Cognitive Sub-groups in ALS:

Neuroanatomical Associations and Theory of Mind Impairments

by

Sneha Raghotham Chenji

A thesis submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Neuroscience

University of Alberta

Abstract

Amyotrophic Lateral Sclerosis (ALS) involves the progressive loss of upper and lower motor neurons. Frontotemporal lobar degeneration (FTLD) has been reported in a considerable proportion of patients, manifesting as cognitive and behavioural impairment in at least 25-50%, with 10-15% patients meeting criteria for co-morbid frontotemporal dementia (FTD). Executive function (EF) impairment is frequently reported while recent studies are identifying impairments in Theory of Mind (ability to decipher cognitive or emotional mental states of individuals).

The Edinburgh Cognitive and Behavioural ALS Screen (ECAS) is emerging as an optimal screening tool to capture impairments and includes a test for ToM. Aim 1 of the thesis was to identify neuroanatomical associations using the ECAS. The study identified EF impairments in a sub-group of patients with ALS. Patients with impaired EF (ALS-exi) displayed a greater extent of frontotemporal white matter degeneration and focal grey matter atrophy, in line with proposed models of pathological spread of phosphorylated TAR DNA-binding protein of 43 kDa (pTDP-43) in ALS. Patients with normal EF (ALS-n) displayed changes in medial prefrontal cortex suggesting the possibility of structural changes in the absence of cognitive decline, or alternatively reflecting pathological changes in the presence of subtle cognitive changes that were not identified on the ECAS. Associations of neuroimaging metrics with EF revealed that extensive loss of white matter integrity contributed to lower EF performance.

Considering the emerging evidence of ToM impairments, aim 2 of the thesis was to identify associations between ToM and EF in ALS. The study revealed that ToM impairments were

present in ALS, whereby patients displayed significantly reduced abilities to infer thoughts and emotions. ALS-exi patients performed worse than ALS-n patients, thus suggesting a link between EF and ToM performance.

To follow-up with the observed ToM impairments, aim 3 of the thesis was to identify structural and functional changes in brain regions associated with ToM deficits. ALS patients and healthy controls in this experiment were a sub-cohort of participants from the experiments for aim 1 and 2. A trend towards ToM impairments and mild atrophy in both grey matter and white matter was noted in ALS patients compared to controls. Functional changes in resting state networks were observed in ALS patients when compared to controls, however these did not reach thresholds for statistical significance. Both ToM and EF had mild associations with reduced grey matter density in the dorsolateral prefrontal cortex (dlPFC), suggesting that a shared neural substrate may be associated with the decline of both the domains.

In summary, the thesis provides evidence of frontotemporal degeneration in cognitive sub-groups identified using the ECAS. ToM impairments were identified in ALS patients and were associated with the degree of EF impairments. Mild associations of ToM and EF with grey matter density in the dlPFC suggests a shared neural substrate.

Preface

This thesis is an original work by Sneha Chenji. The research project of which this thesis is a part, received research ethics approval from the University of Alberta Research Ethics Board, Project Name: "Cognitive Changes in MND", No. Pro00045418, March 24, 2014. Some of the research conducted in this thesis is part of a collaboration under the umbrella of the Canadian ALS Neuroimaging Consortium (CALSNIC) led by Dr. Sanjay Kalra at the University of Alberta. Ethics approval for the collaborative project titled "MRI Biomarkers in ALS" was obtained from the respective sites: University of Alberta (No. Pro0036028, March 15, 2013), University of Calgary (No. REB13-0651, October 23, 2013), McGill University (NEU-13-016, June 23, 2014) and University of Toronto (No. 445-2013, August 26, 2014).

Chapter 3 is a manuscript prepared for submission. I have been involved with data collection, design of experiments and data analysis. Abdullah Ishaque was involved with data processing for diffusion tensor imaging. Dennell Mah was involved with data collection. Dr. Christian Beaulieu, Peter Seres, Dr. Richard Frayne and Dr. Simon Graham were involved with development and harmonisation of MRI protocols across sites. Dr. Wendy Johntson performed neurological examinations for some participants in the study. Dr. Lorne Zinman, Dr. Angela Genge and Dr. Lawrence Korngut are the principal investigators at the ALS clinics in Toronto, Montreal and Calgary respectively, and involved with data collection (neurological examination) for participants in the study. Dr. Sanjay Kalra is the lead principal investigator for the collaborative project and was involved with study design, development of the MRI protocol and data collection.

Chapters 4 and 5 are in preparation for a single manuscript as Study A and B respectively. Chapter 4 includes data from University of Alberta, University of Calgary and McGill University, while Chapter 5 includes data from University of Alberta. I was involved with data collection, design of experiments and data analysis. Dr. Esther Fujiwara helped with data analysis for Chapter 4 (Study A). Dennell Mah was involved with data collection for both Chapters. Abdullah Ishaque was involved with data processing for diffusion tensor imaging for Chapter 4 (Study B). Peter Seres was involved with MRI protocol and data acquisition for Chapter 4. Dr. Wendy Johnston was associated with data collection (neurological examination). Dr. Sanjay Kalra was the principal investigator for the studies and was involved with design of experiments, MRI protocol development and data collection.

Dedicated to my beautiful avva (grandma) and dearest parents

Acknowledgements

I would like to thank a wonderful team of people who have contributed to this work.

My gratitude and many thanks to Dr. Sanjay Kalra for providing me with an opportunity to pursue my PhD. Your guidance and free reign to pursue my interest in brain-behaviour relationships in ALS research are invaluable and I have enjoyed the learning experience.

To my committee members, Dr. Esther Fujiwara and Dr. Nikolai Malykhin, many thanks for your guidance and questions that have shaped my research process. Dr. Esther Fujiwara, a special thank you for teaching me SPSS tricks that made my analysis much easier!

My sincere thanks to Dennell Mah for her immense support with conducting the study. I am grateful to Dennell and my co-workers at the Kalra lab, for being there, be it for brainstorming ideas, their friendship, or for the times when my experiments did not work (which happened often!).

My sincere gratitude to all the participants in the study for taking the time to be a part of our research, this work would not be complete without your participation. I am thankful to the ALS clinic team and the CALSNIC team for their collaborative environments, providing a platform to approach patients and gather data for my study.

I am thankful for the generous funding support received from the Canadian Institute of Health Research (CIHR) Operating Grant (received by Dr. Kalra), the Faculty of Graduate Studies and Research Recruitment Scholarship and the 75th Graduate Student Award and the Faculty of Graduate Studies and Research Travel Award.

I am forever indebted to my parents for their unconditional love and support, I am blessed. My grandmother deserves a special mention for being my inspiration to pursue my interest in brain behaviour relationships. My most favourite people, Ashokappa and Preethima, you both are an epitome of inspiration for me. I look up to you; your unwavering support and love remains with me even if we are countries apart. Last but far from least, to my dear fiancé, I am short of words to express my gratitude for your love, patience and for sponsoring countless meals when I was writing my thesis.

Table of Contents

Abstract ii

Preface iv

Acknowledgements vii

Table of Contents ix

List of Tables xv

List of Figures xvii

List of Abbreviations xix

1. Amyotrophic Lateral Sclerosis: Overview 1

1.1 Epidemiology of ALS 1

1.2 Etiology 4

1.2.1 Genetic Risk Factors 4

1.2.2 Environmental Risk Factors 5

1.3 Clinical Characteristics 6

1.3.1 Prognostic Indicators in ALS 11

1.4 Diagnostic Criteria 11

1.4.1 ALS 12

1.4.2 ALS-FTLD Spectrum 15

1.5 Pathophysiology 22

1.5.1 Glutamate Excitotoxicity 24

1.5.2 Mitochondrial Dysfunction 24

1.5.3 Pathophysiology in ALS-relevant Genetic Mutations 25

1.5.4 RNA Metabolism 26

1.6 Neuropathology 27

1.7 Biomarkers and Neuroimaging Evidence 33
1.7.1 Tissue and Fluid biomarkers 33
1.7.2 Physiological Biomarkers 34
1.7.3 Neuroimaging Biomarkers 35
1.8 Clinical Management 42
1.8.1 Measuring disability 42
1.8.2 Physical weakness and disability 43
1.8.3 Respiratory Function 43
1.8.4 Managing Bulbar Symptoms 44
1.8.5 Nutritional Management 45
1.8.6 Disease Modifying Treatments 46
1.8.7 Monitoring Cognitive and Behavioural Changes 47
1.9 Summary 49
2. Neuropsychometric Profile and Neuroimaging in ALS 50
2.1 ALS-Specific Cognitive and Behavioural Screening Tools 50
2.2 Cognitive Impairment in ALS 51
2.2.1 Executive Functions 52
2.2.2 Social Cognition 68
2.2.3 Language 75
2.2.4 Learning and Memory 80
2.2.5 Visuospatial Functions 84
2.3 Behavioural Impairment in ALS 85
2.3.1 Apathy 86
2.3.2 Other behavioural domains 88
2.4 Longitudinal changes in cognition 90

2.5 Clinical variables and the ALS-FTLD spectrum 91
2.6 Neuroimaging and the ALS-FTLD Spectrum 94
2.6.1 Grey Matter Changes 95
2.6.2 White Matter Changes 99
2.6.3 Functional Changes 102
2.7 Summary 104
2.8 Rationale, and Aims and Hypotheses of the thesis 105
2.9 Supplementary Materials 110
3. Neuroanatomical Associations of the Edinburgh Cognitive and Behavioural ALS Screen (ECAS) 119
3.1 Abstract 121
3.2 Introduction 123
3.3 Methods 124
3.3.1 Participants 125
3.3.2 ECAS and Cognitive Sub-groups 126
3.3.3 MRI acquisition 127
3.3.4 MRI preprocessing 127
3.3.5 Statistical Analysis 129
3.4 Results 129
3.4.1 Demographic and Clinical characteristics 129
3.4.2 Lower ECAS performance in ALS 130
3.4.3 Grey matter degeneration as indicated by VBM 131
3.4.4 Widespread loss of white matter integrity in ALS 132
3.4.5 Frontotemporal associations of grey and white matter with ECAS 132
3.5 Discussion 133

3.5.1 Executive dysfunction detected by ECAS 133
3.5.2 Frontotemporal changes are present in EF normal (ALS-n) patients 134
3.5.3 Greater extent of atrophy in EF impaired (ALS-exi) patients.....135
3.5.4 Neuroanatomical implications of ECAS and MRI metrics.....136
3.5.5 Strengths, Limitations and Future Directions 139
3.6 Conclusions 140
3.7 References.....141
3.8 Tables and Figures.....151
3.9 Supplementary materials 159
4. Executive Functions may Moderate ToM in Amyotrophic Lateral Sclerosis 163
4.1 Abstract.....165
4.2 Introduction.....167
4.3 Methods 169
4.3.1 Participants.....169
4.3.2 Tools.....170
4.3.3 Statistical Analysis.....173
4.4 Results 174
4.4.1 Demographics 174
4.4.2 Group Comparisons 175
4.4.3 ALS Sub-group Analyses.....176
4.4.4 ToM associations with EF and Clinical Variables.....176
4.5 Discussion.....177
4.5.1 ToM impairments are present in ALS and moderated by EF 177
4.5.2 Neuroanatomical Implications 179
4.5.3 Clinical associations with ToM.....180

4.5.4 Strengths and Limitations 181
4.6 Conclusions 181
4.7 References....182
4.8 Tables and Figures....190
4.9 Supplementary materials 194
5. Structural and Functional Associations of ToM in ALS 197
5.1 Abstract....199
5.2 Introduction....201
5.3 Methods 203
5.3.1 Participants....203
5.3.2 Neuropsychometric tests 203
5.3.3 MRI acquisition and analysis....204
5.3.4 ROI selection and Group Comparisons....207
5.4 Results 208
5.4.1 VBM analysis....209
5.4.2 DTI analysis 210
5.4.3 rs-fMRI analysis....210
5.5 Discussion....211
5.6 Conclusions 213
5.7 References....215
5.8 Tables and Figures....220
5.9 Supplementary materials 227
6. General Discussion and Conclusions....230
6.1 Cognitive profile in ALS 232
6.2 Neuroimaging Findings 233

6.3 Clinical Implications.....235

6.4 Strengths, Limitations and Future directions.....236

6.5 Conclusions 239

Bibliography.....240

List of Tables

Table 1.1. Description of LMN and UMN signs at four levels of the CNS (Brooks et al., 2000). 8

Table 1.2. Criteria for clinical diagnosis of FTLD language variants (Gorno-Tempini et al., 2011). 17

Table 1.3. Revised International Criteria for bvFTD (Rascovsky et al., 2011). 18

Table 3.1. Participant characteristics. 151

Table 3.2. Clinical characteristics of the patient group. 152

Table 3.3. MRI protocol 153

Table 4.1. Participant characteristics: mean age, education, gender and clinical variables. 190

Table 4.2. Performance on sub-categories of the ECAS. 191

Table 4.3. Median scores for social cognition (ToM) tests in controls and patients. 192

Table 5.1. Demographic and clinical profile of the participants. 220

Table 5.2. Group comparisons on ECAS and ToM tests. 221

Table 6.1. Summary of aims, hypotheses and results. 231

Supplementary Table 2.1. Brief outline of studies highlighting cognitive performance in ALS. 110

Supplementary Table 2.2. Brief outline of studies of apathy in ALS. 117

Supplementary Table 2.3. ECAS associations with neuropsychometric tests (in-house experiment). 118

Supplementary Table 3.1. Normality tests for the ECAS. 160

Supplementary Table 3.2. Percentage of ALS patients impaired on individual ECAS domains..... 162

Supplementary Table 4.1. Normality of ECAS and ToM scores. 194

Supplementary Table 4.2. Post-hoc Mann-Whitney U-test for the Faux pas test. 195

Supplementary Table 5.1. Summary of voxel-wise correlations between ToM / EF tests and VBM 228

List of Figures

Figure 1.1. Upper and lower motor neuron pathways in the central nervous system. 7

Figure 1.2. ALS-FTLD spectrum as described by Strong et al. (2017). 20

Figure 1.3. Pathogenesis of ALS. 23

Figure 1.4. Neuropathological hallmarks in ALS. 31

Figure 1.5. Sequential spread of pTDP-43 pathology. 32

Figure 3.1. Box plots displaying ECAS performance. 154

Figure 3.2. Performance on tests of executive function in the ECAS. 155

Figure 3.3. Group differences indicating reduced grey matter density in ALS patients. . 156

Figure 3.4. Group differences indicating reduced white matter integrity in ALS patients. 157

Figure 3.5. Grey matter density (GM) and fractional anisotropy (FA) correlations of ECAS verbal fluency and ECAS executive domains. 158

Figure 4.1. Box-plots indicating ToM scores. 193

Figure 5.1. Regions of Interest (ROIs) for MRI analysis. 222

Figure 5.2. ROI-based voxel based morphometry (VBM). 223

Figure 5.3. ROI-based Tract Based Spatial Statistics (TBSS). 224

Figure 5.4. One-sample t-test of seed-based analysis. 225

Figure 5.5. One sample t-test indicating the default mode network (DMN). 226

Supplementary Figure 3.1. Post-hoc comparisons displaying of clinical variables. 159

Supplementary Figure 3.2. Histogram indicating frequency of distribution of scores for the ECAS domain. 160

Supplementary Figure 3.3. Performance on the ECAS per site. 161

Supplementary Figure 4.1. Histogram plot and test statistics for the Faux pas test total score. 196

Supplementary Figure 5.1. Performance on the RME test. 227

S**upplementary Figure 5.2.** ROI-based analysis for AD, RD and MD. 229

List of Abbreviations

3D	Three Dimensional
ACC	Anterior cingulate cortex
ACE	Addenbrooke's Cognitive Exam
ACE-R	Addenbrooke's Cognitive Exam – Revised
AD	Axial Diffusivity
ALS	Amyotrophic Lateral Sclerosis
ALSbi	Amyotrophic Lateral Sclerosis with behavioural impairment
ALScbi	Amyotrophic Lateral Sclerosis with cognitive and behavioural impairment
ALS-CBS	ALS Cognitive and Behavioural Screen
ALScc	cognitively competent ALS patients
ALSci	Amyotrophic Lateral Sclerosis with cognitive impairment
ALSFRS-R	Amyotrophic Lateral Sclerosis Functional Rating Scale - Revised
ALS-FTLD	Amyotrophic Lateral Sclerosis - Frontotemporal Lobar Degeneration
ALSi	ALS with (cognitive) impairments
ALS-motor	ALS patients with only motor symptoms
ALSni	ALS with no impairments
ALS-PDC	Amyotrophic Lateral Sclerosis with Parkinsonism-Dementia Complex
ALSu	ALS with unimpaired cognition
aMCI	amnestic Mild Cognitive Impairment
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid

