALS patients seemed to be biased towards automatic responses, as demonstrated by a greater proportion of express saccades, more variable prosaccade SRTs, and a trend of more errors on antisaccade trials than the controls. It is possible that this automaticity stems from an inability to produce the appropriate task set, and is unlikely a problem linked to saccade execution since saccade metrics were consistent across ALS and control groups. We therefore isolated saccade preparation (or task set) from execution in our fMRI analysis to determine the neurological process which might be influencing this behaviour.

2.3.3 fMRI

fMRI analysis was conducted to compare the activation in key oculomotor areas of the brain during prosaccade and antisaccade tasks (correct saccades only) across the ALS and control groups to determine how brain function might be influencing saccade behaviour. The goal was to investigate whether differences in brain activation during saccade preparation and execution would explain the ALS patients’ bias towards automaticity and their subsequent difficulty with
performing the antisaccade task correctly. More specifically, we predicted differences across ALS and control subjects in the amount of BOLD signal in brain areas involved with executive functions and inhibiting unwanted saccades such as DLPFC and FEF (Pierrot-Deseilligny et al., 1991a; Pierrot-Deseilligny et al., 2003; Rivaud et al., 1994) during pro- and antisaccade preparation. We also expected changes in BOLD signal in other oculomotor regions that modulate SC activity such as SEF, PEF, and CD.

Main contrast analysis

A contrast showing correct prosaccade trials plus correct antisaccade trials was first created (Fig. 2.5) to confirm that both ALS and control participants were able to recruit (or “bring online”) key oculomotor regions of interest (ROIs) known to be involved with producing prosaccades and antisaccades including the DLPFC, FEF, SEF, PEF, and CD (Brown et al., 2007; Connolly et al., 2005; Connolly et al., 2007; Curtis & D'Esposito, 2003b; DeSouza et al., 2003; Everling & Munoz, 2000; Gottlieb, Kusunoki, & Goldberg, 1998; Guitton et al., 1985; Kusunoki, Gottlieb, & Goldberg, 2000; Pierrot-Deseilligny et al., 1991a; Schlag-Rey & Amador, 1997; Watanabe & Munoz, 2009). This contrast was made using the 5th, 6th and 7th time points (see Methods) to show brain activation during the peak hemodynamic response. Significant BOLD activations were seen in all ROIs in both the ALS and control averaged statistical maps.
**Figure 2.5: Anti- and prosaccade contrast map.** Contrast map of prosaccade trials and antisaccade trials added together, cluster size corrected at p < .05 (10 contiguous voxels). Significant BOLD activations were observed in all ROIs ('hot' colours) and are labelled. Talairach coordinate values of planes are given.

Talairach coordinates for the peak activations that exceeded the statistical threshold in each ROI are shown in Table 2. Having established that both ALS and control subjects were able to recruit these key oculomotor areas to perform the prosaccade and antisaccade tasks, we next focused on ROI analysis of BOLD activations during saccade preparation and saccade execution.
Table 2: Talairach coordinates (X, Y, Z) of peak activation in GLM contrast maps for the antisaccade + prosaccade contrast from Fig. 2.5. DLPFC, dorsolateral prefrontal cortex; FEF, SEF, PEF, frontal, supplementary, parietal eye fields; CD, caudate nucleus

Saccade Preparation

To adequately prepare for a pro- or antisaccade, a task-appropriate amount of build-up neural activity (known as preparatory set) is required in brain regions that are responsible for producing saccades (Dorris et al., 1997; Dorris & Munoz, 1998; Everling et al., 1998; Everling et al., 1999; Everling & Munoz, 2000). On correct antisaccade trials, adequate inhibition of the saccade map is required to prevent erroneous prosaccades (Everling et al., 1998; Everling et al., 1999; Everling & Munoz, 2000), and has been linked to increased BOLD signal in DLPFC and FEF.
(DeSouza et al., 2003). Meanwhile, higher levels of pre-target activity in the saccade map are more desirable for producing fast express saccades (Dorris et al., 1997; Everling et al., 1998; Everling & Munoz, 2000) – a behaviour that was observed in the ALS group.

Group-level statistical maps showing significant peak BOLD activations during preparation for pro- and antisaccades were overlaid onto averaged anatomical images and are shown in Figures 2.6A and B, respectively. Talairach coordinates of the peak voxels from the group-level maps that survived cluster correction for multiple comparisons ($p < .05$) and surpassed the significance threshold ($p < .001$) for pro- and antisaccade preparation contrasts are listed in Table 3. Beta weights were extracted from each ROI (including those that fell below the threshold of $p < .001$) and are shown in Fig. 2.7A. The mean peak beta weights (Fig. 2.7B) for each ROI were calculated using beta weights from the 5th and 6th time points shown in grey in Fig. 2.7A.
Figure 2.6: Saccade preparation contrast maps. (A) Contrast map for pro prep created from pro-catch trials subtracting fixation trials, cluster size corrected at $p < .05$ (10 contiguous voxels). The 5th and 6th time points relative to trial onset were used in this subtraction. ROIs are labelled with significant BOLD activations shown as ‘hot’ colours. (B) Contrast map for anti prep created from anti-catch trials subtracting fixation trials, cluster size corrected at $p < .05$ (10 contiguous voxels). The 5th and 6th time points relative to trial onset were used in this subtraction. ROIs are labelled, with significant BOLD activations shown as ‘hot’ colours.
Table 3: Talairach coordinates (X, Y, Z) of peak activation in GLM contrast maps for prosaccade and antisaccade preparation contrasts. Coordinates for significant activations only from Fig. 2.6 are shown. DLPFC, dorsolateral prefrontal cortex; FEF, SEF, PEF, frontal, supplementary, parietal eye fields; CD, caudate nucleus.
Figure 2.7: Region of interest (ROI) analysis for pro and antisaccade preparation. (A) Illustration of mean BOLD signal time course for pro and anti prep trials for all ROIs. (B) Mean peak beta weight values for the 5th and 6th time points from random effects analysis of pro prep and anti prep trials for 125 cubic voxels surrounding the peak activations in regions displaying greater activation during catch trials compared to fixation trials. Left and right hemispheres were averaged. In ROIs where BOLD activation did not surpass the significance threshold (p< .001), thresholds were lowered until beta weights could be obtained. Errors bars represent standard error of the mean (SE).
Mean peak beta weights for saccade preparation in Fig. 2.7B were tested for statistical significance using 2 x 2 ANOVA’s. Significant Task effects and Group-Task interactions are shown in Fig. 2.7B and there were no significant effects of Group to report. SEF showed no main effect of Group ($F(1,12)= .304$, $p= .593$) or Task ($F(1,12)= .793$, $p= .392$), but did show a trend approaching significance for a Group-Task interaction effect ($F(1,12)= 3.957$, $p= .072$). FEF displayed a significant main effect of Task ($F(1,12)= 24.588$, $p< .001$); significantly greater activation was involved with preparing for an antisaccade than for a prosaccade. No main effect of Group ($F(1,12)= 1.319$, $p= .275$) or interaction effects ($F(1,12)= 1.561$, $p= .238$) were found in FEF during preparation. PEF had no main effect of Group ($F(1,12)= .373$, $p= .554$) but showed a significant main effect of Task ($F(1,12)= 5.794$, $p= .035$) where significantly greater activation was present for antisaccade preparation compared to prosaccade preparation. A significant interaction effect between Group and Task ($F(1,12)= 5.319$, $p= .042$) during preparation was observed in PEF, where subsequent analyses revealed that PEF had significantly greater activation when preparing for an antisaccade compared to a prosaccade in ALS subjects only ($p= .019$). DLPFC showed no main effect of Group ($F(1,12)= .391$, $p= .545$), but did display a significant main effect of Task ($F(1,12)= 12.117$, $p= .005$); DLPFC displayed significantly more activation when preparing for an antisaccade compared to a prosaccade. There was a trend of an interaction effect between Group and Task ($F(1,12)= 3.373$, $p= .093$) in DLPFC that failed to reach significance. Much like DLPFC, preparation in CD showed no main effects of Group ($F(1,12)= .026$, $p= .874$) or interaction effects ($F(1,12)= .273$, $p= .612$). A significant main effect of Task was seen ($F(1,12)= 14.708$, $p= .003$) such that preparation for antisaccades involved significantly greater activation in CD than preparation for prosaccades.
Overall, FEF, PEF, DLPFC and CD all showed main effects of Task, where antisaccade preparation BOLD signal change was greater than that of prosaccade preparation. PEF was the only ROI with a significant Group-Task interaction where antisaccade preparation had stronger beta weights than prosaccade preparation in the ALS group only. No significant Group effects were seen between ALS and control subjects.

**Saccade Execution**

BOLD activations during saccade execution were investigated to determine whether the automaticity of ALS patients’ saccade behaviour could be explained by changes in brain activation during the execution of an eye movement. Group-level statistical maps showing significant peak BOLD activations during prosaccade and antisaccade execution were overlaid onto averaged anatomical images and are shown in Figure 2.8 A and B, respectively. Beta weights taken from each ROI (threshold was lowered to extract those where p > .001) are shown in Fig. 2.9A. The mean peak beta weights shown in Fig. 2.9B were calculated for each ROI using beta weights from the 6th and 7th time points shown in grey in Fig. 2.9A (see Methods). Talairach coordinates of the peak voxels from the group-level statistical maps that survived cluster correction and surpassed the significance threshold (p < .001) are listed in Table 4 for prosaccade and antisaccade execution contrasts.
Figure 2.8: Saccade execution contrast maps. (A) Contrast map for pro exec created from prosaccade trials subtracting pro-catch trials, cluster size corrected at p < .05 (10 contiguous voxels). The 6th and 7th time points relative to trial onset were used in this subtraction. Labelled ROIs with significant BOLD activations are shown as ‘hot’ colours. (B) Contrast map for anti exec created from antisaccade trials subtracting anti-catch trials, cluster size corrected at p < .05 (10 contiguous voxels). The 6th and 7th time points relative to trial onset were used in this subtraction. ROIs are labelled, with significant BOLD activations shown as ‘hot’ colours.
### Table 4: Talairach coordinates (X, Y, Z) of peak activation in GLM contrast maps for prosaccade and antisaccade execution contrasts

Coordinates are shown for significant activations only from Fig. 2.8. DLPFC, dorsolateral prefrontal cortex; FEF, SEF, PEF, frontal, supplementary, parietal eye fields; CD, caudate nucleus.

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<th>Group and Region of Interest</th>
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| **Control**                 |          |          |          |    |     |          |          |          |    |     |
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| left DLPFC                  |          |          |          |    |     |          |          |          |    |     |
| right FEF                   | -18      | -13      | 58       | 8.27 | 34  | -27      | -10      | 58       | 7.23 | 22  |
| left FEF                    | -3       | -58      | 52       | 14.30| 629 | -21      | -67      | 49       | 8.25 | 215 |
| SEF                         | 9        | -1       | 67       | 8.38 | 27  |          |          |          |    |     |
| right PEF                   | 24       | -67      | 49       | 9.68 | 183 | 30       | -64      | 58       | 7.36 | 184 |
| left PEF                    | -3       | -58      | 52       | 14.30| 629 | -21      | -67      | 49       | 8.25 | 215 |
| right CD                    |          |          |          |    |     |          |          |          |    |     |
| left CD                     |          |          |          |    |     |          |          |          |    |     |
Figure 2.9: Region of interest (ROI) analysis for pro and antisaccade execution. (A) Illustration of mean BOLD signal time course for pro and anti exec trials for all ROIs. (B) Mean peak beta weight values for the 6th and 7th time points from random effects analysis of pro exec and anti exec trials. Beta weights were extracted from 125 cubic voxels surrounding the peak activations in regions displaying greater activation during saccade trials compared to catch trials. Left and right hemispheres were averaged. In ROIs where BOLD activation did not surpass the significance threshold (p<.001), thresholds were lowered until beta weights could be obtained. Errors bars represent standard error of the mean (SE).
Multiple 2 x 2 ANOVA’s were used to test for significance across the mean peak beta weights for saccade execution that are shown in Fig. 2.9B. Only significant effects of Task or Group-Task interactions are shown in Fig. 2.9B; significant effects of Group are not shown for the purposes of figure clarity. A significant main effect of Group was seen in SEF ($F(1,12)= 4.839$, $p= .050$) such that ALS patients had significantly greater activation in SEF than controls during saccade execution. A trend approaching a main effect of Task was seen ($F(1,12)= 3.287$, $p=.097$) but failed to reach significance. A significant interaction effect ($F(1,12)= 5.560$, $p= .038$) was followed by subsequent analysis that revealed ALS patients had significantly greater activation in SEF during antisaccade execution compared to prosaccade execution ($p= .011$). Additionally, the ALS group had significantly stronger beta weights during the antisaccade task compared to controls ($p= .011$). No significant main effect of Group ($F(1,12)= .986$, $p= .342$) or interaction effect between Group and Task ($F(1,12)= 1.241$, $p= .289$) was found for saccade execution in FEF. However, a significant main effect of Task ($F(1,12)= 12.077$, $p= .005$) was seen in FEF such that antisaccades had significantly greater activation during execution than prosaccades. No significant main effect of Group ($F(1,12)= 1.813$, $p= .205$) or interaction effect between Group and Task ($F(1,12)= .176$, $p= .683$) was found for saccade execution in PEF. However, a significant main effect of Task ($F(1,12)= 5.903$, $p= .033$) was seen in PEF such that antisaccade execution was associated with significantly greater activation than prosaccade execution. Saccade execution in DLPFC had no main effects of Group ($F(1,12)= .288$, $p= .602$), Task ($F(1,12)= .605$, $p= .453$) or interaction effects ($F(1,12)= .001$, $p= .977$). Saccade execution in CD also displayed no main effects of Group ($F(1,12)= 1.164$, $p= .304$), Task ($F(1,12)= 1.577$, $p= .235$) or interaction effect ($F(1,12)= .056$, $p= .817$).
In summary, FEF and PEF displayed effects of Task such that antisaccades had significantly stronger beta weights than prosaccades. ALS patients had significantly more activation in SEF than controls in general, but more specifically, showed interaction effects where ALS subjects had stronger beta weights in SEF than controls on the antisaccade task. Additionally, the ALS group had significantly stronger antisaccades beta weights than prosaccades in SEF.

2.3.4 Structural Analysis

The neurodegenerative nature of ALS is known to cause brain atrophy including reductions in white (Abrahams et al., 2005) and grey matter (Chang et al., 2005; Murphy et al., 2007b) within motor, premotor and frontotemporal regions, in addition to enlarged ventricles (Frank et al., 1997). To investigate whether such changes could be seen within this ALS group, cortical thickness was contrasted between controls and ALS subjects, and is displayed in Fig. 2.10 in terms of the level of significance (q values), rather than in mm of thickness. Areas displaying ‘hot’ colours signify brain regions that had decreased cortical thickness in the ALS brain compared to age-matched controls, and these trends are approaching statistical significance (q’s range 0.16 - 0.12).
Figure 2.10: Significance level of cortical thickness. Cortical thickness in terms of grey matter (GM) compared across ALS and control groups, where ‘hot’ colours represent decreased GM in the ALS brain. Multiple comparisons were corrected for using false discovery rate correction. ROIs overlapping with areas of thinning cortex are labelled. Significant GM thinning is defined by q<.05.

Volumetric estimations of differences in cerebrospinal fluid (CSF; t(30)= .087, p= .931), grey matter (GM; t(30)= -1.080, p= .289) and white matter (WM; t(30)= .986, p= .332) between ALS and controls are shown in Fig. 2.11A, however no statistically significant differences between groups were found. Further investigations were conducted despite the apparent similarities between ALS and control groups in terms of GM volume to determine whether loss of GM followed a pattern typical of frontotemporal dementia (FTD), and whether GM volume
correlated to any behavioural parameters. Estimations of GM volume across the lobes of the brain and cerebellum are shown in Fig. 2.11B. No significant differences were seen between ALS and control GM in the parietal lobes ($t(30) = -1.333, p = .193$), temporal lobes ($t(30) = -0.618, p = .541$), occipital lobes ($t(30) = -0.737, p = .467$), or cerebellum ($t(30) = .050, p = .961$). However, a trend of decreased GM in the ALS brain was apparent in the frontal lobes ($t(30) = -1.703, p = .099$). GM volume was also investigated in more specified areas of each lobe as illustrated in Fig. 2.11C. A trend approaching significance was found in medial frontal ($t(30) = -1.868, p = .072$) and medial temporal cortices ($t(30) = -2.009, p = .054$) suggesting ALS may have decreased GM compared to controls in these areas. GM volume in orbitofrontal ($t(30) = -1.90, p = .850$), lateral prefrontal ($t(30) = -1.514, p = .141$) and lateral temporal cortices ($t(30) = -0.427, p = .672$) showed no significant difference across groups.
Figure 2.11: Volumetric analysis. (A) Volumetric estimations of cerebrospinal fluid (CSF), grey matter (GM) and white matter (WM). (B) Volumetric estimations of GM in the frontal, parietal, temporal, and occipital lobes and cerebellum. (C) Grey matter volume estimations in the medial frontal, orbitofrontal, lateral prefrontal, medial temporal, and lateral temporal areas of the brain. Error bars represent standard error of the mean (SE).