ANART	American version of the National Adult Reading Test
A-ToM	Affective Theory of Mind
ATP	Adenosine triphosphate
aTPJ	anterior Temporopareital Junction
Att-N	Attention Network
AVLT	Auditory Verbal Learning Test
BBI	Beaumont Behavioural Inventory
BCI	Brain Computer Interface
BDI	Beck's Depression Inventory
Bi-PAP	Bi-level Positive Pressure
BNT	Boston Naming Test
BOLD	Blood-oxygen level dependent
bvFTD	behavioural variant Frontotemporal Dementia
C9orf72	Chromosome-9 open reading frame 72
CALSNIC	Canadian ALS Neuroimaging Consortium
CAT-12	Computational Anatomy Toolbox, version12
CATS	Comprehensive Affect Testing System
Cho	Choline
CNS	Central Nervous System
CONN	Connectivity toolbox
CPAP	Continuous Positive Pressure
Cr	Creatinine
CSF	Cerebrospinal Fluid

CST	Corticospinal tract
CT	Computed tomography
C-ToM	Cognitive Theory of Mind
CVLT	California Verbal Learning Test
DAS	Dimensional Apathy Scale
dlPFC	dorsolateral Prefrontal Cortex
DMN	Default Mode Network
DNA	De-oxy Riboncleic Acid
DTI	Diffusion Tensor Imaging
DTI-TK	Diffusion Tensor Imaging – Toolkit
ECAS	Edinburgh Cognitive and Behavioural ALS Screen
EEG	Electroencephalography
EF	Executive function
EIM	Electrical Impedence Myography
EMG	Electromyography
EPI	Echo-Planar Imaging
ERP	Event Related Potential
Exe-N	Executive Network
FA	Fractional Anisotropy
FAB	Frontal Assessment Battery
FDG-PET	Flurodeoxyglucose - Positron Emission Tomography
fMRI	Functional magnetic resonance imaging
FPN	Frontoparietal Network

FrSBe	Frontal Systems Behavioural Screen
FTLD	Frontotemporal Lobar Degeneration
FUS	Fused in Sarcoma
FVC	Forced Vital Capacity
gCC	genu of Corpus Callosum
GDI	Geriatric Depression Inventory
GNT	Graded Naming Test
H&E	Hematoxylin-eosin
HVLT-R	Hopkin's Verbal Learning Test – Revised
IAPS	International Affective Picture System
ICA	Independent Components Analysis
IFOF	Inferior Fronto-occipital Fasciculus
IGT	Iowa Gambling Test
ILF	Inferior Longitudinal Fasciculus
Ino	myo-Inositol
JLO	Judgement of Line Orientation
JP	Judgement of Preference Test
LMN	Lower Motor Neuron
LVR	Lung Volume Recruitment
MCI	Mild Cognitive Impairment
MD	Mean Diffusivity
MiND-B	MND Behavioural Instrument
MMPs	Matrix Metalloproteinases

MMSE	Mini Mental State Exam
MND	Motor Neuron Disease
MND-motor	MND patients with only motor symptoms
MoCA	Montreal Cognitive Assessment
mOFC	medial Orbitofrontal Cortex
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Sprectroscopy
MUNE	Motor Unit Number Estimation
MUNIX	Motor Unit Number Index
NAA	N-acetylaspartate
NIV	Non-invasive Ventillation
NMDA	N-methyl-D-aspartate
PCG	Precentral Gyrus
PEG	Percutaneous Endoscopic Gastrostomy
PET	Positron Emission Tomography
PNFA	Progressive Non-fluent Aphasia
PPA	Primary Progressive Aphasia
pTDP-43	phosphorylated TAR DNA-binding protein of 43 kDa
RAVLT	Rey's Auditory Verbal Learning Test
RD	Radial Diffusivity
RNA	Ribonucleic Acid
rsfMRI	resting state functional Magnetic Resonance Imaging
RSN	Resting State Network

Sal-N	Salience Network
SD	Standard deviation
SemD	Semantic Dementia
SET	Story-based Empathy Task
SLF	Superior Longitudinal Fasciculus
SMN	Sensorimotor Network
SOD1	Superoxide Dismutase-1
SPECT	Single Photon Emission Computed Tomography
SPM-12	Statistical Parametric Mapping, version12
TARDBP	Transactive-region DNA-Binding Protein
TBSS	Tract Based Spatial Statistics
TDP-43	TAR DNA-binding protein of 43 kDa
TIV	Total Intracranial Volume
TMS	Transcranial Magnetic Stimulation
ToM	Theory of Mind
UF	Uncinate Fasciculus
UMN	Upper Motor Neuron
VBM	Voxel Based Morphometry
vmPFC	ventromedial Prefrontal Cortex
VSAT	Visual Series Attention Test
VVT	Visual Verbal Test
WCST	Wisconsin Card Sorting Test

1. Amyotrophic Lateral Sclerosis: Overview

Amyotrophic Lateral Sclerosis (ALS) is a terminal neurodegenerative condition that primarily includes the loss of both upper and lower motor neurons (UMN and LMN respectively). While the clinical features of ALS were recognized as early as 18th century, the disease was named as "*la sclérose amyotrophique*" by French neurologist Jean-Martin Charcot in the 19th century. ALS was historically considered a straight-forward disease with only involvement of the motor nervous system (Turner & Swash, 2015). However van Bogaert (1925) reported presence of 'psychic alterations' in 13 of 31 MND patients. There was emerging evidence of co-morbidity with psychosis-like symptoms that was comparable to another neurological condition involving selective degeneration of the frontotemporal lobes (frontotemporal lobar degeneration, FTLD) leading to speculations about an intersection between the two conditions (Bak, 2010). Indeed, cognitive, behavioural and genetic links establishing a spectrum between ALS and FTLD was confirmed in the 21st century. The following sections discuss details of epidemiology, etiology and risk factors, clinical characteristics, diagnostic criteria, neuropathology, neuroimaging evidence and clinical management of ALS and further elaborate on the ALS-FTLD spectrum.

1.1 Epidemiology of ALS

ALS is a rare disorder with studies indicating variable incidence across provinces in Canada (Wolfson, Kilborn, Oskoui, & Genge, 2009) and the world (Marin et al., 2017). Studies in Canada suggested an incidence ranging from 1.63 per 100,000 in Ontario (year: 1972-1982) (Hudson, Davenport, & Hader, 1986) to 2.4 per 100,000 in Newfounland and Labrador (year: 2000-2004) (Stefanelli et al., 2005). Highest prevalence of motor neuron disease (ALS and

other variants) was noted in Alberta (Worms, 2001), at a rate of 7.38 per 100,000 population (year: 1994-1995) (Svenson, Cwik, & Martin, 1999). It is important to note that these previous studies are not recent and included different methodologies, thus accounting for differing rates of incidence and prevalence in Canada (Wolfson et al., 2009). Further epidemiological studies with superior quality and consistent methodology are required to understand incidence and prevalence rates of ALS in Canada.

A meta-analysis of 44 studies indicated a worldwide crude incidence of sporadic ALS at 1.75 per 100,000 person-years at follow-up (Marin et al., 2017). Gender discrepancy was also noted with higher incidence in men (2.03 per 100,000) than women (1.45 per 100,000) indicating a male to female ratio of 1.4 (Marin et al., 2017). However, another study noted a decreasing male to female ratio from 1.2 in 1925-1984 to 1.0 in 1998 (Sorenson, Stalker, Kurland, & Windebank, 2002). Researchers have argued that improved access to health care and changing lifestyle factors may contribute to higher incidences of ALS in females, however this hypothesis is yet to be confirmed (Logroscino et al., 2008). A homogeneity in ALS incidence was noted for Europe, North America and New Zealand, while Asian populations (China, Japan, Iran, Israel) had heterogenous incidence rates as compared to Europe (Marin et al., 2017). Studies also report differential ALS incidence for ethnic groups; higher incidence was noted among Caucasians (1.48 per 100,000) as compared to African-Americans (0.89) or Asians (0.78) in the United States (Rechtman, Jordan, Wagner, Horton, & Kaye, 2015; Wagner et al., 2015).

Furthermore, studies indicated that age and familial history also influence incidence of ALS. It was reported as highest between the ages of 65-74 years irrespective of gender, with an increasing trend between the ages 25-64 years and a reduced incidence after 80 years of age

(Logroscino et al., 2008). A small proportion of ALS patients, approximately 1.6% (9 of 554 ALS cases), were aged below 30 years in a European study (Logroscino et al., 2008). Also, there is a higher frequency of sporadic ALS as compared to those with a family history. Previous studies had reported the presence of familial ALS in about 10% patients (Kurland & Mulder, 1955). However, more recent studies report smaller proportion of about 5.1% familial ALS patients (Byrne et al., 2011). In Alberta, Canada, one study reported familial ALS in 5.2% of patients (Pfister et al., 2013).

Some high-risk geographical loci for ALS were detected in the Western Pacific countries, with prevalence higher than 50-100 times as compared to other countries (Kurland & Mulder, 1954). These pockets include islands in Guam (one of the Mariana islands), the Kii peninsula and southwest New Guinea, where ALS populations were found to share signs of Parkinsonism and dementia. This form of ALS with Parkinsonism-Dementia complex (ALS-PDC) was speculated to be triggered by environmental factors, such as the accumulation of a neurotoxin (β-methyl-amino-L-alanine) found in cycad flour consumed as a part of the Guam diet (Borenstein et al., 2007; Whiting, 1964). One recent study suggested a change in water supply as a potential contributor to higher ALS-PDC incidence in the Kii peninsula (Kihira et al., 2012). Over the years, a consistent decline in the incidence rate of ALS-PDC has been reported in these Western Pacific countries, possibly due to modernization and changes in diet thereby providing considerable evidence for the influence of environment on this form of ALS (Plato et al., 2003). Despite decreasing incidence, an increased risk among off-spring of ALS-PDC patients was reported, suggesting a possible genetic link to ALS-PDC (Garruto & Yanagihara, 2009). Some studies have suggested that polymorphisms in Microtubule-associated protein tau (MAPT) may increase genetic risk for ALS-PDC

(Sundar et al., 2007), while others have suggested links with Chromosome 9 open reading frame 72 (C9orf72) mutations in the Kii peninsula (O'Dowd et al., 2012) but were not replicated in the Guam islands (Dombroski et al., 2013). Together these findings indicate that in Western Pacific regions, there was a greater influence of environmental factors and a smaller association of genetic factors in the epidemiology of ALS-PDC that require further exploration.

Studies report a mean delay of 12.2 to 12.6 months between symptom onset and diagnosis in ALS (Chio et al., 2013; Logroscino et al., 2008). The median survival was indicated as 2-4 years from onset (Chio et al., 2009; del Aguila, Longstreth, McGuire, Koepsell, & van Belle, 2003). The cumulative probability of survival after diagnosis showed a decreasing trend, ranging from 78% survival at 12 months to 32% at 48 months (Logroscino et al., 2008). Survival beyond 10 years was noted only in 10-20% of ALS patients (Chio et al., 2013).

1.2 Etiology

Genetic mutations are implied as the cause of familial ALS. On the other hand, while genetic mutations are found in a small proportion, the cause of sporadic ALS in a majority of the population remains unknown (Verde, Del Tredici, Braak, & Ludolph, 2017).

1.2.1 Genetic Risk Factors

So far, more than 17 gene variants with Mendelian pattern of inheritance have been identified to be associated with ALS (Strong et al., 2017). The first genetic link to ALS was noted in superoxide dismutase-1 (SOD1) (Rosen et al., 1993). Other important mutations that warrant

mention are in the transactive-region DNA-binding protein gene (TARDBP) encoding for TAR DNA-binding protein of 43 kDa (TDP-43), the fused in sarcoma gene (FUS) and hexarepeat expansions in chromosome-9 open reading frame 72 (C9orf72) have been implied in both ALS and frontotemporal dementia (FTD) (Hosler et al., 2000; Kabashi et al., 2008; Mackenzie, Rademakers, & Neumann, 2010).

Distinct phenotypic differences have been associated with each of the above mutations. The presence of C9orf72 mutations were found to be one of the strongest predictors of cognitive or behavioural impairment in ALS (Turner et al., 2013) and accounted for 41% familial ALS and 5% sporadic cases in a population-based study (Byrne et al., 2012). Another study noted that the presence of C9orf72 mutations was associated with bulbar onset in both familial and sporadic ALS, while TARDBP and SOD1 were associated with upper limb and lower limb onset respectively (Millecamps et al., 2012). Disease duration and survival was shorter in patients with C9orf72 mutations. Considering the implications of these phenotypic differences, identification of genetic variants remains crucial for clinical and research purposes.

1.2.2 Environmental Risk Factors

No definitive environmental factor has been identified to cause ALS, however, several potential risk factors were reported (Verde et al., 2017). These include smoking (Gallo et al., 2009), military service (Horner et al., 2003; Weisskopf et al., 2005), higher physical activity (Scarmeas, Shih, Stern, Ottman, & Rowland, 2002; Turner, 2013), metals and chemicals (Kamel et al., 2012; Sutedja et al., 2009), and cyanotoxins (Bradley et al., 2013; Caller et al., 2009). Higher risk of ALS in soccer players was previously reported in an Italian study

(Chio, Benzi, Dossena, Mutani, & Mora, 2005), but there is a dearth of studies replicating this result in other countries (Verde et al., 2017).

1.3 Clinical Characteristics

As mentioned previously, ALS is a motor neuron disease that involves progressive loss of both upper and lower motor neurons in the central nervous system (CNS). The upper motor neurons (UMN) project axons from the primary motor cortex (precentral gyrus, PCG) to the spinal cord via the corticospinal tract. This pathway relays signals from the UMN to the lower motor neurons (LMN) in the spinal cord, which further sends impulses to the respective muscle groups in either bulbar, respiratory or limb regions to initiate movement. Figure 1.1 displays a schematic diagram of the motor system. Degeneration of the UMN manifests as hyperreflexia and hypertonia, while LMN loss includes hyporeflexia, hypotonia, fasciculations and muscle atrophy. UMN signs in the bulbar region may also result in pseudobulbar affect characterised by uncontrollable episodes of laughter or crying that are incongruent with underlying emotion and situation (Thakore & Pioro, 2017). Brooks, Miller, Swash, and Munsat (2000) outlined a summary of UMN and LMN signs in ALS that may occur at the level of the brainstem, cervical, thoracic or lumbosacral regions of the spinal cord (Table 1.1).

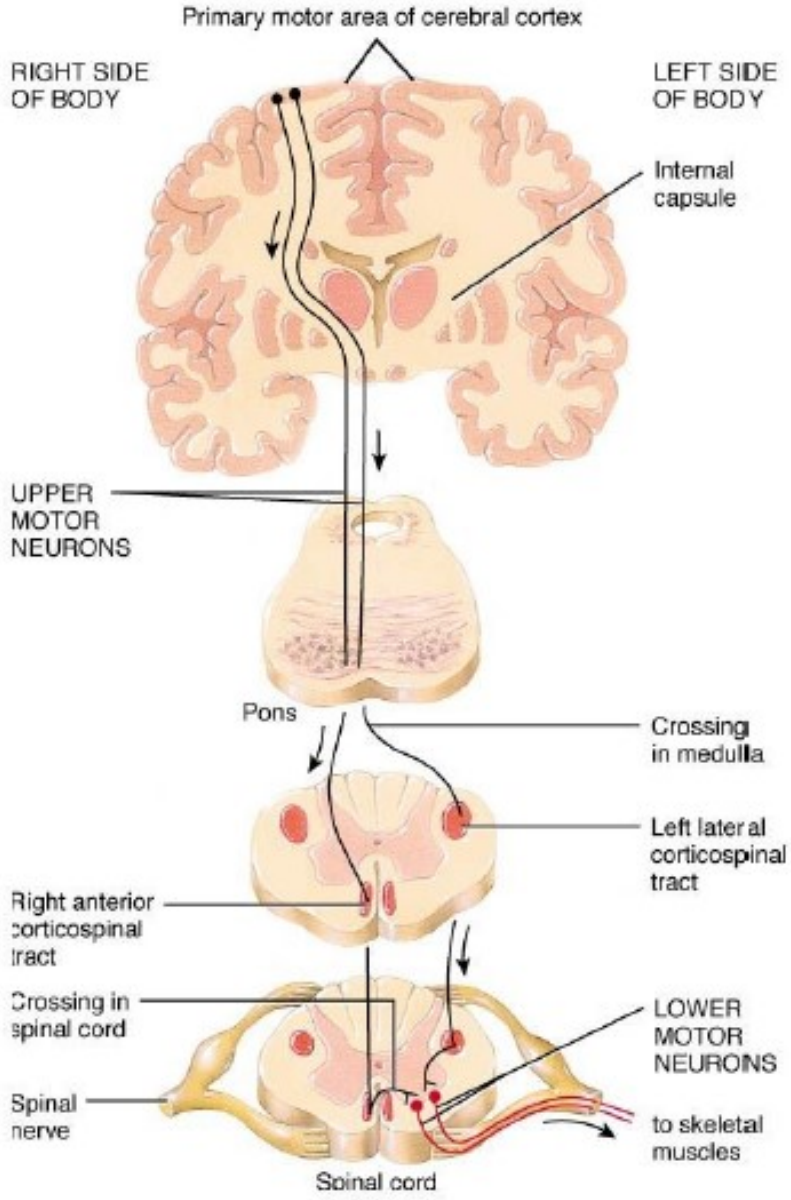


Figure 1.1. Upper and lower motor neuron pathways in the central nervous system.