2.3.5 Correlation Analysis

Saccade performance vs. Neuropsychological scores

Many of the tests administered as part of the neuropsychological battery were sensitive to identifying executive dysfunction, a measure of frontal lobe functioning which can also be tested using the antisaccade task (Hallett, 1978; Munoz & Everling, 2004). Performance on the antisaccade task in terms of SRT and direction errors relies on the intactness of frontal lobe cortical areas which control executive functions (Munoz & Everling, 2004; Pierrot-Deseilligny et
al., 1991a; Pierrot-Deseilligny et al., 2003; Rivaud et al., 1994). For this reason, antisaccade SRTs and direction errors were correlated with neuropsychological test scores from Table 1. All correlations of antisaccade SRTs were found to be not significant ($p's > .111$) (Figure not shown). All correlations of antisaccade direction errors were also found to be not significant ($p's > .062$) (Figure not shown), with the exception of the MoCA score which displayed a significant correlation with antisaccade direction errors ($r(12)= -.686, p=.010$) (Fig. 2.3E), as reported in Section 2.3.2.

**Peak beta weights vs. Saccade performance**

The brain regions selected a priori for our ROIs are known to be critically involved with generating voluntary saccades; their proper functioning establishes the preparatory set that dictates saccade performance in terms of SRT and direction errors (Connolly et al., 2002; DeSouza et al., 2003; Pierrot-Deseilligny et al., 1991a; Pierrot-Deseilligny et al., 2002; Pierrot-Deseilligny et al., 2003). Therefore, mean peak beta weights from each ROI in the antisaccade preparation and execution contrasts were correlated with measures of antisaccade performance such as SRT and proportion of direction errors for both ALS and control groups. Significant correlations only are shown in Fig. 2.12, where data points represent the mean antisaccade SRT for each subject intersecting the mean peak beta weight for that same subject.

When beta weights from antisaccade preparation were correlated with antisaccade SRT, negative correlations were observed across all ROIs (Fig. 2.12A); meaning stronger beta weights were associated with faster SRTs. In particular, PEF beta weights had a significant negative correlation with antisaccade SRT in the control group ($r(12)= -.766, p=.004$), but not the ALS group ($r(12)= -.447, p=.145$). SEF beta weights showed a significant negative correlation with
antisaccade SRT in the ALS ($r(12)=-.594, p=.042$), but not control groups ($r(12)=-.229, p=.475$). Antisaccade preparation beta weights from CD correlated significantly with antisaccade SRTs in the ALS group ($r(12)=-.575, p=.050$), but not in controls ($r(12)=-.076, p=.814$). DLPFC beta weights correlated significantly with antisaccade SRTs in the ALS group ($r(12)=-.583, p=.047$), but not in controls ($r(12)=-.408, p=.187$). The final ROI whose beta weights showed a significant negative correlation with antisaccade SRT in the control group was FEF ($r(12)=-.676, p=.016$), however no correlation was seen in the ALS group ($r(12)=-.440, p=.152$). No significant correlations were found between antisaccade preparation beta weights and antisaccade error rates in the ALS group ($p's>.302$) or controls ($p's>.232$) (Figures not shown).

Peak beta weights from antisaccade execution were also correlated with antisaccade SRTs across all ROIs, however only PEF showed a significant positive correlation in the ALS group ($r(12)=.603, p=.038$), but not for control subjects ($r(12)=.017, p=.958$) (Fig. 2.12B). Meanwhile, all other ROIs showed no significant correlation in ALS ($p's>.111$) or controls ($p's>.101$) (Figures not shown). Antisaccade execution beta weights also showed no significant correlation to the rate of antisaccade direction errors made in the ALS group ($p's>.209$), or controls ($p's>.055$) with the exception of DLPFC. DLPFC anti execution beta weights showed a significant positive correlation with anti error rates in the ALS group ($r(12)=.596, p=.041$) (Fig. 2.12B) such that stronger beta weights were associated with more direction errors; meanwhile controls showed no correlation ($r(12)=.394, p=.206$).
Figure 2.12: Correlations between antisaccade measures (SRT and direction errors) and mean peak beta weights during anti preparation and execution. (A) Mean peak beta weights were extracted from the 5th and 6th time points of random effects analysis for anti prep trials from the 125 cubic voxels surrounding the peak activations in regions displaying greater activation during anti-catch trials compared to fixation trials. (B) Mean peak beta weights were extracted from the 6th and 7th time points of random effects analysis for anti exec trials from the 125 cubic voxels surrounding the peak activations in regions displaying greater activation during antisaccade trials compared to anti-catch trials.

An additional correlation was performed to determine whether the degree of preparatory activation for prosaccade trials was linked to the significantly increased percentage of express saccades made on prosaccade trials by ALS patients. However, no significant correlations were found between prosaccade preparation beta weights from any ROIs and the percentage of express saccades in ALS (p’s > .349) or controls (p’s > .097) (Non-significant correlations not shown in Figures).
Peak beta weights vs. Neuropsychological scores

Because MoCA scores were the only neuropsychological test displaying a significant correlation with antisaccade direction errors made by ALS subjects (Fig. 2.3E), and direction errors showed significant correlations with some ROI peak beta weights in ALS, MoCA scores were therefore the only psychometric test chosen to be correlated with mean peak beta weights from saccade preparation and execution. However, no significant correlations were found between MoCA scores and pro- or anti-saccade preparation and execution beta weights ($p’> .243$) (Non-significant correlations not shown in Figures).

Peak beta weights vs. Grey matter volumes

Because BOLD activation was measured from grey matter, changes in the degree of brain activation in ALS may be attributed to structural degeneration of grey matter. Peak beta weights from all ROIs were correlated with some of the grey matter volumes shown in Fig. 2.11B and C using two-tailed Pearson correlations. Peak beta weights from contrasts of interest (pro prep, pro exec, anti prep, and anti exec) were correlated with grey matter volumes that most closely overlapped with each ROI (Fig. 2.13); frontal lobe GM was correlated against all contrasts of interest for DLPFC and FEF, medial frontal GM was correlated with SEF activation, parietal GM was correlated with PEF activation, and lateral prefrontal GM was correlated with DLPFC activation.
Figure 2.13: Correlations between grey matter (GM) volume and peak beta weights. (A) GM volume estimations from lateral prefrontal cortex (LPF) were correlated with mean peak beta weights of DLPFC extracted from the 5th and 6th time points for anti prep and the 6th and 7th time points for anti exec from the 125 cubic voxels surrounding the peak activation. (B) GM volume estimations from the parietal lobes (PL) were correlated with mean peak beta weights of PEF from the 6th and 7th time points of prosaccade execution trials from the 125 cubic voxels surrounding the peak activation where greater activation was seen during prosaccade trials compared to pro-catch trials. Right and left hemispheres were averaged.

The volume of frontal lobe GM was first correlated with prosaccade preparation and execution beta weights for both DLPFC and FEF, however none of these correlations were significant in the ALS (p’s > .382) or control groups (p’s > .290). Frontal lobe GM was then correlated with antisaccade preparation and execution in FEF which resulted in no significant
findings in ALS ($p$'s > .707) or control groups ($p$'s > .262). However, anti preparation beta weights from DLPFC correlated significantly with frontal lobe GM in controls ($r(12)= .693$, $p= .012$) such that larger GM volume was associated with stronger beta weights; meanwhile no correlation was found in ALS subjects ($r(12)= .086$, $p= .790$) (Figure not shown). DLPFC antisaccade execution beta weights showed a significant negative correlation with ALS frontal lobe GM volumes ($r(12)= -.644$, $p= .024$), where stronger beta weights were associated with less GM volume, but no significant correlation was seen in controls ($r(12)= -.399$, $p= .199$) (Figures not shown).

DLPFC was also correlated with a more specific measure of GM volume, that of the lateral prefrontal cortex, which more closely represents the DLPFC ROI than the estimated GM volume of the entire frontal lobes. For this reason, only the lateral prefrontal cortex correlations with DLPFC are shown in Fig. 2.13. DLPFC anti preparation beta weights of control subjects demonstrated a significant positive correlation with lateral prefrontal GM ($r(12)= .603$, $p= .038$), but ALS beta weights did not ($r(12)= .044$, $p= .893$). GM of lateral prefrontal cortex displayed a significant negative correlation with ALS anti execution beta weights ($r(12)= -.649$, $p= .022$) such that stronger activation occurred with less GM, but did not correlate with control beta weights ($r(12)= -.357$, $p= .255$).

No significant correlations were found between GM volume of medial frontal cortex and SEF for any of the contrasts of interest in ALS ($p$'s > .058) or control groups ($p$'s > .258). The only significant correlation found between PEF beta weights and parietal lobe GM volumes was in the ALS group for anti execution contrasts ($r(12)= -.727$, $p= .007$); meanwhile controls showed no correlation ($r(12)= .304$, $p= .337$). All other contrasts of PEF beta weights (pro prep, anti prep,
anti exec) showed no significant correlation with parietal lobe GM volumes in ALS (p’s > .063) or control groups (p’s > .072).

2.4 Discussion

The objective of this study was to investigate the neural correlates of cognitive impairment in ALS: 1) using a battery of neuropsychological tests to detect subtle cognitive and behavioural deficits related to executive function; 2) using measures of behavioural performance on voluntary saccadic eye movement tasks controlled by frontal regions of the brain; 3) using functional imaging to determine if ALS patients showed significant differences in activation in oculomotor brain regions compared to controls and whether this influenced their flexible control of saccade behaviour, and 4) to determine if the pattern of extramotor cortical degeneration in ALS overlaps with areas that are involved with controlling saccades and executive functions. We found that, compared to controls, the ALS patients displayed signs of cognitive impairment, and were biased towards automated responses as demonstrated by their inclination towards making more express saccades and their increased rate of direction errors on antisaccade trials.

Due to physical restrictions associated with imaging ALS patients, only a subset completed the imaging portion of the protocol; this lack of statistical power left us mostly with trends to interpret our data. Functionally, there were trends in the ALS group that suggested decreased preparatory activation in all ROIs for prosaccades, and increased activation for antisaccade preparation compared to controls, which suggests the inability to establish the appropriate task set perhaps as a result of executive dysfunction. A trend of widespread cortical degeneration was seen in the ALS patients, and overlapped with oculomotor and executive brain areas. We
suggest that the pattern of cortical thinning in oculomotor and executive control regions may contribute to the cognitive impairment seen in ALS patients, which may cause them to be more prone to distractibility, as shown by their automatic saccade behaviour and their difficulty establishing the appropriate task set in terms of preparatory brain activation.

2.4.1 Influence of Cognitive Impairment

As a group, ALS patients varied considerably in their cognitive performance on neuropsychological evaluations, suggesting a continuum of impairments in this small sample size (Table 1). However, on average, we found that ALS patients displayed mild cognitive impairment, based on their poor performance on the MoCA and verbal fluency evaluations, in addition to behavioural fluctuations, as demonstrated by their borderline emotional lability. Based on previous findings, we can assume that poor performance on the MoCA and verbal fluency assessments are likely a result of ALS patients displaying impairments in executive function, working memory, attention and concentration (Abrahams et al., 2000; Nasreddine et al., 2005).

We suspect that ALS patients with labile affect, which is presumed to result from damage to the brainstem and/or frontotemporal regions (Moore et al., 1997; Poeck, 1969), could represent a subset of patients with frontotemporal dementia (FTD)-like pathology (Merrilees et al., 2010; Neary et al., 1998; Neary et al., 2000; Strong et al., 1999; Talbot et al., 1995; Wilson et al., 2001). A large body of evidence supports a continuum between FTLD and ALS, in which deficits of varying severity in behavioural conduct and executive control are characteristic (Abe et al., 1997; Heidler-Gary & Hillis, 2007; Merrilees et al., 2010; Neary et al., 1990; Neary et al., 1998; Neary et al., 2000; Ringholz et al., 2005; Strong et al., 1999).
Both verbal fluency and emotional lability deficits appear relatively early on in ALS and remain consistent over time even in non-demented patients (Abrahams et al., 2005), which could explain why we were able to detect these changes in our small sample that mostly consisted of relatively new diagnoses. It is possible that other significant deficits would have been identified in other cognitive domains if this group of patients had been more progressed in their disease, as previous studies found worsening cognitive performance as ALS progressed (Strong et al., 1999).

Although no other psychometric tests in our study revealed any abnormalities of ALS subjects as a group, individually, some patients had worse cognitive profiles than others. In supplementary analyses, it was revealed that a number of neuropsychological test scores correlated significantly with one another (Table 5). For example, patients who performed well on the MoCA had better judgment and reasoning scores (as shown by the positive correlations), were better at word generation (N.B., low VF scores are associated with better word generation), and had fewer depressive tendencies (as shown by the negative correlations). Numerous other correlations were significant, but in general, the patients who showed signs of impairment in one area tended to perform poorly on other cognitive tests as well. This suggests a pattern of overall cognitive and behavioural impairment that is reminiscent of the ALS-FTD spectrum (Neary et al., 1998), in that it affects a broad range of top-down processes.
Out of the cognitive domains tested, the common thread between successful performance on the MoCA, COWA and CNS-LS can be attributed to successful top-down processing, such as intact executive function, attentional control, and response inhibition. Because ALS patients did not perform well on these measures, we can assume that their top-down processes are not performing optimally. We therefore investigated their performance on saccade tasks that were designed to probe these executive functions.

### 2.4.2 Saccades

Behaviourally, there was large variability in our data, especially within the ALS group. This variability was likely introduced because disturbances to eye movements in ALS are thought to lie on a continuum, much like the proposed continuum of cognitive impairment in ALS, where...
normal eye movements would exist at one end of the spectrum, while the uncommon occurrence of ophthalmoplegia (or paralysis of the extraocular muscles) in highly progressed patients would exist at the other end of the spectrum (Averbuch-Heller et al., 1998; Donaghy et al., 2011). Despite this variability, clear trends emerged from our small sample size.

In general, antisaccade SRTs were longer than prosaccade SRTs (Fig. 2.3A), and more direction errors were made on antisaccade compared to prosaccade trials (Fig. 2.3D). This behaviour is believed to reflect the additional executive processing required of the antisaccade task (e.g., inhibition and top-down attentional control) (Munoz & Everling, 2004). Differences between ALS and control groups for prosaccade and antisaccade mean SRTs and direction errors did not reach significance, but did reveal a trend that is consistent with DLPFC and FEF damage or dysfunction (Guitton et al., 1985; Pierrot-Deseilligny et al., 1991a; Pierrot-Deseilligny et al., 2002; Pierrot-Deseilligny et al., 2003; Rivaud et al., 1994). ALS patients had somewhat longer antisaccade SRTs than controls (Fig. 2.3A), and slightly elevated antisaccade error rates (Fig. 2.3D), both of which have been previously demonstrated in ALS (Donaghy et al., 2010a; Donaghy et al., 2010b; Donaghy et al., 2011; Evdokimidis et al., 2002; Shaunak et al., 1995). The ALS patients’ impaired performance on producing correct antisaccades cannot be attributed simply to a lack of understanding the task, because antisaccade errors were corrected in all patients. This suggests that they understood the task instructions, but were simply unable to suppress the visual grasp reflex in order to prevent erroneous prosaccades on antisaccade trials, perhaps due to insufficient inhibition from higher level cortical areas such as DLPFC (Guitton et al., 1985).

Because no group effects were observed for mean SRT, it is possible that this measure alone was not sensitive enough for detecting differences between the ALS and control groups.
However, express saccades appeared to provide a more sensitive measure; ALS patients made significantly more express saccades than controls on prosaccade trials (Fig. 2.3C). Express saccades have not been explicitly studied in ALS before, but the rate at which these ALS subjects were making express saccades was quite striking. Based on neural recordings in the monkey, express saccades are elicited when high levels of pre-target activity combine with visual responses in saccade-related neurons of the SC (Dorris et al., 1997; Dorris & Munoz, 1998; Everling et al., 1998; Everling et al., 1999). During the gap period, pre-target preparatory activity is elevated and conditions are optimal for express saccade generation, making suppression of an unwanted saccade on an antisaccade trial very difficult, unless sufficient inhibition from the frontal lobes were to be exerted on saccade neurons in SC (Everling et al., 1998; Everling et al., 1999; Everling & Munoz, 2000). Therefore, the ALS patients' bias towards automatic express saccades may have stemmed from high levels of motor preparation activity, which when combined with poor executive control, likely impeded their ability to suppress the automatic response when performing the antisaccade task.