Table 1.1. Description of LMN and UMN signs at four levels of the CNS (Brooks et al., 2000).

General features	CNS Regions			
	Brainstem	**Cervical**	**Thoracic**	**Lumbosacral**
LMN signs - weakness - atrophy - fasciculations	- jaw, face - palate - tongue - larynx	- neck, arm, hand - diaphragm	- back - abdomen	- back, abdomen - leg, foot
UMN signs - pathologic spread of reflexes - clonus - spasticity	- clonic jaw jerk - gag reflex - exaggerated snout reflex - pseudobulbar features - forced yawning - pathologic DTR - spastic tone	- clonic DTRs - Hoffman reflex - pathologic DTRs - spastic tone - preserved reflex in weak limb	- loss of superficial abdominal reflexes - pathologic DTRs - spastic tone	- clonic DTRs - extensor plantar response - pathologic DTRs - spastic tone - preserved reflex in wasted limb

Note: UMN = Upper motor neuron, LMN = Lower motor neuron, DTR = Deep tendon reflex,

Symptom onset and degeneration in the motor pathways is variable in ALS patients, subsequently resulting in a spectrum of clinical features (Swinnen & Robberecht, 2014). Symptom presentation can occur either in the limbs, bulbar and respiratory regions exclusively or in a combination of two or more regions. Limb onset (also referred to as spinal onset) is the most frequent form of ALS, with two-thirds of patients presenting with initial complaints of muscle weakness and wasting in either distal or proximal parts of the limbs (Wijesekera & Leigh, 2009). Additional symptoms may include cramps and fasciculation, with a possibility of gradual spasticity in the weak limbs affecting dexterity and gait. Bulbar-onset is reported in one-third of patients with dysarthria and dysphagia as presenting symptoms. Most bulbar-onset patients also develop simultaneous limb symptoms within two years of onset and display relatively poor prognosis (~2 years mean survival) as compared to limb-onset patients (Swinnen & Robberecht, 2014). Respiratory-onset is extremely rare and noted in only 3-5% of patients (Chio et al., 2011). Presenting symptoms include orthopnea or dyspnoea with mild or possibly absent limb and bulbar signs. The prognosis for respiratory onset is extremely poor with mean survival of only 1.4 years (Shoesmith, Findlater, Rowe, & Strong, 2007). Considering the recent developments in the ALS-FTLD spectrum, cognitive onset is another contemplation in the field, with cognitive and behavioural changes preceding motor symptoms. The ALS-FTLD spectrum is increasingly recognised with about 13% of cases displaying simultaneous symptoms for both ALS and FTLD (Saxon et al., 2017).

In addition to variability in onset, patients may show other symptoms like extrapyramidal signs (bradykinesia, tremors, etc.), and sensory dysfunction (decreased vibration, discrimination and blunting of temperature variation) (Brooks et al., 2000; Desai & Swash,

1999; Wijesekera & Leigh, 2009). Identifying these features helps understand disease patterns and explore possibility of ALS mimics through differential diagnosis. This enables delineating conditions that may be treatable, as opposed to ALS which is terminal. For instance, multifocal motor neuropathy is an autoimmune condition presenting with progressive and asymmetric limb weakness that can be reversed with immunoglobulin treatment (Zarei et al., 2015). Other ALS mimics include cervical spondylotic myelopathy, Kennedy disease and post-polio syndrome. In ALS patients with no overt dementia, cognitive and behavioural changes are being increasingly recognised in about 25-50% of cases (Goldstein & Abrahams, 2013; Phukan et al., 2012). Deficits are predominantly in executive functions that includes cognitive processes such as planning, set-shifting and decision making (Goldstein & Abrahams, 2013), while apathy is reported as the most frequent behavioural change (Lillo, Mioshi, Zoing, Kiernan, & Hodges, 2011). Shorter survival times have been indicated in patients with cognitive and behavioural impairments (Elamin et al., 2011; Hu et al., 2013).

The presentation and spread of symptoms can be mixed in ALS patients, with some patients displaying greater LMN signs, while others may exhibit more UMN signs. Pure LMN signs represent Progressive Muscular Dystrophy (PMA), while pure UMN signs are indicative of Primary Lateral Sclerosis (PLS), both of which are classified as motor neuron diseases. Studies have indicated that 30% PMA patients tend to develop UMN signs within 18 months of symptom onset, thereby transferring their diagnosis to ALS (Hardiman, van den Berg, & Kiernan, 2011; Visser et al., 2007; Zarei et al., 2015). In PLS patients, 77% develop LMN signs within 3-4 years since symptom onset and are re-diagnosed as ALS (Hardiman et al., 2011). Identifying these transitions in patients is crucial as they influence prognosis in

patients (Swinnen & Robberecht, 2014). The median survival for PMA patients was found to be 56 months (~ 4.6 years), while survival for PLS patients is around 20 years since onset (Zarei et al., 2015).

As the disease progresses, fatigue and loss of ability to perform daily activities is common in ALS patients (Kiernan et al., 2011). Most patients develop dysphagia that leads to dietary changes, subsequently resulting in weight loss and possible malnutrition, factors which are associated with poor prognosis. End-stage ALS is characterised by respiratory compromise, especially dyspnoea at rest is indicative of imminent death.

1.3.1 Prognostic Indicators in ALS

Several factors such as biology, clinical features and environmental aspects appear to moderate survival and disease prognosis in ALS. Shorter survival was associated with female gender (del Aguila et al., 2003), older age (del Aguila et al., 2003; Eisen, Schulzer, MacNeil, Pant, & Mak, 1993; Haverkamp, Appel, & Appel, 1995), poor nutritional status (Desport et al., 1999), bulbar and upper limb onset (Mandrioli, Faglioni, Nichelli, & Sola, 2006), shorter time between symptom onset to diagnosis (del Aguila et al., 2003), loss of functional abilities (Appel, Stewart, Smith, & Appel, 1987), and decline in pulmonary function (Armon & Moses, 1998). Environmental aspects such as agricultural occupation and residence in mountain regions have also been implicated with poor prognosis and shorter survival (Mandrioli et al., 2006). However, access to health care and socioeconomic status could play a critical role in influencing survival in these environmental circumstances.

1.4 Diagnostic Criteria

1.4.1 ALS

The revised El Escorial criteria were collectively approved by the World Federation of Neurology Research Committee on Motor Neuron Diseases in 1998 (Brooks et al., 2000). It is widely applied for the diagnosis of ALS requiring the following:

"(A) the presence of:

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

together with:

(B) the absence of

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

Any one of the following categories of diagnosis is assigned to patients based on clinical and laboratory investigations:

1. *Clinically Definite ALS*. In this category, patients display UMN and LMN signs in at least three regions, of which one set of signs could be in the bulbar region.
2. *Clinically Probable ALS*. For this category, patients show both UMN and LMN signs in at least two regions, typically with UMN signs rostral to LMN signs.
3. *Clinically Probable ALS - Laboratory-supported*. Patients are classified in this category if they present with clinical UMN signs in only one region, while LMN signs are supported by electrophysiological findings in at least two regions.
4. *Clinically Possible ALS.* This category is assigned to patients who show UMN and LMN signs in only one region or show LMN signs without evidence of laboratory supported results in two regions.

The electrophysiological findings provide confirmation for LMN loss in regions with and / or without clinical evidence, while also excluding possibility of other diseases. Typically, they include techniques such as electromyographic (EMG) and nerve conduction examinations. Evidence for active denervation is indicated by fibrillation potentials and positive sharp waves on the EMG examination, while chronic denervation is indicated by large motor unit potentials, reduced interference pattern with high firing rates and unstable motor unit potentials (Brooks et al., 2000). Another characteristic EMG feature for ALS are fasciculation potentials. However, distinction between benign fasciculations and abnormal potentials in other disorders such as motor neuropathies are essential. For nerve conduction examination in ALS, normal or near normal motor and sensory nerve conduction times are required. If nerve conduction studies are abnormal, they may indicate the presence of other

disorders of the peripheral nerve, neuromuscular junction or ALS mimics. More recent studies outline specific features to distinguish ALS-related fasciculations from benign fasciculations (de Carvalho et al., 2008). While the authors acknowledge that fasciculations alone reduce diagnostic specificity of the muscle tested, they underscore the importance of providing early diagnosis in patients.

Some studies recognise that the major caveat to the revised El Escorial criteria is the emphasis on clinical findings, while electrophysiological evidence is provided in the absence of clinical evidence in a limb. A recent study highlighted the importance of using both clinical and electrophysiological evidence to provide accurate diagnosis (Awaji criteria) (de Carvalho et al., 2008). This suggestion eliminates the category "*Clinically Probable ALS Laboratory-supported*" in the assignment of diagnosis. Despite the higher sensitivity of diagnosis using the Awaji criteria especially for bulbar patients (Costa, Swash, & de Carvalho, 2012; Geevasinga et al., 2016), the revised El Escorial criteria remains the gold standard for diagnosing ALS so far.

1.4.2 ALS-FTLD Spectrum

Motor symptoms often take precedence in ALS clinics. However, with increasing evidence for cognitive and behavioural symptoms shared with FTLD, a clinical and genetic spectrum between ALS and FTLD has been proposed (Strong et al., 2017). FTLD collectively refers to heterogenous neurological disorders characterised by selective involvement of the frontal and temporal lobes. The International Consensus Criteria for FTLD were proposed by Neary et al., (1998) and outlined three sub-types: behavioural variant frontotemporal dementia (bvFTD), progressive non-fluent aphasia (PNFA) and semantic dementia (SemD). All three FTLD sub-types are marked by subtle onset and gradual progression of symptoms, however, each sub-type involves distinct core features. BvFTD is marked by profound changes in personality and social conduct, including apathy, disinhibition, stereotypical and perseverative behaviours. Both PNFA and SemD are disorders of language, in which the former is characterised by dysfunction in expressive language while the latter is a disorder of naming and comprehension. Recently the classification for FTLD language variants were improved (Gorno-Tempini et al., 2011). Patients are now initially diagnosed with Primary Progressive Aphasia (PPA) and then classified into three variants (nonfluent or agrammatic, semantic and logopenic) based on specific speech and language features. The updated criteria for clinical diagnosis of FTLD language variants are summarised in Table 1.2. An updated and more detailed criteria for bvFTD diagnosis were suggested by Raskovsky et al., (2011) summarised in Table 1.3.

FTLD is reported to be the leading cause of dementia in patients below 65 years of age (Rabinovici & Miller, 2010). In ALS, bvFTD is the most prevalent form of dementia with 10-15% meeting criteria for this diagnosis, while PNFA and SemD is relatively rare (Lomen-

Hoerth et al., 2003). Around 78% of ALS-FTLD cases present with ALS-bvFTD compared to 1% of patients with ALS-PNFA, 3% with ALS-SD or a combination of ALS-bvFTD-SemD and ALS-bvFTD-PNFA (Saxon et al., 2017).

Table 1.2. Criteria for clinical diagnosis of FTLD language variants (Gorno-Tempini et al., 2011).

Primary Progressive Aphasia (PPA)

Inclusion: criteria 1–3 must be answered positively

1. Most prominent clinical feature is difficulty with language
2. These deficits are the principal cause of impaired daily living activities
3. Aphasia should be the most prominent deficit at symptom onset and for the initial phases of the disease

Exclusion: criteria 1–4 must be answered negatively for a PPA diagnosis

1. Pattern of deficits is better accounted for by other nondegenerative nervous system or medical disorders
2. Cognitive disturbance is better accounted for by a psychiatric diagnosis
3. Prominent initial episodic memory, visual memory, and visuoperceptual impairments
4. Prominent, initial behavioral disturbance

Nonfluent / Agrammatic variant PPA

At least one of the following core features must be present:

1. Agrammatism in language production
2. Effortful, halting speech with inconsistent speech sound errors and distortions (apraxia of speech)

At least 2 of 3 of the following other features must be present:

1. Impaired comprehension of syntactically complex sentences
2. Spared single-word comprehension
3. Spared object knowledge

Semantic variant PPA

Both of the following core features must be present:

1. Impaired confrontation naming
2. Impaired single-word comprehension

At least 3 of the following other diagnostic features must be present:

1. Impaired object knowledge, particularly for low frequency or low-familiarity items
2. Surface dyslexia or dysgraphia
3. Spared repetition
4. Spared speech production (grammar and motor speech)

Logopenic variant PPA

Both of the following core features must be present:

1. Impaired single-word retrieval in spontaneous speech and naming
2. Impaired repetition of sentences and phrases

At least 3 of the following other features must be present:

1. Speech (phonologic) errors in spontaneous speech and naming
2. Spared single-word comprehension and object knowledge
3. Spared motor speech
4. Absence of frank agrammatism

Table 1.3. Revised International Criteria for bvFTD (Rascovsky et al., 2011).

I. Neurodegenerative disease:

The following symptom must be present to meet criteria for bvFTD

A. Shows progressive deterioration of behaviour and/or cognition by observation or history (as provided by a knowledgeable informant).

II. Possible bvFTD

Three of the following behavioural/cognitive symptoms (A-F) must be present to meet criteria.

A. Early behavioural disinhibition (one of the following symptoms must be present):
   A.1. Socially inappropriate behaviour
   A.2. Loss of manners or decorum
   A.3. Impulsive, rash or careless actions

B. Early apathy or inertia (one of the following symptoms must be present):
   B.1. Apathy
   B.2. Inertia

C. Early loss of sympathy or empathy (one of the following symptoms must be present):
   C.1. Diminished response to other people's needs and feelings
   C.2. Diminished social interest, interrelatedness or personal warmth

D. Early perseverative, stereotyped or compulsive / ritualistic behaviour (one of the following symptoms must be present):
   D.1. Simple repetitive movements
   D.2. Complex, compulsive or ritualistic behaviours
   D.3. Stereotypy of speech

E. Hyperorality and dietary changes (one of the following symptoms must be present):
   E.1. Altered food preferences
   E.2. Binge eating, increased consumption of alcohol or cigarettes
   E.3. Oral exploration or consumption of inedible objects

F. Neuropsychological profile (all the following symptoms must be present):
   F.1. Deficits in executive tasks
   F.2. Relative sparing of episodic memory
   F.3. Relative sparing of visuospatial skills

III. Probable bvFTD

All the following symptoms must be present to meet criteria.

A. Meets criteria for possible bvFTD
B. Exhibits significant functional decline
C. Imaging results consistent with FTD (one of the following must be present):
   C.1. Frontal and/or anterior temporal atrophy on MRI or CT
   C.2. Frontal and/or anterior temporal hypoperfusion or hypometabolism on PET or SPECT

IV. bvFTD with definite FTLD pathology

Criterion A and either criterion B or C must be present to meet criteria.

A. Meets criteria for probable bvFTD
B. Histopathological evidence of FTLD on biopsy or at post-mortem
C. Presence of a known pathogenic mutation

In ALS, about 25-50% ALS patients show cognitive and behavioural impairments that do not meet criteria for FTLD, however, they do raise concerns for cognitive and / or behavioural deficit. The recognition of this overlap between ALS and FTLD is relatively recent and the hypothesis of ALS-FTLD continuum has been revisited and established over the years (Bak, 2010). Initial consensus criteria were proposed by Strong et al., (2009) and recently revised (Strong et al., 2017) to aid identification of cognitive and behavioural sub-types in ALS. Impairments are typically defined at 2 standard deviations (SD) below healthy controls' average performance on cognitive and behavioural tests (Abrahams et al., 2000; Strong et al., 2017), though studies have employed lenient criteria at 1.5SD below control means. The following sub-sections elaborate on criteria for cognitive (ALSci) and behavioural impairment (ALSbi). The revised Strong criteria for neuropsychological categorization are illustrated in Figure 1.2.

1.4.2.1 ALS with cognitive impairment (ALSci)

This category is assigned to patients who show impairments in executive functions (EF) (including social cognition) and / or language.

Executive impairment in ALS (ALS-exi) is defined as:

(1) Impaired verbal fluency (letter) which is controlled for motor and / or speech impairments, OR

(2) Impairment on at least two non-overlapping measures of Executive functions, which may include social cognition.

Language impairment in ALS is defined as impairment on two non-overlapping tests, which could include pragmatic function.

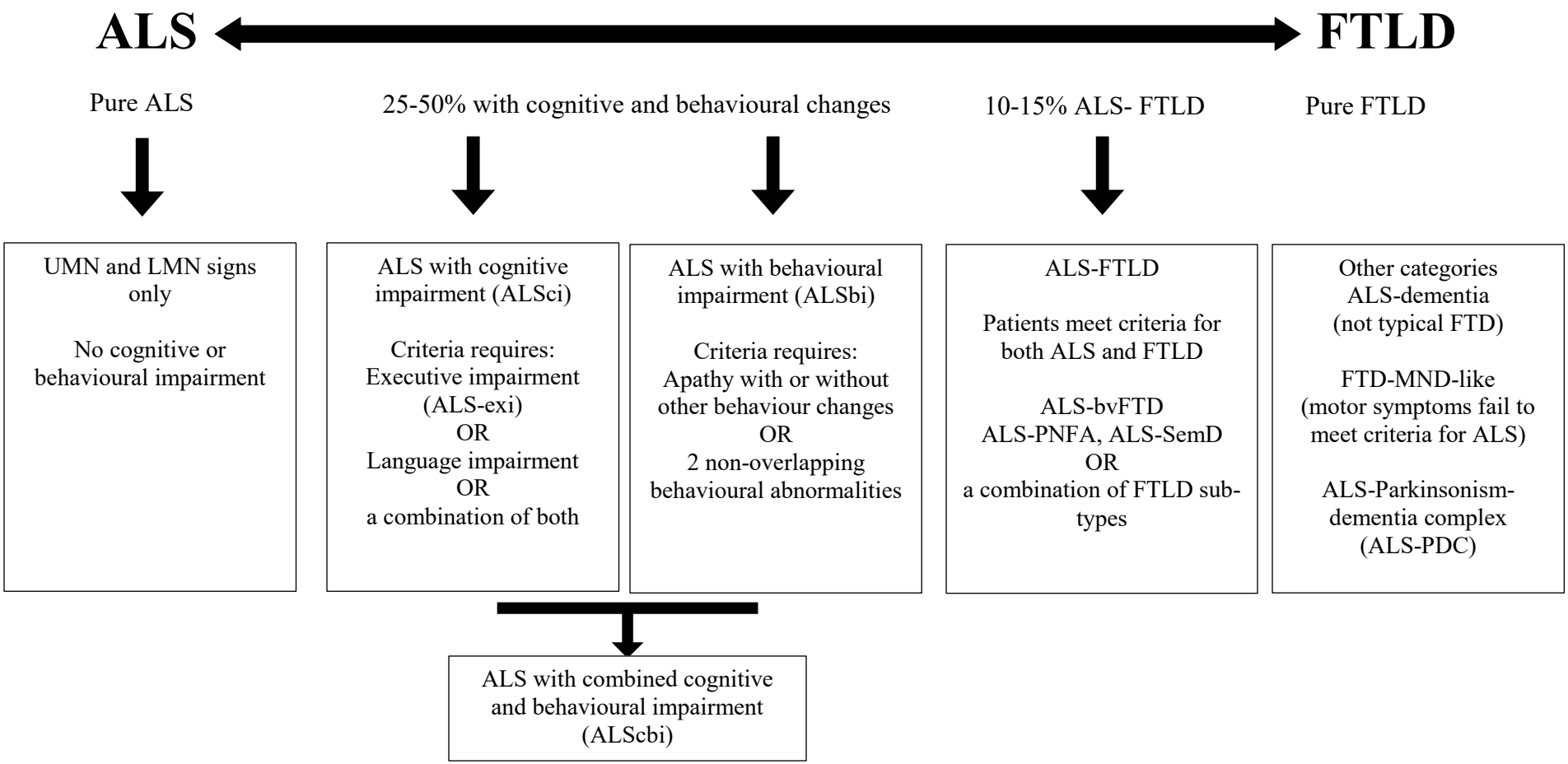


Figure 1.2. ALS-FTLD spectrum as described by Strong et al. (2017).

It is important to note that in the revised Strong criteria, social cognition is included alongside criteria for EF impairments in their recommendations (see section 1.4.2.1). Social cognition deficits have been identified in FTLD and have become a recent focus in ALS. In FTLD, impaired social cognition may be present even when EF is normal. Strong et al. (2017) recognise that while some studies show associations between EF and social cognition in ALS, others do not. A detailed literature review regarding the possible link between social cognition and EF in ALS, along with the rationale for the current thesis is provided in Chapter 1.

1.4.2.2 ALS with behavioural impairment (ALSbi)

This category is assigned to patients who do not meet criteria for bvFTD and yet show significant behavioural impairments. ALSbi is defined by:

(1) the identification of apathy with or without other behaviour change, OR

(2) the presence of two or more of the following behavioural symptoms:

(a) disinhibition, (b) loss of sympathy and empathy, (c) perseverative, stereotyped or compulsive behaviour, (d) hyperorality or dietary change, (e) loss of insight, (f) psychotic symptoms (somatic delusions, hallucinations, irrational beliefs).

These behavioural characteristics, specifically 2a-d were derived from the FTLD criteria (Table 1.3). The revised Strong criteria recommends the use of patient and informant (caregiver) responses to determine loss of insight, previously reported in ALS (Woolley, Moore, & Katz, 2010).

For patients who meet criteria for both ALSci and ALSbi, but not FTLD, assigning a category of ALS with combined cognitive and behavioural impairments (ALScbi) has been

suggested. Further details on the recommended tests and literature in the ALS-FTLD spectrum are discussed in Chapter 2.

1.5 Pathophysiology

The exact pathogenic basis for ALS remains elusive. However, multifactorial disruptions in inter-related pathways have been underscored to lead to the development of ALS-related symptoms (Kiernan et al., 2011). The current perspective suggests an initial involvement of genes, environment and developmental factors which subsequently lead to molecular changes which in turn express as clinical symptoms after a certain pathophysiological threshold has been exceeded (Figure 1.3). As indicted in section 1.2, genetic and environmental factors have been noted as early substrates of ALS. Among the suggested pathophysiological mechanisms, glutamate excitotoxicity, generation of free radicals, mutant SOD1 enzymes, mitochondrial dysfunction and impaired axonal transport through accumulation of intracellular aggregates have gained considerable attention (Turner et al., 2013). More recent studies have provided evidence for prion-like spread of intracellular protein aggregates in ALS which are considered the leading cause for spread of the disease.(Braak et al., 2013; Brettschneider et al., 2012; Fatima, Tan, Halliday, & Kril, 2015).

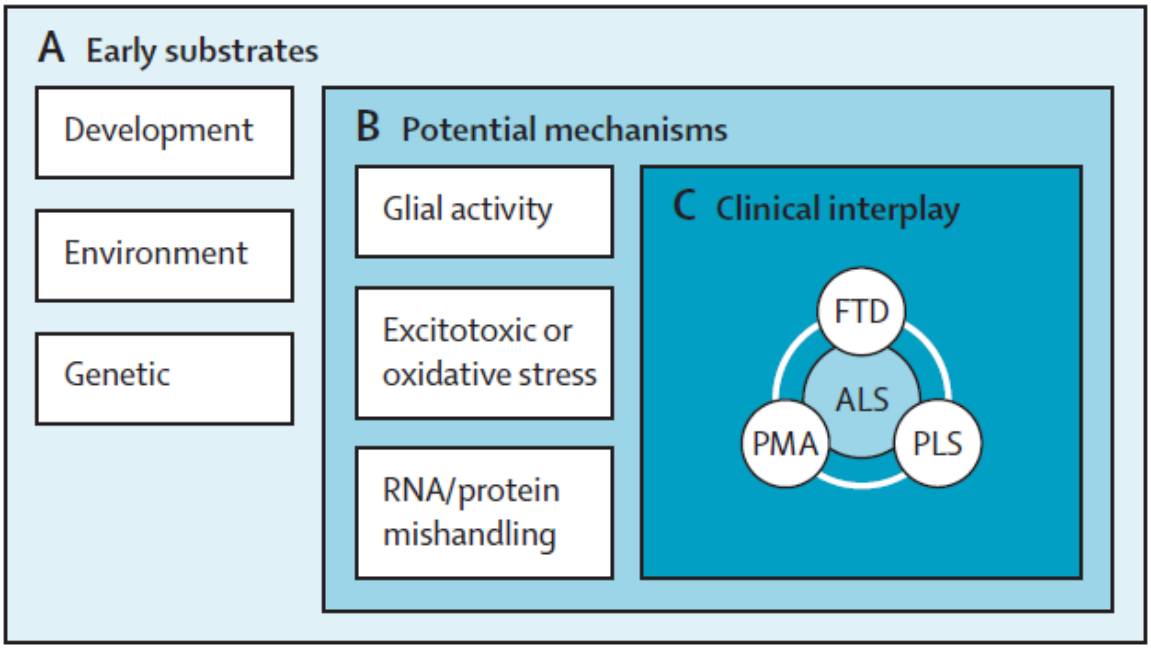


Figure 1.3. Pathogenesis of ALS.
Reprinted from "Controversies and priorities in amyotrophic lateral sclerosis" by Turner et al, 2013, *Lancet Neurol, 12,* pg. 319. Copyright (2013) by Elsevier. Reprinted with permission.

1.5.1 Glutamate Excitotoxicity

Glutamate is the major excitatory neurotransmitter in the CNS which is synthesized and packaged in the presynaptic neuron, ready to be released during synaptic transmission. It binds with both ionotropic N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid (AMPA) receptors located on the post-synaptic neurons in the brain. In ALS patients and transgenic SOD1 mouse models (with induced SOD1 mutations), evidence of reduced astroglial glutamate transporter has been indicated to cause an increase in extracellular glutamate, subsequent over stimulation and excitotoxic neurodegeneration (Lin et al., 1998; Trotti, Rolfs, Danbolt, Brown, & Hediger, 1999). Excess glutamate leads to excitotoxic degeneration in two distinct ways: (1) through activation of calcium-dependent enzymatic pathways that increase sodium and calcium ion influx, or (2) by generation of free radicals subsequently leading to oxidative stress (Ferraiuolo, Kirby, Grierson, Sendtner, & Shaw, 2011; Forsberg, Andersen, Marklund, & Brannstrom, 2011).

1.5.2 Mitochondrial Dysfunction

Mitochondria are membrane-bound organelles of the cell that play a significant role in energy production for the cell, cellular respiration, calcium homeostasis and control of apoptosis. Abnormalities in ALS mitochondria have been noted at both morphological and functional levels, which may lead to either abnormal ATP production for energy, poor calcium homeostasis, axonal transport and apoptosis (Boillee & Cleveland, 2008; Chung & Suh, 2002).

The above mitochondrial contributions re-affirm the multifactorial nature of pathogenesis in ALS. While the exact point of origin remains unknown, the mechanism of disease spread is another widely debated topic in ALS. Studies on glutamate excitotoxicity suggests a "dying-forward" mechanism of anterograde degeneration, whereby UMN neurons drive LMN loss via excitotoxic processes (Hensley et al., 2006; Maher & Davis, 1996). On the other hand, cellular processes in the LMN and at level of neuromuscular junctions suggest a "dying-back" or retrograde mechanism supported by observations of synaptic denervation prior to the onset of motor degeneration. An independent process involving both mechanisms has also been proposed (Kiernan et al., 2011). More recent findings suggest prion-like propagation of pTDP-43 deposits along axons via anterograde transport (Braak et al., 2013).

1.5.3 Pathophysiology in ALS-relevant Genetic Mutations

Section 1.2 briefly discussed various genes implied in ALS, specifically related to ALS and FTLD. The current section elaborates on the mechanism of dysfunction of genetic mutations thought to be involved in ALS, including SOD1, TARDBP, FUS, and C9orf72. SOD1 mutations have been associated with an increase in free radicals, a consequence of toxic gain of function of the mutated SOD1 enzyme, leading to cell death (Bruijn et al., 1997; Bruijn et al., 1998; Bruijn, Miller, & Cleveland, 2004; Liu, Althaus, Ellerbrock, Becker, & Gurney, 1998). They may also lead to instability of molecular structure and misfolding of the SOD1 peptide, leading to formation of intracellular aggregates that disrupt axonal transport and cell functions (Bruijn et al., 1998; Zetterstrom, Andersen, Brannstrom, & Marklund, 2011). However, more recent research has revealed that SOD1 disruptions of axonal transport may emerge independently as the axons survive despite long-term transport deficits (Marinkovic et al., 2012).

TARDBP mutations have been noted to trigger the formation of TDP-43 protein aggregates in the cytoplasm of patients with ALS, while for healthy neurons, TDP-43 protein aggregates in the nucleus (Sreedharan et al., 2008; Van Deerlin et al., 2008). TDP-43 plays a key role in binding of deoxy-ribonucleic acid (DNA) and ribonucleic acid (RNA) strands and may subsequently impair RNA processing. FUS mutations have been noted independent of SOD1 and TDP-43 mutations and have been reported to be involved with processing of small regulatory RNAs, RNA maturation and splicing (Vance et al., 2009). C9orf72 has been noted to encode for a potential guanine exchange factor for an un-known G-protein (Taylor, 2017). C9orf72 mutations have been associated with abnormal microglia and neuroinflammation, thereby providing evidence for involvement of non-neuronal processes in ALS (O'Rourke et al., 2016).

1.5.4 RNA Metabolism

The above genetic mutations further influence RNA regulation and metabolism, thereby leading to more recent hypotheses that ALS could be a disorder of RNA metabolism with hallmark features indicating the presence of intracellular inclusions in both neuronal and glial cells (Droppelmann, Keller, Campos-Melo, Volkening, & Strong, 2013; Strong, 2010). As mentioned in the previous sub-section, TDP-43 and FUS have been associated with RNA regulation, with TDP-43 affecting multiple steps in the RNA metabolism, and FUS altering RNA-binding pattern (Verma & Tandan, 2013). Another line of work suggests that these alterations influence RNA metabolism via stress granules (Droppelmann et al., 2013). Stress granules are aggregations of proteins and RNAs in the cytoplasm that are generated in response to cellular stress and are noted to arrest translational processes. In ALS, it is speculated that interactions between environmental stress and genetic mutations modify

RNA-binding proteins post-translationally, thereby further altering the formation of stress granules that result in the formation of protein aggregates (Droppelmann et al., 2013). These then travel via endo- and exocytosis to adjacent motor neurons, leading to spread of the disease.

1.6 Neuropathology

The classic neuropathological features of ALS include motor neuron loss, astrogliosis and the presence of intraneuronal inclusions in the degenerating neurons and glia (Wijesekera & Leigh, 2009). These are typically microscopic changes with no significant gross abnormalities. Atrophy of the precentral gyrus may be noted in some cases, while significant atrophy of the frontal and temporal cortices is indicative of an intersection with FTLD. At the neuromuscular level, biopsies have revealed muscle atrophy of both Type I and Type II fibres in ALS (Baloh, Rakowicz, Gardner, & Pestronk, 2007).

Neuron loss is observed at both the level of the brain and spinal cord (Figure 1.4A-D). Degeneration and a reduction in motor neurons have been noted using hematoxylin-eosin (H&E) stains in the primary motor cortex (Betz cells), cranial motor nuclei of the brainstem and anterior horn of the spinal cord (Ellison et al., 2012; Hammer, Tomiyasu, & Scheibel, 1979; Nihei, McKee, & Kowall, 1993; Saberi, Stauffer, Schulte, & Ravits, 2015). Axonal loss is observed in the lateral and anterior columns of the spinal cord (Ellison et al., 2012). Other microscopic changes include presence of Bunina bodies which are oval eosinophilic intracellular inclusions with a size of 3-6 μm (Piao et al., 2003; Tomonaga, Saito, Yoshimura, Shimada, & Tohgi, 1978). The biological significance of Bunina bodies is yet to be established. However, studies have reported selective presence of Bunina bodies in ALS,

observed in motor neurons of the brainstem and spinal cord (Figure 1.4E-F), but not in Betz cells of the motor cortex, oculomotor neurons or the Onuf nuclei (Okamoto, Hirai, Amari, Iizuka, et al., 1993; Okamoto, Mizuno, & Fujita, 2008; Sasaki & Maruyama, 1993).