Interestingly, the ALS group showed a trend of making slightly fewer direction errors on prosaccade trials than controls (Fig. 2.3D). Other studies that have examined automatic saccade behaviour in ALS have failed to find differences between patient and control groups (Averbuch-Heller et al., 1998; Evdokimidis et al., 2002; Shaunak et al., 1995), with the exception of bulbar-onset ALS patients, who displayed longer prosaccade SRTs than spinal-onset ALS patients and controls (Donaghy et al., 2010a). It is possible that bulbar-onset patients had greater saccadic impairments due to the greater pathological involvement of the brainstem, which houses key oculomotor structures (Scudder et al., 2002; Sparks, 2002). In the current study however,
patients from whom we acquired behavioural data were all spinal-onset cases, and they performed better than control subjects on the prosaccade task. This is the behaviour we would expect if ALS patients had high levels of preparatory activation related to motor planning during the prosaccade task, because it would be highly unlikely for them to exert sufficient top-down inhibition to suppress the prosaccade to instead produce an erroneous antisaccade. Rather, it is more likely that the decreased proportion of errors made by ALS patients on the prosaccade task was due to their inclination towards the visual grasp reflex. Control subjects may have occasionally made anticipatory errors where they predicted which side of the screen the target would appear, rather than simply responding to the target with a visual grasp.

Both ALS and control groups had significantly more variable reaction times for prosaccades than antisaccades, as determined by CV_{SRT} (Fig. 2.3B). High intrasubject variability is common within aging and clinical populations (Cameron et al., 2011; Peltsch, 2011); however, antisaccade SRTs were more consistent across ALS and controls, meanwhile prosaccade SRTs were significantly more variable in ALS patients. This discrepancy between tasks is likely due to the more complex processing steps required for the antisaccade task, which would produce a smaller range of feasible reaction times (Munoz & Everling, 2004). In contrast, planning of a prosaccade may take a number of oculomotor pathways, some of which are more direct than others and could make the reaction times more variable. The significantly higher percentage of express saccades made by ALS patients than controls may have been driving the significantly greater variability we observed with ALS prosaccade CV_{SRT}.

ALS patients who failed to suppress the visual grasp reflex during antisaccade trials also performed poorly on the MoCA (Fig. 2.3E). Antisaccade direction errors in ALS are often
associated with ‘distractibility’ and are thought to reflect a lack of executive and attentional control, and abnormal scores on tests of executive function have previously been shown to correlate with distractibility in ALS (Donaghy et al., 2010a; Evdokimidis et al., 2002). We postulated that the behaviour displayed by ALS patients was characteristic of distractibility, likely as a result of impaired executive functioning, and that neuroimaging techniques would detect differences in function of oculomotor brain areas.

2.4.3 Neural Circuitry

We hypothesized that the automatic nature of the ALS patient’s saccadic responses, primarily characterised by a higher proportion of express saccades, may be associated with greater preparatory activity related to motor planning in the SC. Therefore, we investigated the functionality of regions within the frontal, parietal and subcortical areas that are known to mediate activity in the SC (Everling & Munoz, 2000; Hikosaka et al., 2000; Hikosaka et al., 2006; Huerta et al., 1986; Huerta & Kaas, 1990; Paré & Wurtz, 2001), to examine how the functioning of this neural circuitry may explain saccade behaviour. More specifically, we wanted to establish whether differences in activation, in terms of BOLD signal change across Task and Group, could be attributed to differences in saccade preparation or execution.

Our fMRI analyses revealed that in FEF, PEF, DLPFC and CD, significantly greater preparatory BOLD activation was recorded for antisaccades compared to prosaccades in general, and was consistent with previous findings (Brown et al., 2007; Connolly et al., 2005; Curtis & D’Esposito, 2003b; Curtis & Connolly, 2008; DeSouza et al., 2003; Ford et al., 2005; Luna et al., 1998; Sweeney et al., 1996). This difference in BOLD activation is thought to reflect the differences in processing of information (i.e., suppressing automatic responses) (Munoz & Everling, 2004)
and/or differences in difficulty (i.e., attentional load) (Kimmig et al., 2001) between the two saccade tasks. SEF was the only ROI that did not have significant differences in activation between the two tasks; however, upon examination of these findings, it appears that this was driven by the control subjects who showed little difference in activation between prosaccade and antisaccade trials, rather than the ALS subjects.

BOLD signal is thought to reflect the input and processing of information from inhibitory and excitatory neural projections from other regions (Logothetis, 2003; Logothetis & Wandell, 2004). Because ALS patients had significantly greater preparatory activation in PEF for antisaccades compared to prosaccades, we can speculate that increased BOLD signal change in PEF may be related to increased input from other areas such as FEF, SEF, and DLPFC (Connolly et al., 2002; DeSouza et al., 2003; Pierrot-Deseilligny et al., 2002). This increased input from other ROIs might serve to prevent prosaccade generation via suppression of parietal activity (Pierrot-Deseilligny et al., 1991a).

The high frequency with which ALS patients produced express saccades may be explained by preparatory activation in PEF. Increased parietal signalling during the first half of the gap period has been shown to predict PEF activation in the second half of the gap, where greater activity increased express saccade probability (Hamm, Dyckman, Ethridge, McDowell, & Clementz, 2010). This presents an apparent incongruence between our findings of slightly decreased prosaccade preparatory activation in PEF within the ALS compared to controls, despite the ALS patients making substantially more express saccades. The discrepancy between our findings and those of Hamm et al. (2010) may be explained by: 1) the relatively poor temporal resolution of BOLD fMRI, where gap-related activation cannot be specifically looked at, compared to the
superior resolution that was achieved with EEG by Hamm and colleagues; 2) the fact that we did not explicitly model express saccades, and therefore the prosaccade BOLD response for ALS patients reflects all prosaccade latencies; and 3) the mean peak beta weights upon which we based our statistical analyses were taken from time points that corresponded to the peak of the hemodynamic response for all prosaccade latencies, which might not correspond to the appropriate hemodynamic response for the express saccade epoch. It is possible that performing sub-analyses by modelling express saccades into our fMRI data in the future might allow us to pull out differences in the mean peak hemodynamic response between ALS and controls associated with express saccade preparation only. Alternatively, including more functional imaging data by collecting more patients might alter our findings, such that PEF preparatory activation might be greater in ALS patients than controls.

Differences in BOLD signal change between ALS and control groups did not reach statistical significance for saccade preparation. However, trends of slightly decreased prosaccade and slightly increased antisaccade preparatory activation in ALS patients compared to controls were observed in all ROIs. To our knowledge, there is an absence of research investigating differences between ALS and control subjects in terms of cortical activation during the antisaccade and prosaccade tasks. This raises an important research question: whether our paradigm using BOLD fMRI provides a sensitive enough measure for detecting changes to brain function in an ALS population that are relatively not progressed. Perhaps in a more progressed group of patients, or if a larger sample size were to be collected, we would find significant differences in BOLD signal change between ALS and controls.
When interpreting the trend of decreased prosaccade preparatory activation that we found in ALS patients compared to controls, we must consider how changes in cerebral perfusion (in terms of regional cerebral blood flow; rCBF) might influence the BOLD signal measured in ALS patients. Decreased rCBF in extramotor areas including frontal, temporal, and parietal regions has been well documented in ALS populations, where greater reductions in perfusion were found in patients displaying cognitive impairment (Abrahams et al., 1996; Abrahams et al., 2004; Kew et al., 1993a; Talbot et al., 1995). However, we cannot attribute decreased BOLD signal change in ALS during prosaccade preparation to reduced rCBF because ALS patients showed somewhat increased activation during antisaccades. This indicates that these differences between ALS and controls are unlikely a result of a global decrease in perfusion, since BOLD signal varied with the task at hand. Conversely, insufficient forced vital capacity (FVC % predicted) can be neglected as a potential reason for reduced BOLD signal change in the ROIs, because all patients were screened based on a FVC of 60% or higher in order to take part in the MRI experiment.