Non-neuronal cell involvement in ALS has also been demonstrated. Reactive astrogliosis is a neurological hallmark alongside degenerating motor neurons (Boillee, Vande Velde, & Cleveland, 2006; McGeer & McGeer, 2002; Yamanaka et al., 2008). Activated microglia is another feature of gliosis and has been found to be associated with UMN degeneration (Lasiene & Yamanaka, 2011). Microglial activation is associated with the release of proinflammatory cytokines which can accelerate neurodegenerative processes (e.g., by the release of reactive oxygen thereby increasing oxidative stress)

Over the years, neuropathological studies have provided increasing evidence of proteinopathy in ALS (Figure 1.4G-L). Ubiquitin-positive inclusions were one of the first to be reported in ALS (Leigh et al., 1988; Lowe et al., 1988) and were later identified in FTLD patients (Bergmann, Kuchelmeister, Schmid, Kretzschmar, & Schroder, 1996; Ikeda et al., 2002; Jackson, Lennox, & Lowe, 1996), thus providing an initial link between ALS and FTLD. They have been noted in neurons of the frontal cortex, temporal cortex, hippocampus and striatum in ALS and FTLD cases independently (Kawashima et al., 1998; Okamoto, Hirai, Amari, Watanabe, & Sakurai, 1993; Okamoto, Hirai, Yamazaki, Sun, & Nakazato, 1991; Piao et al., 2003; Wightman et al., 1992). Adding to this finding, TDP-43 was discovered as the main component of ubiquitin inclusions in both ALS and FTLD patients, which established the link between the two neurodegenerative conditions (Arai et al., 2006; Neumann et al., 2006). TDP-43 is typically found in the nucleus of normal neurons and glial cells. However, in ALS and FTLD cases pathological TDP-43 aggregates are seen in the

cytoplasm, either caused by their translocation from nucleus to cytoplasm or an impaired cytoplasm-to-nucleus shuttling process (Geser et al., 2011; Mackenzie et al., 2011; Neumann et al., 2006; Thorpe, Tang, Atherton, & Cairns, 2008). More recent studies have disputed these findings, suggesting that TDP-43 is present in other neurological populations as well as in aging populations (Geser, Lee, & Trojanowski, 2010; Higashi et al., 2007). Instead, a phosphorylated band of TDP-43 (pTDP-43) likely from posttranslational modification was found to be exclusively present in ALS and FTLD but absent in the neurons of healthy or aging populations (Arai et al., 2006; Braak, Ludolph, Thal, & Del Tredici, 2010; Davidson et al., 2007; Hasegawa et al., 2008).

Based on the burden of pTDP-43 burden, a neuropathological staging system has been proposed (Braak et al., 2013; Brettschneider et al., 2013). The model included 4 stages with increasing pTDP-43 burden and spread to other brain regions at higher stages (Figure 1.5). Stage 1 features mild pTDP-43 burden in the motor cortex, brainstem and spinal motor neurons. Stage 2 involves mild-moderate pTDP-43 burden, with spreading into the middle frontal gyrus, reticular formation and precerebellar nuclei. Stage 3 displays moderate burden of pTDP-43 with further spread into the basal ganglia, prefrontal cortex, postcentral cortex and striatum. Stage 4 involves severe pTDP-43 burden with involvement of the hippocampal formation. More recently, a neuroimaging study confirmed this model in the white matter of ALS patients, in-vivo, using diffusion tensor imaging (DTI) (Müller et al., 2016).

(Fatima et al., 2015; Müller et al., 2016) independently validated the staging system proposed by Brettschneider et al. (2013), however the authors question the possibility of pTDP-43 in deep white matter regions such as the internal capsule of the corticospinal tract (CST) suggested by neuroimaging studies (Fatima et al., 2015; Müller et al., 2016). The

authors report that the presence of oligodendroglial pTDP-43 in cortical white matter but not in deep white matter regions such as the CST or other white matter tracts such as corpus callosum and the cingulum bundles. This suggests that oligodendroglia may not propagate pTDP-43 spread in deep white matter, and any changes in tracts such as the CST or corpus callosum may represent an overall loss of structural integrity, secondary to pathology (Fatima et al., 2015).

A few studies have also investigated pathological features associated with cognitive impairment in ALS. Brettschneider et al. (2012) reported higher microglial activation and TDP-43 burden in ALS-exi patients in the middle frontal and superior or middle temporal gyri, compared to ALS without EF impairments. These measures, but not tau and Aβ pathologies, were found to highly correlate with EF impairment. Another recent study suggested that a trend-level association between pTDP-43 and cognitive impairment, with more substantial associations to macroscopic changes such as synaptic loss (Henstridge et al., 2018).

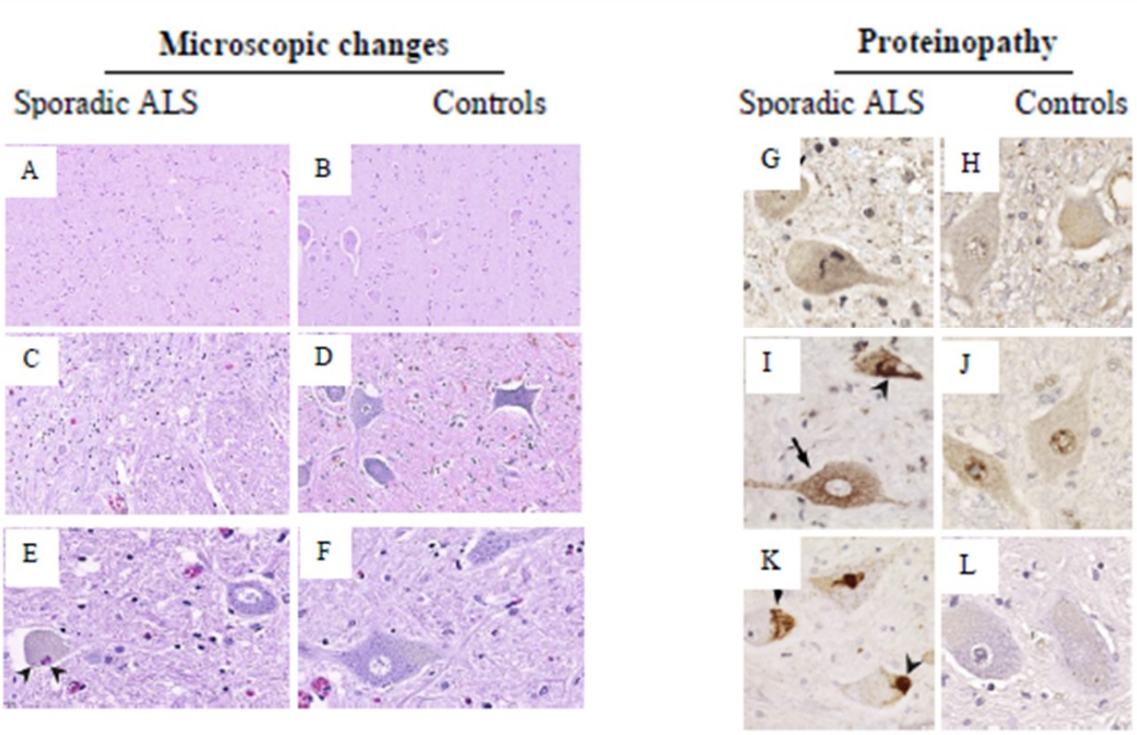


Figure 1.4. Neuropathological hallmarks in ALS.
Panels (A-D) indicate loss of motor neurons and astrogliosis in sporadic ALS as compared to controls in the motor cortex (A vs. B) and the anterior horn of the spinal cord (C vs. D). Panel E-F displays bunina bodies (indicated by arrows) in the cytoplasm of motor neurons. Neuropathological inclusions are indicated in panels (G-L) displaying Ubiquitin inclusions (G vs. H), TDP-43 inclusions (I vs. J) and pTDP-43 inclusions (K vs L) in spinal motor neurons of ALS patients but not controls.

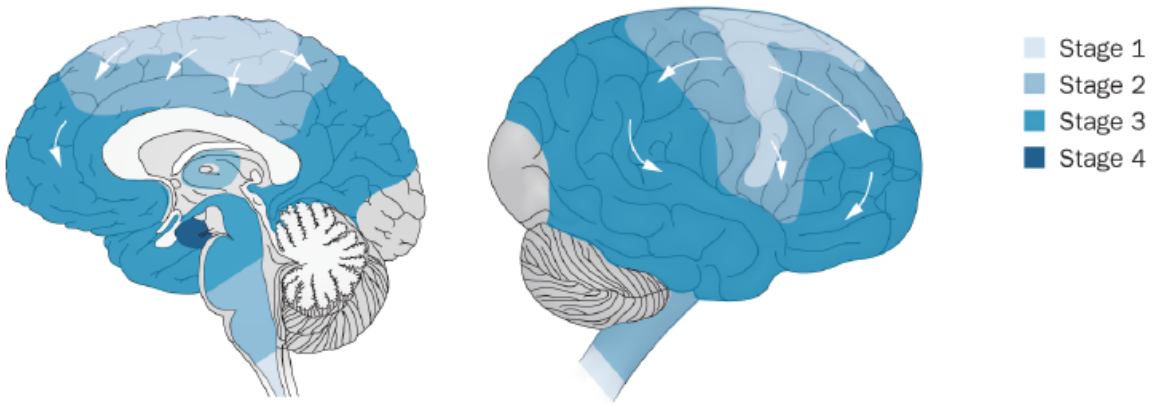


Figure 1.5. Sequential spread of pTDP-43 pathology.
Stage 1 includes regions of initial impact in the agranual neocortex (motor, premotor and supplementary motor cortices), bulbar and spinal somatomotor neurons. Stage 2 depicts pathology in reticular formation, precerebellar nuclei and portions of the thalamus. Stage 3 highlights the involvement of postcentral cortex and striatum. Stage 4 depicts the involvement of entorhinal cortex, hippocampal area and dentate fascia.

1.7 Biomarkers and Neuroimaging Evidence

There is an increasing need to identify the pathological changes in ALS in-vivo to facilitate diagnosis and monitor disease progression for effective decision-making and care-planning (Chen & Shang, 2015). Biomarker is a term that refers to any quantifiable 'biological marker' of medical signs which is measured accurately and reproducibly (Strimbu & Tavel, 2010). Candidate biomarkers have been identified for tissues and biofluids, electrophysiological and neuroimaging measures in ALS.

1.7.1 Tissue and Fluid biomarkers

Biochemical biomarkers such as tissue and biofluids enable investigations of specific cellular or signaling alterations that may help in diagnosis or monitoring disease course. Key biomarkers have been identified in blood, skeletal muscle and cerebrospinal fluid (CSF) in ALS patients.

Blood-based biomarkers such as serum albumin and creatinine levels are used as reliable markers of clinical severity and are especially used to define prognosis when diagnosing ALS (Chio et al., 2014). Studies have indicated that creatinine loss is associated with ALS progression and has the potential to serve as a candidate biomarker in ALS (Patin et al., 2015). Skeletal muscle biopsies were also used to identify progressive denervation and atrophy and have been found to be associated with disability in ALS (Jokic et al., 2005). Analysis of the muscle transcriptome have revealed increased levels of proteins such as smad1, 5, 8 mRNA and elevated smad phosphorylation levels, indicative of skeletal muscle injury. Both increased levels of smad proteins and their phosphorylation have been suggested as potential biomarkers for ALS (Si et al., 2014).

CSF is the most common biofluid for discovering biomarkers, reflecting not just systemic but CNS-relevant pathophysiological alterations in ALS. CSF biomarkers have been suggested to index: (1) blood brain barrier dysfunction such as increased matrix metalloproteinases (MMPs) (Ilzecka, Stelmasiak, & Dobosz, 2001), (2) neuroaxonal degeneration such as higher neurofilament light chains and reduced p-tau / t-tau protein levels (Grossman et al., 2014; Tortelli et al., 2012; Wilke, Deuschle, Rattay, Maetzler, & Synofzik, 2015), (3) reduced neuroprotective factors such as decreased cystatin C which is a marker for neurodegeneration and repair of the CNS (Pasinetti et al., 2006), (4) increased inflammation response and microglial activation such as higher levels of interleukin-6 and interleukin-8 (Tarasiuk, Kulakowska, Drozdowski, Kornhuber, & Lewczuk, 2012) or CHIT-1 enzyme (Varghese et al., 2013), and (5) decreased erythoprotein levels (Brettschneider, Widl, Ehrenreich, Riepe, & Tumani, 2006).

1.7.2 Physiological Biomarkers

The presence of physiological markers of UMN and LMN function enable distinguishing ALS from other neurological conditions. Most commonly, electromyography is used to identify LMN loss as characterised by the presence of fibrillation potentials and positive sharp waves and is often investigated at the time of diagnosis in ALS. However, its limited sensitivity (60%) in ALS has contributed to the emergence of other physiological markers such as motor unit number estimation (MUNE) that have been associated with survival (Felice, 1997). Recent studies have suggested an improved technique, motor unit number index (MUNIX) to be a reliable marker of LMN loss in ALS (Neuwirth et al., 2015). Emerging technology includes electrical impedance myography (EIM) to assess integrity and structure of muscle (Rutkove, 2009) and muscle ultrasound (Dengler, 2012). Signs of

UMN are a challenge to track in ALS. Most clinicians rely on neurological examination of reflexes. One suggested UMN marker is using transcranial magnetic stimulation (TMS). It enables investigation of subclinical UMN dysfunction and distinguishes between ALS and possible variants (Vucic, Cheah, Yiannikas, & Kiernan, 2011). However, these techniques are experimental at the current time and require further validation as a reliable biomarker for differential diagnosis in ALS.

1.7.3 Neuroimaging Biomarkers

With advances in medical imaging techniques such as computed tomography (CT) scans and magnetic resonance imaging (MRI), the above pathological changes were supported by gross changes in brain structure and function using neuroimaging since the 1980s. CT scans initially identified morphological alterations in the skeletal muscles of the limbs (Kuther, Rodiek, & Struppler, 1987) and visual inspection of atrophy in cortical and ventricular regions (Poloni, Capitani, Mazzini, & Ceroni, 1986).

MRI enables in-vivo investigation of structural and functional changes in diseased and healthy populations. It makes use of magnetic properties of protons to derive high-contrast resolution structural images. Initial MRI scans reported hyperintensities along the CST on T2-weighted images (Goodin, Rowley, & Olney, 1988). However, T2 hyperintensities are not specific to ALS and thus studies have explored advanced MRI techniques to identify in-vivo markers of disease progression in ALS. The application of MRI techniques to identify structural and functional brain changes is an important aspect of the current thesis. In addition to providing literature support, the sub-sections below describe the MRI techniques applied in the thesis.

1.7.3.1 Voxel Based Morphometry (VBM)

VBM is an advanced approach that applies computational techniques to investigate structural brain changes. It enables identifying differences in the local composition of brain tissue (density or volume) after the images are registered to a standard reference template to discount macroscopic differences in shape (Ashburner & Friston, 2000). The technique involves segmentation of T1-weighted scans into grey and white matter, normalisation of scans to a standard reference template and applying Gaussian smoothing for further analysis. Grey matter density is used to measure the average concentration of the tissue represented within the voxel relative to other tissue types. The values for grey matter density range from 0 to 1. A slight variation to the pipeline includes multiplying the normalised grey matter maps with respective Jacobian determinants of the deformation field to retain macroscopic properties of brain structure (volume of local tissue). This procedure is termed as modulation and involves alterations of tissue intensity to preserve local volumes (higher intensity indicates greater tissue volume). These smoothed grey matter maps are used in statistical analysis to detect density or volume differences.

However, studies have identified some factors that could confound detection of true grey matter density or volume change. Motion artifacts and subsequent mis-registrations to the standard reference template may influence segmentations and result in classification differences. Another factor may be reductions in relative intensity of grey matter and white matter in participants (e.g. one group has lower grey matter intensity as compared to other group). These factors may indicate significant difference on statistical analysis, but these

differences may not be due to true reductions in grey matter density or volume. Nevertheless, advances in registration techniques and application of multiple comparisons corrections have enabled minimising false-positive findings in VBM analysis.

With advances in structural imaging analysis, evidence of reduced grey matter density and volume using voxel based morphometry (VBM), and reduced cortical thickness have been reported in motor cortices of ALS patients(Agosta et al., 2016; Agosta et al., 2012; Chang et al., 2005). Extra-motor regions including atrophy in medial prefrontal cortex, dorsolateral prefrontal cortices and temporal regions in ALS-FTLD spectrum have also been reported (Agosta et al., 2016; Agosta et al., 2012; Chang et al., 2005).

1.7.3.2 Diffusion Tensor Imaging (DTI)

DTI techniques rely on diffusion of water molecules within axonal tracts to determine specific properties of degeneration such as loss of axonal integrity or demyelination (Song et al., 2003). In MRI, diffusion coefficient (also referred to as apparent diffusion coefficient) reflects the interaction of diffusing molecules such as water with cellular structures over a given time. In the absence of barriers, water diffusion is the same in all directions (isotropic diffusion) while in the presence of highly oriented barriers diffusion may be greater in a specific direction (anisotropic diffusion). In white matter, water diffusion is greater parallel to the length of axonal tracts than perpendicular. Additionally, the presence of axonal membranes, microtubules and myelin sheath restrict diffusion of water perpendicular to the axonal tract (Beaulieu, 2002).

These properties are measured as fractional anisotropy (FA), axial diffusivity (AD), radial diffusivity and mean diffusivity (MD). FA is an index reflecting the degree of diffusion

directionality. It represents the overall integrity of white matter with values ranging from 0 (isotropy) to 1 (anisotropy). While FA values enable identification of microstructural white matter changes, but they are not specific to biological substrates (axonal or myelin loss). Reduced FA values could be attributed to either reduced parallel diffusivity or increased perpendicular diffusivity. Instead, AD and RD provide information on biological substrates of white matter changes. AD is an index for parallel diffusion and represents axonal integrity. RD is an index for perpendicular diffusion and reduced RD has been associated with demyelination (Song et al., 2003). MD is the apparent diffusion coefficient and represents the average of eigenvalues in the x, y and z directions. Some confounding factors that alter DTI metrics include thermal / physiological noise, artifacts such as eddy currents or head motion, partial volume averaging between tissues (e.g. grey matter, CSF and white matter boundaries) and regions of crossing white matter fibres (Alexander, Lee, Lazar, & Field, 2007). DTI preprocessing includes corrections for eddy currents, ringing artifacts and motion (EPI distortions) to improve quality of the scan. Further analysis of the preprocessed images includes techniques such as tractography to select specific regions of interest such as the corticospinal tract (CST) or projecting highest DTI values (FA, AD, RD or MD) to a skeleton representing the core white matter structure for further voxel-wise analysis.

White matter changes such as reductions in white matter volume (Abrahams, Goldstein, et al., 2005) and reduced integrity of the CST using DTI has also been reported. Studies have identified reduced fractional anisotropy (FA) in the CST, corpus callosum and longitudinal association tracts such as superior longitudinal fasciculus (SLF), inferior longitudinal fasciculus (ILF), inferior fronto-occipital fasciculus (IFOF) and uncinate fasciculus (UF)

(Agosta et al., 2010; Dimond et al., 2017; Menke et al., 2014; Sarro et al., 2011; Zhang et al., 2011).

1.7.3.3 Functional Magnetic Resonance Imaging (fMRI)

The development of fMRI enabled investigation of in-vivo brain function. The technique uses magnetic properties of blood-oxygenation to identify regions of brain activity. Deoxyhaemoglobin is paramagnetic and induces magnetic local inhomogeneities, while oxyhaemoglobin is diamagnetic and produces little effect. An increase in concentration of deoxyhaemoglobin reduces image intensity, while a decrease in deoxyhaemoglobin increases image intensity (Heeger & Ress, 2002). The variations in blood-oxygen level dependent (BOLD) signals are captured in an fMRI scan using a T2* weighted sequence. During regional brain activations, there is an initial decrease in signal intensity due to deoxygenation which is followed by an increase in signal intensity owing to a surge oxygenated blood supply (Toronov et al., 2003). This rapid response is the basis of haemodynamic response and the increased local blood flow occurs after a delay of approximately 2 seconds of neural activation. Initial models suggested that the BOLD signal is dependent on venous blood volume (Buxton, Wong, & Frank, 1998), but more recent studies have highlighted complex relationships suggesting contributions from arterial blood volume, oxidative metabolism and neurovascular coupling (Gauthier & Fan, 2018). These regional brain activations are also found at rest (Biswal, Yetkin, Haughton, & Hyde, 1995) and subsequently several resting state networks (RSNs) have been identified (Allen et al., 2011). RSNs can be derived using a-priori regions to interest (e.g. precentral gyrus for sensorimotor networks [SMN]). Advances in exploratory techniques such as Independent Components Analysis (ICA) have also enabled model-free investigation of RSNs where the

effects of interest are less understood or not predicted accurately (Beckmann, DeLuca, Devlin, & Smith, 2005).

A single resting state fMRI (rs-fMRI) scan lasts for 5-10 minutes and typically involves acquisition of T2* images contiguously. The preprocessing steps for rs-fMRI analysis require alignment of images at all time-points, registering the images to a standard reference template and smoothing the data to improve signal-to-noise ratio. The smoothed rs-fMRI images are usually used in further analysis such as seed-based correlation analysis or ICA.

Functional changes using blood oxygen level dependent (BOLD) signal for both task-based and resting-state paradigms have been reported, with functional magnetic resonance imaging (fMRI) studies indicating either reduced activity in the motor cortex (Mohammadi et al., 2009), increased activity in the motor cortices as well as dorsolateral prefrontal cortices (Agosta et al., 2013) or no significant differences (Chenji et al., 2016) in ALS patients as compared to healthy controls.

1.7.3.4 Other Neuroimaging Techniques

Functional changes were initially identified using positron emission tomography (PET) studies indicating cortical glucose hypometabolism in ALS (Dalakas, Hatazawa, Brooks, & Di Chiro, 1987) including regions of the motor cortex (Hatazawa, Brooks, Dalakas, Mansi, & Di Chiro, 1988), premotor and supplementary motor areas, anterior cingulate cortex (ACC), paracentral lobule, superior and inferior parietal cortex (Kew, Leigh, et al., 1993). Furthermore, widespread attenuation of cerebral blood flow as indicated by PET scans were reported for ALS patients with impaired verbal fluency in bilateral medial prefrontal cortices as compared to controls and in parahippocampal regions, thalamus and rostral ACC as

compared to ALS patients with normal verbal fluency (Kew, Goldstein, et al., 1993). There were no abnormalities noted in the Broca's area, a region typically associated with verbal fluency. Kew et al., (1993) suggested that their findings align with degeneration of libo-thalamic-cortical pathways, however the direction (driven cortically or via sub-cortical structures) of such functional loss could not be inferred.

In-vivo markers of cerebral degeneration were also identified using magnetic resonance spectroscopy (MRS), a technique which permits quantification of cerebral tissue metabolites. N-acetylaspartate (NAA) over total creatinine (Cr) and total choline (Cho) ratios are markers of neuronal integrity (Chen & Shang, 2015) while myo-inositol (Ino) is regarded as a glial marker (Kalra, Hanstock, Martin, Allen, & Johnston, 2006). Studies have identified decreased NAA-ratios and increased Ino-ratios in the motor cortex in ALS (Block et al., 1998; Kalra et al., 2006; Pioro, Antel, Cashman, & Arnold, 1994). This pattern was also noticed in medial prefrontal cortex suggesting extra-motor changes in ALS patients (Usman et al., 2011).

1.7.3.5 Current Application of MRI in ALS

Clinical neuroimaging has primarily been used to eliminate other neurological causes (such as cerebrovascular disease, multiple sclerosis, etc.) prior to assigning the diagnosis of ALS. Over the years, studies are increasingly striving towards identifying an optimal neuroimaging biomarker for ALS to enable early diagnosis and monitor prognosis of the disease (Kalra & Arnold, 2003). Studies have reported association between (1) grey matter atrophy and faster disease progression (Agosta et al., 2012; Mezzapesa et al., 2013), (2) loss of white matter integrity, UMN burden and disease progression (Agosta et al., 2016; Woo et

al., 2014), and (3) increased functional connectivity with greater disability (Chenji et al., 2016). While studies have made advances in identifying imaging biomarkers, further translational studies validating these markers at the level of a single subject are required.

1.8 Clinical Management

Currently, there are no effective therapeutic drugs to combat ALS, and thus it remains a terminal disease. Clinical care and management of ALS is focused on treating symptoms and providing supportive therapy to enable autonomy, prolong survival and improve quality of life for patients. Multidisciplinary care with a coordinated team of neurologists, physical therapists, speech therapists, occupational therapists, respiratory therapists, social workers, dietitians, and nursing care managers has been suggested to enhance health care delivery in ALS (Andersen et al., 2012; Miller et al., 2009; Mitsumoto et al., 2014; van den Berg et al., 2005).

1.8.1 Measuring disability

Activities of daily living and changes over time are measured using clinical scales such as the Appel ALS rating scale (Appel et al., 1987) and the ALS Functional Rating Scale – Revised (ALSFRS-R; (Cedarbaum et al., 1999). The Appel score is determined from five sub-scores measuring bulbar, respiratory, muscle strength, lower extremity and upper extremity function. A sub-score of 6 for each domain indicates minimal dysfunction, while a sub-score of 30-36 points indicate maximal dysfunction. Lower total Appel scores indicate minimal disability (a score of 30 was obtained by healthy controls), while higher scores indicated greater disability (a score of 164 indicated maximum impairment).

The ALSFRS-R is more commonly used to measure disability in both clinical and research settings. The ALSFRS-R is a questionnaire-based scale that includes 12 items assessing motor and respiratory abilities when performing activities of daily living (Cedarbaum et al., 1999). It incorporates four domains of function: gross motor tasks (walking, climbing, turning in bed), fine motor tasks (handwriting, handling utensils, dressing and hygiene), bulbar function (speech, swallowing and salivation) and respiratory function (dyspnea, orthopnea and respiratory insufficiency). The maximal score on the ALSFRS-R is 48, indicating normal function, while lower scores indicate greater disability. A linear drop of 1 point per month is expected in ALS patients. A loss of greater points or a plateau in the score over time are indicative of faster or slower progression, respectively.

1.8.2 Physical weakness and disability

Frequent falls, inability to walk, dress or maintain routine hygiene habits are observed in varying degrees of intensity in ALS. Providing patients with adequate orthotics such as ankle foot brace or adaptive aids such as a walking frame and wheelchair are important aspects of care delivery. Musculoskeletal cramps, pain, fasciculations and spasticity are managed by physiotherapy, intake of non-steroidal anti-inflammatory drugs, muscle relaxants (such as baclofen and botulinum toxin), anticonvulsants (gabapentin) and opioid drugs. With increasing disability, patients may experience pain due to immobility which would require re-positioning and pressure care such as use of pressure-relieving cushions and mattress (Zarei et al., 2015).

1.8.3 Respiratory Function

Respiratory muscle weakness is noted in ALS patients as a secondary symptom to progressive motor neuron degeneration and results in reduced ventilation. It is manifested as dyspnea during walking or talking, orthopneoa, nocturnal hypoxia resulting in disturbed sleep, morning headaches, fatigue, depression, poor concentration and nocturia. Respiratory failure is the primary cause of mortality in ALS (Wijesekera & Leigh, 2009) and effective management ensures better quality of life in patients. Spirometry techniques are employed to assess respiratory function. Forced Vital Capacity (FVC), the total volume of air exhaled during a forced breath, is a common measure used to detect respiratory decline. The recommended threshold for FVC is 70 percent reference, below which the patient may develop respiratory failure.

Respiratory support involves exercises for lung volume recruitment (LVR), non-invasive ventilation (NIV) or invasive ventilation (tracheotomy). LVR was found to significantly improve coughing and pulmonary function in ALS and is considered effective treatment for improving lung function (Cleary, Misiaszek, Kalra, Wheeler, & Johnston, 2013). Bi-level positive pressure devices (Bi-PAP) is the recommended NIV option; continuous positive pressure (CPAP) was not useful in ALS patients (Radunovic, Mitsumoto, & Leigh, 2007). NIV is usually initiated for intermittent nocturnal use, to reduce nocturnal hypoventilation and is eventually increased to include daytime and / or continuous use as the disease progresses.

1.8.4 Managing Bulbar Symptoms

Dysarthria or slurred speech in ALS is a consequence of weakness in the laryngeal muscles. Use of augmentative and alternative communication aids and strategy is essential to help

patients with their daily communication needs (Kraat, 1990). With advancement of technology, the current communication aids typically include use of erasable writing devices, speech-to-text application on cellular devices or tablet computers. More recent advances have enabled the use of brain computer interface (BCI) for patients who are in a "locked-in" state in ALS (Nijboer et al., 2008). However, the acceptance and use of these devices have been found to be varied among patients and could be influenced by differential communication needs over time. Some patients are unable to adapt to the technology and may display a lack of acceptance to use these devices (Ball, Beukelman, & Pattee, 2004). Educating caregivers and family members regarding strategies and symptomatology may help in providing a support to the communication needs of patients.

Bulbar symptoms in patients also include sialorrhea (excessive watery saliva) or thickened saliva. Sialorrhea is managed by the intake of drugs such as anticholinergic antidepressants (amitriptyline), anticholinergic drugs (e.g. glycopyrronium bromide), botulin toxin injections, and radiation of salivary glands. For patients with thick saliva, recommendations include natural remedies (intake of papaya), adequate hydration and saline nebulisers (nebulised N-acetylcysteine). Use of mouth care products and suctioning of the mouth is often recommended for both conditions (Kiernan et al., 2011; Zarei et al., 2015).

1.8.5 Nutritional Management

Dysphagia (or difficulty swallowing food and liquids) is noted in most ALS patients, although at varying points of their disease course. This leads to choking, aspirations, restrictions in nutrition, dehydration and weight loss in patients. Insufficient caloric intake in patients has been found to augment weakness and fatigue (Miller et al., 2009). Initial

changes in intake include alteration of food consistency for effective swallowing, minimising choking and aspirations. With higher severity of dysphagia, enteral feeding devices such as a percutaneous endoscopic gastrostomy (PEG) is prescribed (Kasarskis et al., 1999). PEG is a surgical procedure which is usually recommended for patients whose weight is lower than 10% of their pre-diagnostic weight and has been noted to help stabilize weight loss in ALS patients alongside providing adequate nutrition (Zarei et al., 2015).

1.8.6 Disease Modifying Treatments

There are two drugs, riluzole and edaravone, that are currently FDA-approved for treating ALS. Riluzole is an anti-glutamate agent with beneficial effects in ALS reported in 1994 (Bensimon, Lacomblez, & Meininger, 1994). The mechanism of action was proposed to include both inhibition of glutamate release as well as inactivation of voltage-gated Na^+ channels (Cheah, Vucic, Krishnan, & Kiernan, 2010). Magnetic resonance spectroscopy (MRS) studies indicated a 6% increase of the N-acetylaspartate/Choline (NAA/Cho) ratio, a neuronal marker, in the primary motor cortex of ALS patients after 3 weeks of drug administration, while a 4% reduction of this ratio was noted in untreated patients, indicating some neuroprotective effects in the CNS (Kalra, Cashman, Genge, & Arnold, 1998). Riluzole intake has also been found to reduce hyperexcitability of the cortex indicated by TMS and Na+ conductance in the axons of the peripheral nervous system (Vucic et al., 2013). These findings suggest that riluzole plays a modulatory effect on glutamate excitability at both the central and peripheral levels. However, no long-lasting effects of survival have been reported, with studies indicating increase in survival by only 2-3 months on a dose of 100mg per day (Miller, Mitchell, & Moore, 2012). Adverse effects for riluzole

include fatigue, nausea, dizziness and diarrhoea and elevated liver enzyme levels (Gordon, 2011). These effects are reversed when the drug is discontinued.

Edaravone, an antioxidant, was approved by the FDA in 2017, after a 22 year long-wait since the introduction of riluzole (Rothstein, 2017). It is a free radical scavenger that helps eliminate lipid peroxides and hydroxyl radicals. This reduced effect on oxidative stress was confirmed in a Phase-II clinical trial of 20 ALS patients with a dose-varying administration of edaravone (Yoshino & Kimura, 2006). A reduced ALSFRS-R score and 3-nitrotryosine level (3NT, marker for oxidative stress) in the CSF was reported at the end of six-month treatment period in patients administered with a higher dose (60mg). The Phase-III trial showed an initial failure to differentiate between treated from untreated groups (Abe et al., 2014). However, a repeat trial with stringent criteria (ALSFRS-R $\geq$ 24 and FVC $\geq$ 80%, disease duration $\leq$ 2 years) indicated reduced ALSFRS-R changes in the treatment group, suggesting beneficial effects of edaravone over a 24-week period (Writing & Edaravone, 2017). There appear to be limitations such as a select sub-group of ALS patients with mild disability, short trial duration, lack of onset-subgroups, inconvenient drug administration that raise practical concerns for effective prescription in a clinical setting (Hardiman & van den Berg, 2017). Further studies on edaravone are warranted to address these limitations.