Another possible explanation for why we did not find significant differences between ALS and controls in these ROIs could be a result of cortical reorganization (for review see: (Lulé, Ludolph, & Kassubek, 2009)). There has even been evidence of shifts in BOLD signal activity in ALS compared to controls, where ALS patients recruited more anterior areas of the pre-motor cortex when performing upper limb movement tasks (Konrad et al., 2002). Despite findings that cortical reorganization is related to upper motor neuron pathology (Tessitore et al., 2006), this neural plasticity is not necessarily unique to ALS; evidence of anterior shifts in activation have been shown in patients with other neurological conditions, such as stroke, to compensate for
loss in motor function (Weiller, May, Sach, Buhmann, & Rijntjes, 2006). If reorganization occurred within this group of ALS patients, then perhaps peak BOLD activations would lie outside of the ROI locations that were chosen a priori, thereby explaining why our ROI analyses were inconclusive in terms of identifying group differences in activation. There is doubt, however, as to whether frontal lobe functions can undergo reorganization; it has been suggested that areas of executive control do not have the same redundancy as motor areas, thereby making compensation unlikely (Lulé et al., 2009). Therefore, it seems improbable that areas such as DLPFC were shifted in terms of the peak activations during the cognitively demanding antisaccade task in the ALS brain compared to controls.

We found significantly greater activation in FEF and PEF during antisaccade execution compared to prosaccades in general (Fig. 2.9B). Previous findings suggested that increased activation in FEF and PEF indicates they are involved with generating antisaccades (Ettinger et al., 2008), likely through their projections to SC (Leigh & Zee, 1999). Interestingly, increased activity in the monkey lateral intra-parietal area (which corresponds to the human PEF) has been recorded during antisaccades compared to prosaccades (Gottlieb & Goldberg, 1999). However, it is not known whether antisaccade-related activation in parietal areas reflects activity related to saccade generation, or whether it represents visual attention and/or visuospatial remapping of the target location for a saccade response (Brown, Goltz, Vilis, Ford, & Everling, 2006).

We did not anticipate finding differences between ALS and control subjects in terms of BOLD signal change during saccade execution, because there were no significant differences in saccade metrics between groups in terms of amplitude and velocity for pro- and anti-saccades. Surprisingly, we found that ALS patients, but not controls, showed significantly greater
activation in SEF during execution of antisaccades compared to prosaccades. SEF’s involvement during antisaccade execution has been demonstrated previously in human imaging studies (Ettinger et al., 2008). SEF is thought to be mostly involved with the execution of volitional saccades, such as antisaccades (Coe et al., 2002; Curtis & D’Esposito, 2003b), and pre-learned saccade sequences, which are believed to rely on the same neural resources as voluntary saccades (Petit et al., 1996). Neural recordings in nonhuman primates also support these findings, where SEF neurons discharged more often during onset of antisaccades than prosaccades (Schlag-Rey & Amador, 1997).

Greater activations in dorsal oculomotor areas such as PEF, FEF, and SEF have been associated with tasks requiring greater attentional demand, such as newly learned saccade sequences (Grosbras et al., 2001), or in this case, greater activation was associated with the antisaccade task. Therefore, the significantly greater antisaccade BOLD signal change in SEF during antisaccade execution might reflect a higher attentional demand required by the ALS patients than the controls. This may be signifying greater task difficulty, rather than genuine changes in functional performance, since ALS patients and controls at resting state previously showed no differences in fMRI activation patterns (Mohammadi et al., 2009). Greater activation during saccade execution in ALS has been found in areas associated with motor learning, such as the basal ganglia, cerebellum (Han & Ma, 2006; Konrad et al., 2002) and brainstem (Konrad et al., 2002). Our study did not find significant differences in CD during saccade execution, and we did not explicitly image the brainstem and cerebellum. It is assumed that changes to BG function in ALS are related to a loss of predominantly UMs (Tessitore et al., 2006), but most patients in our study displayed both upper and lower motor neuron deficits.
In summary, while fMRI neuroimaging provides a valuable clinical tool for investigating neural changes in ALS, it remains speculative as to whether changes in brain function during saccade tasks are sensitive enough to provide any diagnostic value, or whether these changes represent individual phenomena that vary between subjects. We predict that increasing the sample size of our functional data might make this measure of brain function more sensitive to differences between neurodegenerative and healthy groups.

2.4.4 Structural Integrity

There has been substantial evidence pointing to abnormalities in brain regions that lie outside of the motor cortex in ALS, where cognitively impaired patients displayed greater extramotor involvement (Abrahams et al., 1996; Abrahams et al., 2000; Abrahams et al., 2005; Chang et al., 2005; Guedj et al., 2007; Neary et al., 2000). We found trends of widespread loss of cortical thickness in terms of GM in ALS patients compared to age-matched controls which appeared to overlap with many of the oculomotor ROIs (Fig. 2.10). Changes in cortical volume in frontal and temporal regions have been said to underlie changes in brain function in ALS patients without cognitive impairments (Abrahams et al., 2003). Therefore, these structural changes may influence the differences we previously described in functional and behavioural performance on oculomotor tasks in the ALS group compared to controls.

Despite previously recorded differences between ALS and controls on measures of cerebrospinal fluid (CSF), grey matter (GM) or white matter (WM) volume (Abrahams et al., 2005; Chang et al., 2005; Frank et al., 1997; Murphy et al., 2007b; Thivard et al., 2007), we did not find significant differences between groups (Fig. 2.11A). It is possible that differences in GM, WM and CSF would arise if we separated ALS patients who displayed more severe cognitive
impairment in our sample from those who were less impaired, since greater atrophy has been documented in ALS patients with comorbid cognitive abnormalities (Murphy et al., 2007a; Murphy et al., 2007b).

We also found trends of extramotor atrophy in ALS patients in terms of decreased GM volume in frontal, medial frontal, and medial temporal regions (Figs. 2.11B, C). This appeared to follow a frontotemporal pattern described previously (Kato et al., 1993; Yoshida, 2004), which is believed to spread with disease progression and is a hallmark of frontotemporal dementia (FTD) (Neary et al., 1998). Even though our patients did not strictly meet the diagnostic criteria for FTD, they did display a continuum of impairments based on neuropsychological tests that are consistent with impaired frontal lobe functions. We can therefore infer that worsened cognitive function, more severe disease progression, and loss of structural integrity are highly interconnected. It is possible that if our sample contained more highly progressed patients, this atrophy would extend from anterior to posterior areas, and may reach statistically significant group differences.

As such, our structural data show promising preliminary findings of frontotemporal atrophy; however it remains unclear as to whether the behavioural and functional changes we observed in ALS can be attributed directly to pathological structural changes, or whether they represent functional compensation as a result of increased task difficulty.

2.4.5 Functional Compensation

Cortical degeneration can result in various compensatory mechanisms that make up for motor function loss in ALS, such as recruitment of surrounding cortical areas and even cortical reorganization (Lulé et al., 2009). Our functional results from oculomotor ROIs can be looked at
in the context of functional compensation, and we propose potentially two compensation mechanisms were at work: compensation due to task difficulty vs. structural damage.

Behaviourally, it appears as though the antisaccade task was considerably more difficult than the prosaccade task for the ALS patients, where greater difficulty would require more cognitive effort exerted to perform the task correctly (Schoenfeld et al., 2005). It has been suggested that functional compensation in ALS may be achieved via recruitment of existing cortical resources to make up for the additional task difficulty (Schoenfeld et al., 2005), which would explain the trends we saw of increased BOLD signal change for antisaccade preparation. One could also argue that difficulty with the antisaccade task in ALS patients was derived from GM loss in oculomotor and frontal areas that prevent unwanted reflexive saccades, such as DLPFC (Guitton et al., 1985).

Although our cortical thickness analyses showed trends of decreased GM in the parietal lobes, it seems improbable that this lesion was substantial enough to merit functional compensation in PEF during preparation; while ALS patients did show a compensation-like increase in BOLD signal for antisaccades, activation during prosaccade preparation closely resembled that of control subjects. Because lesions to PEF have behavioural consequences such as longer prosaccade SRTs (Pierrot-Deseilligny et al., 1991a), and our ALS subjects showed no impairment to prosaccade latencies, it seems more plausible that increased activation during antisaccade preparation in PEF is related to ALS patients compensating for the increased task difficulty, rather than compensation due to structural damage.

We therefore investigated correlations between GM volumes and BOLD signal responses to determine whether a compensatory mechanism was at work (Fig. 2.13). Because BOLD signal is
mostly measured from GM, reduced cortical thickness may explain why we saw functional
differences between ALS and controls. We found significant correlations within DLPFC, where
increased antisaccade preparatory activation correlated with increased lateral prefrontal GM in
control subjects; meanwhile, ALS subjects appeared to be approaching a negative correlation. It
is possible that this positive correlation within control subjects signifies a lack of compensation
required, since reduced GM volumes (perhaps as a result of atrophy associated with normal
aging) were not associated with increased functional compensation. Even though the ALS
patients did not show a significant negative correlation, they may still be compensating slightly
in comparison to controls. We also found significant negative correlations for pro- and
antisaccade execution where reduced GM volumes in areas that house oculomotor ROIs were
associated with increased activation in those same ROIs within the ALS group only. This appears
to represent functional compensation due to reduced GM in ALS patients compared to controls,
in lateral prefrontal and parietal regions.