1.8.7 Monitoring Cognitive and Behavioural Changes

The severity of physical impairments in ALS often take focus in a clinic, compared to the cognitive and behavioural changes, which also often lack formal assessment and monitoring. The increasing evidence of cognitive and behavioural changes in ALS calls for incorporation of screening for these impairments in a clinic setting. Information gathered from cognitive

screening tools will enable ALS clinics to prioritize the level of impairment and facilitate education of caregivers regarding these changes. While emotional lability and pseudobulbar affect (PBA) are widely recognised and treated using drugs such as amitriptyline, benzodiazepines and quinidine sulfate (Zarei et al., 2015), cognitive and behavioural changes are subtle and are not yet treatable in ALS. Cognitive screening tools developed specifically for ALS such as the Edinburgh Cognitive and Behavioural ALS Screen (ECAS) and the ALS Cognitive and Behavioural Screen (ALS-CBS) help in screening for ALS-specific impairments (Abrahams, Newton, Niven, Foley, & Bak, 2014; Woolley, York, et al., 2010).

Presence of cognitive and behavioural impairments might be indicative of treatment adherence, decision making in everyday life and the need for additional strategies of communication in affected patients (Goldstein & Abrahams, 2013; Meier, Charleston, & Tippett, 2010). Formal diagnosis of cognitive and behavioural impairments would also enable care providers assist patients in making end-of-life decisions at much earlier stages in the disease course. Anxiety has been reported to be higher in patients at the time of diagnosis, with significantly higher reports of anxiety in female patients (Cui et al., 2015). It remains crucial to identify other confounding and/or comorbid factors that may contribute to cognitive impairment. This includes comorbid neurological (e.g. cerebrovascular disease, pre-existing or concurrent head injury), systemic (e.g. hypothyroidism, diabetes), pharmacological (e.g. substance abuse) or psychiatric (e.g. severe depression, anxiety or psychosis) conditions. Additional clinical factors such as pseudobulbar affect, respiratory insufficiency, disrupted sleep, delirium, pain, fatigue and medications (such as psychotropic or narcotic analgesic medications) should be considered (Strong et al., 2009). Further details

on the neuropsychological profile, cognitive assessments and associated frontotemporal deficits are discussed in Chapter 2.

1.9 Summary

ALS is a terminal neurodegenerative disorder that extends beyond the motor system to include frontotemporal changes which overlap with FTLD. The current chapter provided an overview of ALS from a clinical, genetic and pathological perspectives. Additionally, cognitive and behavioural symptoms add to the complexity in the condition and may influence prognosis. A review of literature highlighting the neuropsychological and neuroimaging profile of ALS-FTLD spectrum is provided in Chapter 1.

2. Neuropsychometric Profile and Neuroimaging in ALS

Chapter 1 provided an outline of the ALS-FTLD spectrum and elaborated on the diagnostic criteria suggested for recognizing cognitive and behavioural changes in ALS. Based on the criteria, subgroups included ALS with cognitive impairment (ALSci), behavioural impairment (ALSbi), a combination of the two (ALScbi) and a smaller subset that meet criteria for FTLD. Independent studies in ALS have defined these cognitive sub-groups using a myriad of acronyms such as ALSi, ALS-ci, ALS-plus, MND-plus for the cognitively impaired groups and ALSu, ALS-cc, ALS-ni, ALS-motor, MND-motor for the cognitively unimpaired groups. For purposes of this Chapter, all cognitively impaired ALS sub-groups are referred to as 'ALS-ci' and ALS patients with no cognitive impairments are referred to as 'ALS-only'.

The current chapter discusses various domains of cognitive and behavioural impairment (such as executive functions, apathy, etc.) reported in the literature for the ALS-FTLD spectrum, with primary focus on non-demented ALS patients. The clinical profile, prognosis and neuroimaging findings of the cognitive and/or behavioural sub-groups are also discussed. Gaps in the literature were identified and a rationale, aims and hypotheses for the current thesis are outlined.

2.1 ALS-Specific Cognitive and Behavioural Screening Tools

Most studies investigating cognitively impaired groups in ALS have employed extensive neuropsychometric testing. Popular cognitive screens such as the Addenbrooke's Cognitive Examination - Revised (ACE-R) (Mioshi, Dawson, Mitchell, Arnold, & Hodges, 2006), Montreal Cognitive Assessment (MoCA) (Nasreddine et al., 2005) and Frontal Assessment

Battery (FAB) (Dubois, Slachevsky, Litvan, & Pillon, 2000) do not accommodate motor disability or dysarthria/anarthria noted in ALS patients. These screening tools are weighted towards specific cognitive domains such as executive functions (FAB) or Memory (ACE-R). They fail to address broader changes noted in FTLD associated with ALS (Goldstein & Abrahams, 2013). Over the years, studies have developed ALS-specific cognitive and behavioural screening tools such as the ALS Cognitive and Behavioural Screen (ALS-CBS) (Woolley, York, et al., 2010), MND Behavioural Instrument (MiND-B) (Mioshi, Hsieh, et al., 2014), ALS-FTD questionnaire (ALS-FTD-Q) (Raaphorst et al., 2012).

The Edinburgh Cognitive and Behavioural ALS Screen (ECAS) is the most recent and comprehensive screening tool for various cognitive changes in ALS including language, memory and social cognition (Abrahams et al., 2014). It has been reported to have good to excellent internal consistency, moderate to high sensitivity and high specificity (Abrahams et al., 2014; Lulé et al., 2015; Niven et al., 2015; Pinto-Grau et al., 2017). More recent studies have introduced three different ECAS versions for longitudinal analysis (Crockford et al., 2018; Crockford et al., 2018). Relatively few studies have investigated neuroimaging associations of this screening tool (Keller et al., 2017) or attempted to identify cognitive stages in association with ALS-related changes in white matter (Lulé et al., 2018).

Considering the extensive investigations into cognitive and behavioural impairments in ALS, the subsequent sections review the literature on cognitive and behavioural changes in ALS assessed using neuropsychometric tests and attempt to provide a comprehensive picture on the nature of cognitive and behavioural impairments in ALS.

2.2 Cognitive Impairment in ALS

The spectrum of cognitive impairment varies in severity for patients. Studies have reported impairment between 25%-50% in ALS patients (Goldstein & Abrahams, 2013; Phukan et al., 2012). The varying degree of impairment in studies is primarily influenced by tests used for identifying deficits and possible bias due to motor impairments (Beeldman et al., 2016; Goldstein & Abrahams, 2013). Small to medium effect sizes have been noted for the domains of executive functions, language, verbal memory and more recently, social cognition (Beeldman et al., 2016; Bora, 2017; Raaphorst, de Visser, Linssen, de Haan, & Schmand, 2010b). Each of these domains are elaborated further in the sections below and a brief overview of studies included in this section are provided in Supplementary Materials

Supplementary Table *2.1*.

2.2.1 Executive Functions

Executive functions (EF) refers to a broad set of higher-level cognitive processes that enable control and regulation of thoughts, actions and behaviours (Friedman & Miyake, 2017). They are used to describe various cognitive abilities such as maintaining and / or monitoring of working memory contents (updating), switching between tasks (flexibility and shifting) and resisting automatic responses (inhibition). One task that employs these cognitive abilities is verbal fluency, as it requires intrinsic generation of words beginning with a specific letter, ability to monitor words that have been generation, shift from one word to the next and inhibit repetition of words or generation of words that do not fit with the rules specified (Mitrushina, Boone, Razani, & D'Elia, 2005). Verbal fluency is considered the most sensitive marker of EF impairment in ALS (Abrahams et al., 2000; Beeldman et al., 2016; Phukan et al., 2012; Strong et al., 2017). EF also requires intact attention and working

memory, processes that have also been reported to be impaired in ALS (Abe et al., 1997). Higher order tasks such as planning and decision making use lower order EF abilities such as attention, working memory, cognitive flexibility, and inhibition to evaluate situations, orient thoughts and execute actions to achieve specific goals (Owen, 2005). Impaired planning and decision making have been reported in ALS patients (Abrahams et al., 1997). This section further describes impairments in verbal fluency, lower level of EF (attention, working memory, cognitive flexibility, set-shifting and inhibition) as well as higher order EF abilities (planning and decision making) impaired in ALS patients.

2.2.1.1 Verbal Fluency

Verbal fluency is a timed task that involves generation of as many intrinsic responses as possible for either specific letters (e.g. F, A, S, etc.) or categories (e.g. animals, fruits, vegetables etc.). There are minimal external cues or triggers for the verbal fluency task and it relies on processes such as initiating responses using effective retrieval strategies (e.g. words only beginning with letter F), updating working memory to track the words generated and inhibiting repetition of responses. These processes are considered to rely on a central executive (Baddeley & Della Sala, 1996) and / or a supervisory attention system (Norman & Shallice, 1986) that enable effective executive control. Additionally, verbal fluency may also rely on processes such as phonological loops to access words with similar sounds and may depend on age-related language abilities such as semantic knowledge (Fisk & Warr, 1996). In ALS patients, Abrahams et al., provided evidence for deficits in the central executive or supervisory attention system (Abrahams et al., 2000). The authors compared functions such as intrinsic response generation (verbal fluency), phonological loop functions (e.g. recall for phonologically similar vs. dissimilar letters) or simple word retrieval (e.g.

sentence completion task) in 21 ALS patients and 25 healthy controls. Impaired verbal fluency was revealed, while phonological loop functions and simple word retrieval remained intact in ALS, implying dysfunction of the central executive or the supervisory attention system.

Verbal fluency responses can be generated either orally or by writing and both versions of the tasks have been administered to ALS patients in previous studies (Abe et al., 1997; Abrahams et al., 1997; Abrahams et al., 1996; Abrahams et al., 2000; Kew, Goldstein, et al., 1993; Kew, Leigh, et al., 1993). Since verbal fluency tests are timed, motor impairments in ALS would confound true verbal fluency deficits (Abrahams et al., 1996; Strong et al., 2017). Dysarthria or hand weakness may slow the number of words generated in the given time to perform oral or written version of the task, respectively, with low scores reflecting not necessarily cognitive but motor dysfunction. Correction for motor impairments was suggested by Abrahams et al., (1996) by applying a simple algorithm, termed verbal fluency index (VFI). For this algorithm, a second condition was introduced in which participants were timed as they copied the list of words they generated previously. As outlined below, the VFI represents the average time taken to mentally generate words, corrected for the time required to write them down, such that higher scores represent longer mental word retrieval times indicating EF impairment.

$$\text{Verbal Fluency Index (VFI)} = \frac{\text{Time for generation condition} - \text{Time for copy condition}}{\text{Total number of items generated}}$$

Several studies have consistently shown verbal fluency deficits in ALS using the VFI, especially for letter fluency (Abrahams et al., 1996; Abrahams et al., 1997; Abrahams et al.,

2004; Abrahams, Goldstein, et al., 2005; Abrahams, Leigh, & Goldstein, 2005; Abrahams et al., 2000; Phukan et al., 2012; Stukovnik, Zidar, Podnar, & Repovs, 2010).

The performance on category fluency is less consistent with some studies reporting significant differences between ALS patients and controls (Abe et al., 1997; Abrahams et al., 2000; Hanagasi et al., 2002; Phukan et al., 2012), while others reported comparable performance (Talbot et al., 1995). While some studies have reported differences between ALS patients and controls for the category "animals" (Abe et al., 1997; Abrahams et al., 2000; Hanagasi et al., 2002), others have reported differences in other categories such as "fruits", "vehicles" or "supermarket goods" (Abe et al., 1997; Rottig et al., 2006).

Another study revealed deficits in strategies employed for the verbal fluency task (Lepow et al., 2010). Two strategies are considered to play a role in the verbal fluency task: clustering (accessing related words) and switching (changing from one cluster to another) (Troyer, Moscovitch, & Winocur, 1997). Clustering requires access to long-term memory to retrieve related words and is mediated by the temporal lobe, whereas switching has been associated with frontal-lobe functions (Troyer, Moscovitch, Winocur, Alexander, & Stuss, 1998). In a group comparison between 49 ALS patients and 25 healthy controls, Lepow et al., noted significant differences in clustering and switches for both letter and category fluency (Lepow et al., 2010). A marked decrease in clusters and switches was revealed when patients stratified into cognitive sub-groups, with performance decreasing in the order: controls > ALS intact > ALS mild > ALS-FTD (Lepow et al., 2010). Letter fluency typically relies on EF processes associated with the frontal lobe, while category fluency relies on semantic knowledge and may rely more on temporal lobe processes. As demonstrated in Lepow et al., (2010), these processes may be influenced by other aspects such as language and memory

processes. Thus, the study by Lepow et al., (2010), suggests the possible role of both frontal and temporal lobe dysfunction contributing to verbal fluency deficits in ALS.

2.2.1.2 Attention and Working Memory

Attention broadly refers to the ability to intiate, maintain or shift focus on incoming information (Riccio, Reynolds, Lowe, & Moore, 2002). The mode of information could either be sensory (visual, auditory, tactile) or cognitive (specific thoughts). Working memory further uses these incoming inputs to enable effective processing and manipulation of information. Three subsystems of working memory have been proposed: (1) phonological (auditory) loop for rehearsal and manipulation of linguistic information, (2) visuospatial sketchpad for manipulating visual and spatial information, and (3) episodic buffer that enables interaction between the first two sub-systems. All the three sub-systems are regulated by a central controller called the central executive (Baddeley, 2000; Baddeley & Della Sala, 1996). As mentioned in the previous section (pg. 51 and 52), dysfunctional central executive was associated with impaired verbal fluency in ALS.

Both attention and working memory deficits in ALS have been attributed to dysfunctions in the frontal lobes. Abe et al., administered tests of memory, visuo-constructive ability, attention and EF to 26 ALS patients (Abe et al., 1997). Here, the authors described verbal fluency and digit span tests as measures of attention, while Wisconsin Card Sorting Test (WCST) was used as a measure of EF. Participants were found to cluster into three groups based on cognitive testing scores, whereby decreasing mean z-scores for attention and EF were noted in the order: Group 3 (n=12) > Group 2 (n=6) > Group 1 (n=8). Single-photon emission computed tomography (SPECT) analysis indicated reduced isotope uptake (i.e.

blood flow) in Groups 1 and 2, classified based on neuropsychometric scores, thereby providing evidence for frontal hypoperfusion accompanying more severe deficits. Pathological findings in 3 of 8 patients in Group 1 indicated spongy degeneration and neuronal loss in the frontal lobes, with one patient showing presence of ubiquitin-immunoreactive cystoplasmic inclusions. On the other hand, 3 of 12 patients in Group 3 showed no significant pathology in the frontal regions.

Hanagasi et al. (2002) noted significant differences in performance for tests of attention such as digit span backwards test and continuous performance test (assessing selective attention to infrequently occurring stimulus). The latter indicated differences in accuracy of responses, but not in latency of responses. This suggests that there are deficits in attention despite comparable response times between patients and controls, suggesting the involvement of frontal networks in ALS patients.

Chari et al., (1996) administered series of 5 tests assessing visual attention and memory in 50 MND, 50 controls and 23 patients with spinal cord injury but no cerebral dysfunction. These tests were uncorrected for motor impairments. On the visual working memory task (matching to sample) the MND group performed worse than controls on the simultaneous matching condition while delayed matching showed no significant differences. In the visual attention task (visual search matching to sample), the MND group performed significantly lower than the control group, at the largest set (8 items), while performance was comparable at smaller sets (1, 2 and 4 items). However, this result must be interpreted with caution as score on the largest set correlated with motor disability. The authors argued that attention deficits rather than motor disability were driving the results since no specific associations to motor functions were noted for the smaller sets. Considering that patients showed significant

impairments on the simultaneous matching and visual search matching conditions (requiring attention abilities) but not on the delayed matching condition (mnemonic ability), the authors suggested that fronto-striatal dysfunction (contributing to the attention deficit) may be involved as opposed to temporal dysfunction (usually associated with mnemonic ability).