Functional compensation has been shown to progressively adapt with the ongoing loss of
function in ALS (Lulé et al., 2007); therefore larger differences in brain activation between ALS
and control subjects as a result of compensation may become apparent with more severe
disease progression. Disease progression is also associated with greater spread of atrophy in
cortical and subcortical regions (Nair et al., 2010; Thivard et al., 2007), which would also
promote compensation to make up for a lack of structural integrity. Regardless, it is difficult to
say conclusively with the current data whether structural changes caused greater task difficulty
and subsequent functional compensation, or whether task difficulty compensation is
independent of structure.
2.4.6 Limitations and Future Directions

There are several limitations associated with conducting multidisciplinary research of this nature within a fragile clinical population such as ALS. The most challenging physical restriction, which led to a number of recruited patients being unable to complete the entire experiment, was the impaired respiratory capacity that accompanies ALS. Patients who had a FVC of 60% predicted or lower were unable to complete the fMRI portion of the experiment as a safety precaution. One patient had a sufficient FVC, but had experienced sialorrhea and had difficulty breathing when lying down in the MRI, so the experiment was aborted. Other physical restrictions that prevented us from doing the MRI session included failed safety screening (i.e., metal implants), and claustrophobia. Along a similar vein, further physical limitations were experienced as a result of disease progression; more severe progression is usually associated with worsened FVC, therefore we were unable to study brain function or structure in highly advanced patients. Because of this, we were unable to gain insight on whether and/or how cognitive decline worsens with progression, and how this influences brain pathology.

Although our research provides interesting insight into executive dysfunction in ALS, it would be beneficial to recruit more ALS and control subjects to complete the entire protocol in order to have 25 – 30 participants in each group. This should allow us to improve statistical power in our measures. With the current sample size, we saw large variability in the behavioural (Fig. 2.3) and functional data (Fig. 2.7, 2.9) that may have resulted from the continuum of cognitive profiles within this ALS group. Future analyses to separate patients who are cognitively impaired from those who are not (or less impaired) on measures of behaviour and brain function (in terms of BOLD signal) might reveal less variability within these sub-groupings. If our
preliminary results are strengthened by adding new data, and we are able to confidently identify the neural correlates of cognitive decline in ALS, we may have a model system to use for critical evaluation of future therapies and interventions that will be developed for ALS.
Chapter 3

General Discussion

This research was conducted as the first to investigate cognitive impairment in ALS using: neuropsychological assessments to evaluate cognitive and behavioural deviances; saccadic eye movement tasks to assess changes in behavioural control; functional imaging to characterize how underlying neural correlates of saccade control are altered in ALS; and structural imaging to determine whether brain atrophy displayed frontotemporal and oculomotor involvement. We predicted that ALS patients would be impaired on tests of executive function, including the antisaccade task. We also proposed that impaired behavioural performance would correspond to changes in functional activation during antisaccade preparation and cortical atrophy in frontal lobe and oculomotor regions.

We provided evidence that ALS patients displayed cognitive impairments, and that the antisaccade task may prove as a reliable tool for detecting such impairments in ALS (Fig. 2.3E). ALS patients were biased towards express saccades and, consequently, had slightly increased antisaccade error rates, both which may have been precipitated from high levels of activation related to motor preparation in the superior colliculus (Figs. 2.3C, D) (Dorris et al., 1997; Dorris & Munoz, 1998; Everling et al., 1998; Everling et al., 1999). Our functional imaging data revealed trends that ALS patients had increased preparatory activation in oculomotor regions for antisaccades, perhaps to functionally compensate for the added task difficulty and their impaired executive control. Decreased grey matter in ALS patients revealed a pattern of frontotemporal pathology, and overlapped with saccades control areas. We speculate that the
loss of cortical resources in frontal and temporal regions in ALS may underlie the resulting functional changes (Abrahams et al., 2003), such as compensation, to counteract the executive dysfunction and added task difficulty of voluntary saccades for ALS patients. However, it appears as though this compensation is perhaps insufficient, since voluntary saccades were somewhat slower and resulted in slightly more errors in the ALS group compared to controls.

3.1 Clinical Relevance

Cognitive impairment in ALS is still a relatively new facet of the disease, and despite its high prevalence (Ringholz et al., 2005), it is not an official symptom for diagnosis. However, cognitive decline in ALS has been widely documented in the literature, and in some cases can be detected before any motor symptoms of ALS (Guedj et al., 2007; Mackenzie et al., 2005; Murphy et al., 2007a). Therefore a patient’s cognitive status might be an important diagnostic or screening tool for those at risk of developing the disease (i.e., family history). Cognitive tests alone, however, may not provide the most reliable diagnosis, and therefore should be combined with other tools such as neuroimaging (Barson et al., 2000; Strong et al., 1999), which are often inaccessible and costly. Saccade tasks may provide an appropriate tool to complement neuropsychological tests in identifying changes to cognition and behaviour. Based on our findings, changes in saccade performance in ALS may correspond to specific functional and structural changes, which could possibly provide an alternative supplementary diagnostic tool to neuroimaging. We not only found that antisaccade errors are closely associated with executive dysfunction in ALS, but also that antisaccade reaction times were related to functional changes that corresponded to a particular pattern of brain atrophy (i.e. DLPFC and PEF).
Our findings have provided promising preliminary data, which, if expanded upon to create a larger and more reliable data set, might provide a sensitive and accessible way of quantifying neurological changes that are characteristic of ALS pathology. Saccades may also be an appropriate tool for assessing cognitive function in highly progressed patients who have become ‘locked in’ due to loss of motor function. Even in severely progressed ALS patients, extraocular muscles are somewhat resistant to motor neuron loss (Okamoto et al., 1993). This preservation would allow us to quantify changes in saccade behaviour, and subsequently infer changes to brain function and structure in severely progressed ALS patients who are unable to lie supine in an MRI due to respiratory difficulties. Studying patients who vary in disease severity and progression might allow us to map out a trajectory of ‘expected saccadic impairments as a result of progression’, which could prove to be a valuable prognostic tool when assessing a patient’s life expectancy.

This study provides important measures of brain structure and function in ALS that are distinct from impairments seen in other neurodegenerative disorders. For example, patients with Parkinson’s disease (PD) also have a neurodegenerative pathology that affects their cognition and motor skills. ALS patients in our study and PD patients from the study of Cameron and colleagues (2011) displayed similar saccade impairments such as increased difficulty with the antisaccade task, and a bias towards automatic responses. However, ALS patients appeared to make even more express saccades than PD patients, and the nature of functional changes in PD were highly distinguishable from those seen in ALS. PD patients, both on and off medications, had decreased preparatory activation in a number of ROIs during the antisaccade task (Cameron et al., 2011), whereas ALS patients showed trends of increased preparatory
activation. This suggests that different pathological and compensatory processes are at work, and that brain function and behaviour are influenced differently within these neurodegenerative diseases. It is possible that upon further data collection of ALS saccade behaviour, we could establish greater statistical power to unveil more subtle differences between ALS and PD patients’ performance on saccade tasks.

3.2 Limitations

Additional limitations we experienced in the current study were predominantly of two streams: 1) pragmatic limitations; and 2) data-related limitations. The major pragmatic limitation encountered was the location in which this research took place. This research was conducted in Kingston, Ontario, a city with a population of approximately 150,000 people, and ALS patients were recruited from two neuromuscular clinics associated with Kingston hospitals. The prevalence of ALS is said to be 6 per 100,000 people (Mitchell & Borasio, 2007). Since many surrounding areas do not have ALS specialists, many patients from smaller municipalities (excluding Toronto and Ottawa) travel to Kingston for specialized health care. Based on this limited pool of subjects, we were only able to obtain consent from 21 eligible ALS patients for this study. Since all ALS patients in this study were from Kingston or the surrounding areas, we may have introduced an unavoidable sampling bias based on geographical location.

The limitations we experienced related to data were: 1) a non-random sample; 2) effects of medications; 3) not modelling fixation, and 4) altered express saccade epoch. The participants who completed our study were not randomly sampled; participation in this study depended upon the patient: 1) living within the Kingston region; 2) giving informed consent; 3) not meeting any exclusion criteria; and 4) successful data acquisition. Additionally, the sample was
heterogeneous in terms of symptom onset, disease duration and severity. Due to the small cohort of patients we successfully ran, we did not have sufficient statistical power to categorize patients based on their disease duration, onset, or severity of symptoms. As a result, we cannot draw any conclusions about how the type of onset or disease severity influenced behaviour, brain function, or structure. The patients we recruited were almost exclusively spinal-onset, because most bulbar-onset patients experience early decline in their respiratory function, making them poor candidates for completing the MRI protocol. Bulbar-onset, however, is also associated with greater executive dysfunction than spinal-onset (Abrahams et al., 1997; David & Gillham, 1986; Lomen-Hoerth et al., 2003; Massman et al., 1996), which perhaps would have provided us with statistically significant findings when compared to control results.

Due to our limited access to ALS patients, we did not consider neurologically irrelevant health afflictions as exclusion criteria. Patients who were included in the final analyses did not display any current depression or anxiety symptoms (based on medical history and scoring on the HADS), and had no other known neurological confounds. However, almost all patients were taking medications (see Table 1), some which were for ALS symptom management/ treatment such as Baclofen™ and Riluzole™, while others were for unrelated health conditions (i.e., diabetes, chronic obstructive pulmonary disease, high cholesterol, and gastric acid reflux). Antianxiety and antidepressant medications were being taken by a number of patients, not due to pre-existing clinical anxiety or depression disorders, but rather for managing the anxiety and depression associated with having a terminal disease. We recognize that these medications have powerful neurological effects which could potentially influence the oculomotor network; however such investigations have not been explicitly studied in ALS. Eye movements have been
studied in patients with schizophrenia on and off antipsychotic medications, where antipsychotics did not influence the rate of antisaccade errors, but did increase antisaccade SRTs in non-medicated patients compared to medicated patients and controls (Hutton et al., 1998). We must therefore interpret our results with caution. Some patients were also taking antihypertensive medications, which may have influenced BOLD signal changes. While we cannot exclude the possibility that these medications influenced our fMRI data, previous findings support that beta-blocking antihypertensives did not affect cerebral blood flow, cerebrovascular reactivity, or cognitive performance (Heinke, Zysset, HundGeorgiadis, Olthoff, & von Cramon, 2005).

One saccade parameter that was not specifically looked at in this study, and which may have caused a limitation in the interpretation of our data, was fixation. Evidence of saccadic intrusions during fixation have been shown in ALS patients, where intrusions were greater in amplitude (Donaghy et al., 2009) and frequency (Shaunak et al., 1995) for ALS patients compared to controls. However, it was reported that the size of the intrusions were not significantly different across ALS and controls when a fixation cue was present (Donaghy et al., 2009). With the exception of the 200 ms “gap” in our paradigm, there was always a cue present when subjects were asked to fixate; therefore this should not be of concern for our data. Provided that we did not explicitly look at fixation in the current study (and fixation was not modelled in our fMRI analyses), we cannot confirm that fixation abnormalities were absent in this ALS subject pool. If abnormalities were present in the patient group, the baseline for all fMRI analyses might be inconsistent across ALS and control groups. Future studies should perhaps consider modelling fixation into the general linear model, so that brain activation

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caused by saccadic intrusions during fixation can be isolated and removed from the contrasts, rather than being concealed within the baseline measure of fixation.

When the behavioural parameters were chosen for this study, the express saccade epoch was based on what had been documented in the human and monkey literature: reported express saccade latencies tend to fall within 90 – 140 ms (Dorris et al., 1997; Fischer & Boch, 1983; Fischer & Weber, 1993; Munoz et al., 1998). Interestingly, however, we have observed from previously conducted research in the Munoz lab (unpublished data) that saccadic reaction times are often elongated when subjects are lying supine in an MRI compared to when they are sitting upright performing saccade tasks. Prosaccade reaction times follow a bimodal distribution, where express saccades are the first peak, and regular latency saccades are the second peak. Based on the data we collected from ALS patients lying supine, their express saccade epoch appeared to span from 90 – 160 ms, and therefore our epoch was adjusted slightly from that reported in the human and monkey literature where subjects were sitting upright. We therefore should exercise caution when making comparisons to other studies (such as those looking at the frequency of express saccades in PD patients) where a different express saccade epoch was used (i.e., (Cameron et al., 2011)).

3.3 Future Directions

In future research, it would be extremely valuable to do longitudinal large-scale studies to determine how ALS patients’ cognitive abilities deteriorate over time and how this influences their saccadic performance and corresponding functional and structural integrity. Evaluations of cognitive performance have been shown to decline over time in bulbar-onset ALS patients (Strong et al., 1999), so it is plausible that antisaccade performance (which correlates with
cognitive abilities) would also change with time. Based on the time constraints of a Master’s program, I was unable to conduct longitudinal research within this cohort of patients. Additionally, some of the patients within the sample progressed so rapidly after the first data collection session that a follow-up session would not have been possible due to respiratory and physical decline. However, I did have the opportunity to follow-up with one patient who repeated the protocol 9 months after his initial participation in the study. Unfortunately, findings from this one subject were inconclusive; only very subtle differences were seen across neuropsychological, behavioural, functional and structural measures. It is therefore difficult to conclude anything from this one follow-up since this patient might be particularly slow at progressing. Perhaps if a third follow-up was conducted a number of months later, or if I had been able to do follow-ups with more patients from this sample, I would have found more substantial differences. This would be an extremely valuable arena to explore, as it could provide important insight on the influence of disease progression on the ALS brain.

3.4 Summary and Conclusions

The results of our study demonstrate that, even with a small sample size, cognitive impairments on tests of executive function can be detected in ALS patients. These deficits were associated with more direction errors made on the antisaccade task, which is designed to probe executive control. Surprisingly, ALS patients were biased towards rapid automatic responses that were likely caused by increased preparatory activation in SC as a result of inadequate suppression from higher-order cortical areas (Dorris et al., 1997; Guitton et al., 1985; Munoz & Everling, 2004). We found evidence of functional compensation in ALS patients, which might reflect greater task difficulty (perhaps as a result of cognitive impairment) or may signify
compensation for a lack of grey matter. To our knowledge, this study marks the first to combine psychometric evaluations, saccade tasks, functional and structural imaging techniques to study neurological changes in ALS. Our preliminary data are promising, and further data collection may bring the trends we observed closer to statistical significance, allowing us to confidently identify the neural correlates of cognitive decline in ALS. If, however, we fail to identify a neural substrate for cognitive impairment in ALS with a larger data set, we will have to explore other cognitive tasks that probe executive functions that lie beyond the oculomotor system.


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Appendix A

El Escorial World Federation of Neurology Revised Criteria for ALS Diagnosis. (Brooks, 1994; Brooks et al., 2000).

The diagnosis of ALS requires the presence of:

1) evidence of lower motor neuron (LMN) degeneration by clinical, electrophysiological or neuropathologic examination,
2) evidence of upper motor neuron (UMN) degeneration by clinical examination, and
3) progressive spread of signs within a region or to other regions, as determined by history or examination

Together with the absence of:

1) electrophysiological or pathological evidence of other disease processes that might explain the signs of LMN and/or UMN degeneration, and
2) neuroimaging evidence of other disease processes that might explain the observed clinical and electrophysiological signs

“Clinically Definite ALS is defined on clinical evidence alone by the presence of UMN, as well as LMN signs, in the bulbar region and at least two spinal regions or the presence of UMN and LMN signs in three spinal regions.

Clinically Probable ALS is defined on clinical evidence alone by UMN and LMN signs in at least two regions with some UMN signs necessarily rostral to (above) the LMN signs.

Clinically Probable ALS – Laboratory-supported is defined when clinical signs of UMN and LMN dysfunction are in only one region, or when UMN signs alone are present in one region, and LMN signs defined by EMG criteria are present in at least two regions, with proper application of neuroimaging and clinical laboratory protocols to exclude other causes.

Clinically Possible ALS is defined when clinical signs of UMN and LMN dysfunction are found together in only one region or UMN signs are found alone in two or more regions; or LMN signs are found rostral to UMN signs and the diagnosis of Clinically Probable ALS – Laboratory supported cannot be proven by evidence on clinical grounds in conjunction with electrodiagnostic, neurophysiologic, neuroimaging or clinical laboratory studies. Other diagnoses must have been excluded to accept a diagnosis of Clinically Possible ALS.

Clinically Suspected ALS may be suspected in many settings, where the diagnosis of ALS could not be regarded as sufficiently certain to include the patient in a research study. Hence, this category is deleted from the revised El Escorial Criteria for the Diagnosis of ALS.”