Christidi et al., (2012) investigated selective attention using the Stroop test (interference condition) in 22 non-demented ALS and 22 healthy controls. Selective attention refers to the ability to highlight important stimuli while suppressing awareness of competing distractors (Lezak, Howieson, Loring, & Fischer, 2004). The authors acknowledge that while Stroop is not an exclusive test of selective attention and has been primarily used as a test of EF for studying inhibition in the literature. ALS patients in Christidi et al., (2012) were found to perform significantly lower in the inhibition portion of the Stroop task, compared to controls (task performance was adjusted for motor impairments).

Massman et al., (1996) administered a battery of tests on 146 ALS patients of which Verbal Series Attention Test (VSAT) (Mahurin, Velligan, & Miller, 1998) was used to assess attention related processes. The VSAT includes a series of tests such as forward and reverse generation of arithmetic series, days of the week, months of the year, number – letter sequencing and auditory vigilance for a spoken target letter. Comparison of patient performance against the normative data revealed lower scores for patients, despite above-average IQ scores, thereby indicating likely attention deficits in ALS patients. The time-dependent nature of completing the VSAT was found to be associated with dysarthria; patients with dysarthria showed higher rates of impairment. However, since patients with dysarthria could successfully complete other time-based tasks in the battery of tests (e.g. fluency) the authors suggested that attentional deficits were not entirely due to motor

impairments and may be associated with underlying neuroanatomical substrates such as frontal lobe dysfunction.

Attention and working memory deficits were further confirmed by other studies including population-based samples (Phukan et al., 2012). These deficits were noted to be comparable between patients with ALS and bvFTD patients, while both groups performed worse than controls, thereby providing evidence for a continuum between ALS-FTD spectrum (Lillo, Savage, Mioshi, Kiernan, & Hodges, 2012).

2.2.1.3 Cognitive Flexibility, Set-Shifting and Inhibition

Cognitive flexibility is the ability to adjust one's behaviour according to the demands of the environment, enabling an individual to disengage from a previous task, reconfigure a new response and implement it to the task at hand (Dajani & Uddin, 2015). Typically, cognitive flexibility is assessed by switching between rules or mental sets such as letter-number sequencing or card sorting tests like the WCST which probe the ability to identify and/or follow different task rules. Inhibition refers to the ability to suppress automatic or prepotent responses (Miyake et al., 2000), and has been investigated in ALS using tests such as the Stroop test and Hayling Sentence Completion test.

The trail making test (TMT) includes two parts containing numbers or letter-number combinations distributed across letter-sized pages. The first part (also referred to as TMT-A) includes numbers from 1-26, while the second part (TMT-B) includes numbers 1-13 and letters A-L. Participants are typically required to trace a line between each of the numbers or number-letters in an ascending order (e.g. 1, 2, 3 etc., or 1-A, 2-B, 3-C, etc.). The time required to connect all numbers (TMT-A) or all numbers and letters (TMT-B) on the page

reflects the performance score in the TMT. Early studies noted that ALS patients showed significantly higher completion times for both TMT-A and TMT-B (Hartikainen, Helkala, Soininen, & Riekkinen, 1993). Difference in cognitive processing times as indicated by difference in completion time between TMT-B and TMT-A were also significantly higher, indicating particularly prolonged time to process letter-number switching, i.e. cognitive flexibility in ALS patients. Similar impairments in TMT were noted in larger groups of ALS patients more recently (Lomen-Hoerth et al., 2003; Witgert et al., 2010). However, in these studies, cognitive flexibility was not controlled for motor impairments on the task. More recent studies have adapted the TMT to include only the number-letter sequencing (TMT-B) and modified the score to reflect the number of errors as opposed to completion time, thereby effectively eliminating the motor dependency on the task, a correction that is also adapted by cognitive screening tests for ALS (Abrahams et al., 2014; Woolley, York, et al., 2010).

The Wisconsin Card Sorting Test (WCST) (Anderson, Damasio, Jones, & Tranel, 1991) requires participants to sort cards based on specific sorting rules or principles. Each card shows one of four types of symbols (e.g., diamonds, circles) in groups of one to four each, printed in one of four colours (red, green, yellow, blue), such that cards can be sorted by shape, number, or colour. These sorting rules are not explained to the participant and throughout the task rules switch without warning. Thus, participants must identify and then switch card sorting rules. This shift in mental set is thought to reflect cognitive flexibility. Studies have indicated either no significant differences (Lulé et al., 2005) or differences in the number of correctly identified sorting rules as compared to controls (Abrahams et al., 1997). Moreover, a meta-analysis indicated a medium effect size for differences between the

patient and control groups using the WCST (Beeldman et al., 2016). The WCST administration time is relatively long and thus may not be optimal for testing in clinic settings. Studies have attempted to investigate cognitive flexibility using other tests as described below.

Evans et al. (2015) investigated cognitive flexibility in 56 patients (47 ALS and 9 ALS-FTD) and 29 healthy controls using the Visual-Verbal test (VVT) (Feldman & Drasgow, 1951). This is a brief non-motor (untimed task in which participants identify features such as shape, size, orientation or fill lines that are similar among three of four geometric figures. Subsequently, in the second trial, participants are asked to identify a different feature shared by the same set of geometric designs. This is repeated for ten sets of geometric designs. Thus, the VVT requires that participants disengage from the first set of designs and identify a second set of shared features, requiring inhibitory control and set-shifting. ALS patients as a group performed significantly worse on the second trial, but not the first trial indicating impaired cognitive flexibility. Error in responses for the second trial indicating failed cognitive flexibility was noted for about 48% of patients. Each of the sub-groups of pure ALS patients and ALS-FTD patients also underperformed relative to the control group. Impaired performance on this task was associated with Mini Mental State Examination (MMSE), as well as other executive measures namely, verbal fluency and digit span backwards. No differences were noted based on site of onset (e.g. limb vs. bulbar patients). While the test displays simple use for clinical purposes, some ceiling effects on the maximum score (10 points) may over-estimate impairments.

The Stroop test (Comalli, Wapner, & Werner, 1962) measures inhibition of prepotent responses and includes three conditions. For example, in the Victoria version whereby the

participant is required to name the color of the items displayed for three conditions: (i) dots, (ii) color-congruent words (e.g. the word “Red” displayed in red font), and (iii) color-incongruent words (e.g. the word “Red” displayed in blue font). The final condition is also referred to as the Interference condition whereby higher number of errors and time to complete the task are typically recorded. Impairments in the Stroop test as indicated by longer time and higher errors for the incongruence condition have been reported in ALS patients (Hanagasi et al., 2002; Phukan et al., 2012), while one study indicated no such differences in the patient group (Raaphorst, de Visser, Linssen, de Haan, & Schmand, 2010a). Modified versions of the Stroop test have also been employed. For instance, Amato et al. (2013) assessed 32 non-demented ALS patients, 10 non-demented PLS patients, and 27 healthy subjects on a modified version of the Stroop test, whereby the participants provided non-verbal identification of congruence or incongruence of color-word by pressing one button for the former (choice C) and another button for the latter (choice I). The time to identify congruent and incongruent stimuli were recorded. In a separate condition, the time taken to detect the presence of stimulus, irrespective of stimulus type, was recorded as a control condition. Since a non-verbal test was employed, attention during the task was monitored after every 10-15 trials by asking participants to identify the type of stimulus (congruent or incongruent) in the previous trial. Group comparisons revealed more errors in ALS patients compared to PLS and controls. Notably, the reaction time in the ALS group was comparable to controls, while PLS patients showed fewer errors but longer reaction times. The combination of reduced reaction time and higher error rate in ALS patients was interpreted as dysfunction in conflict-monitoring processes.

Lillo, Savage, et al. (2012) compared inhibition in 23 ALS patients, 20 bvFTD patients and 20 controls using the Hayling's Sentence Completion test (Shallice & Burgess, 1996). The test includes two sections containing phrases in which the last word is to be completed by the participant. In the first section of the test, participants are required to provide a word that finished the sentence as quickly as possible (e.g. "*He posted the letter without a…**stamp***"). In the second section of the test, participants are required to provide an unrelated word that is not associated with anything that could reasonably complete the sentence (e.g. "*The captain wanted to stay with the sinking…**apple***"). Thus, the second part of the test requires inhibition of an automatic, semantically coherent response, a response pattern that is established in the first half of the test. Both ALS and bvFTD patient groups had higher number of errors compared to the control group in Lillo, Savage, et al. (2012), with most severe deficits observed in the bvFTD group.

Studies have also employed eye-movement tasks to study frontal lobe contributions to inhibition in ALS, specifically using the anti-sacchades eye-movement. Saccades are rapid eye movements that bring an object to the fovea of the eye for clearest vision (Donaghy, Thurtell, Pioro, Gibson, & Leigh, 2011). Studies have described abnormalities in reflexive and memory-guided saccades in ALS (Donaghy et al., 2010; Leveille, Kiernan, Goodwin, & Antel, 1982; Ohki et al., 1994; Shaunak et al., 1995). Slower saccades were noted, especially in bulbar-onset ALS patients (Donaghy et al., 2010). Of interest are anti-sacchades in ALS. Anti-saccades involve volitional movement of the eye away from a peripheral stimulus and are typically associated with frontal eye-fields and the dlPFC, although the functional involvement of these regions may differ slightly. Activity in the right dlPFC was found to be higher during anti-saccade task, a region involved with working memory (Ford, Goltz,

Brown, & Everling, 2005). Errors in anti-saccades were reported to be higher in ALS, with patients showing higher responses of looking towards the target instead of away from the target. Anti-saccadic errors were also found to be associated with the Stroop test in 44 ALS patients (Donaghy et al., 2010). Thus, frontal lobe (especially dlPFC) involvement in ALS may influence anti-saccades.

2.2.1.4 Planning and Decision Making

Planning and decision making are higher order EF processes that rely on lower level component processes such as updating, set-shifting and inhibition to enable goal-oriented behaviours (Lezak et al., 2004). Abrahams et al. (1997) assessed planning using a computerised version of Tower of Hanoi test (Morris et al., 1988) in 52 ALS and 28 healthy controls. The task involves representation of three discs of varying sizes slotted into three rods. In Abrahams et al. (1997), a target (goal) arrangement and a response arrangement were displayed on a touch sensitive screen and participants were required to rearrange the discs in the response arrangement to match the target arrangement in either 4, 5 or 6 moves. Participants were to complete the arrangement in as few moves as possible, moving one disc at a time and only placing smaller discs on top of the larger discs. To control for motor impairments, planning time was used as the time taken between the presentation of the target arrangement and the initiation of the first move. Group comparisons revealed that ALS patients with pseudobulbar affect displayed much shorter planning times on complex trials (involving placement of disc away from the target to complete minimum number of moves) with a tendency to fail to solve trials as effectively compared to ALS patients without pseudobulbar affect and the control group.

In the same study, Abrahams et al. (1997) used the random movement joystick task (Deiber et al., 1991) to assess decision making. The task involved two conditions wherein participants had to make movements with a joystick in either of four directions. The first condition involved random generation of movements when cued with an auditory stimulus; participants were instructed to not repeat movements in any specific pattern. In the second condition, participants were required to make a movement in a fixed direction based on instructions displayed on a computer screen. Fifty trials were administered and the difference in time taken to make random movements compared to fixed movements was used as an assessment of decision time. Group comparisons indicated a trend whereby ALS patients generated less random movements compared to controls, even though the decision times between the groups were comparable. Abrahams et al., (1997) suggested that the generation of random movements are similar to verbal fluency and rely on intrinsic processes, which have been associated with bilateral activation of the dorsolateral prefrontal cortex (dlPFC) (Deiber et al., 1991). Thus, the slight impairment noted in making random joystick movements could be attributed to frontal lobe dysfunctions, specifically the dlPFC.

Lillo, Savage, et al. (2012) (23 ALS patients, 20 bvFTD patients and 20 controls) used the Iowa Gambling task (IGT) (Bechara, Damasio, Damasio, & Anderson, 1994) to compare risk-based decision making across groups. The IGT is thought to be an ecologically valid test, to assess real-life risk-based decision-making abilities, using reward, punishment, and decision outcome uncertainty. Briefly, in the IGT four decks of cards (A, B, C and D) are presented to participants, whereby the primary goal is to win fictitious money by drawing a single card from these card decks over 100 trials. Each draw will lead to a win or a loss of game money. Undisclosed to the participant, decks A and B (high risk) will lead to large

immediate wins, but even larger losses over the course of the task, while decks C and D (low risk) win less money but result in a positive net-balance over time. These contingencies are learnt solely through feedback (win, loss) after each card draw. Intact IGT performance is indicated by learning to prefer decks C and D over decks A and B as the task goes on. Patients with focal ventromedial frontal lesions fail to learn these associations and continue with more risky moves using decks A and B (Bechara et al., 1994). In Lillo, Savage, et al. (2012), bvFTD patients' IGT performance was significantly more risky in trials 60 to 100 compared to performance of controls, and only in trial 100 compared to ALS patients. ALS patients and controls showed no significant difference on the IGT. In another study, significantly risky IGT performance was reported in a group of 19 non-demented ALS patients (trials 60-100) compared to 20 healthy controls (Girardi, Macpherson, & Abrahams, 2011). This suggests higher severity of decision making impairments in bvFTD patients, though some decision-making deficits may also be present in ALS patients possibly associated with ventromedial frontal dysfunctions.

Meier et al. (2010) employed other ecologically valid tests to measure planning and decision-making abilities in 18 ALS patients and 18 healthy controls. The One-touch Stockings of Cambridge was used to assess spatial planning. The task is a computerised adaptation of Tower of London task, which is a variant of the Tower of Hanoi (Shallice, 1982). In the One-touch Stockings of Cambridge, two sets of three colored balls, held in stockings are presented, one on the top and another on the bottom of the screen. Participants are required to rearrange the balls in the bottom set to match the pattern in the top set, after initial demonstration by the experimenter. To control for motor impairments, in the actual task, participants are required to calculate the minimum number of moves (ranging from 1-6) to

achieve the pattern instead of manually rearranging the balls. More attempts at reaching the correct pattern were noted in ALS patients as compared to healthy controls in Meier et al. (2010). There were no interaction effects of group or task difficulty level (1-3 versus 4-6 moves) between patients and controls.

Meier et al. (2010) also included a Holiday Apartment task adapted from (Fellows, 2006) and was used to assess decision making abilities in ALS patients. This task focuses on application of mental heuristics and strategy to reach a decision in the context of known and limited options of availability and information. Participants are instructed to imagine that they were going on a 4-week holiday and that they should choose an apartment (catered for physical disability) that would suit them best and are encouraged to examine as much as information as possible before making their decision. Decisions are to be made between 2, 4 or 6 apartments with 4, 6 or 7 attributes, respectively, that are presented as a grid (e.g., 2 x 4 grid for a decision trial including two apartments with four attributes). The pattern of search across the grid measures indicated pattern of information acquisition, and search strategy was classified as attribute based (horizontal search looking at attributes across apartments) or apartment based (vertical search looking at an apartment for almost all attributes). There were no differences between patients and controls for amount of information viewed or time taken to reach a decision. However, ALS patients differed significantly in their search index for complex trials (6 x 7 grid). ALS patients favoured choosing an apartment more often that the attributes. This finding remained significant even when performance in verbal fluency was used as a covariate, suggesting that decision making was not EF dependent. The authors suggest that this aspect of apartment-based search is similar to the reports of patients with ventromedial prefrontal cortex (vmPFC) damage such as in (Fellows, 2006).

2.2.2 Social Cognition

Considering the ALS-FTLD continuum and the higher prevalence of bvFTD in ALS, the domain of social cognition has recently gained more attention, with meta-analyses indicating moderate-high effect size of social cognition deficits in ALS patients compared to healthy controls (Beeldman et al., 2016; Bora, 2017). Social cognition is an umbrella term that encompasses several important processes involved in understanding of and responding in social situations. It includes abilities such as identifying emotions, understanding thoughts or feelings of oneself and others (theory of mind, ToM), and perceiving and responding to complex processes such as humor and sarcasm (Frith & Frith, 2012). The Diagnostic and Statistics Manual for Mental Disorders - fifth edition (DSM-5) introduced social cognition as one of the six categories important for normal neurocognitive functioning (Henry, von Hippel, Molenberghs, Lee, & Sachdev, 2016). ALS patients are reported to have poor emotion perception, particularly in identifying emotions from facial expressions and identifying mental states (ToM).

2.2.2.1 Emotion Perception and Processing

Emotions refer to both a state of feeling accompanied by physical and physiological changes such as changes in facial expression, changes in tone / prosody and body language (relaxed / tense posture), increase or decrease in heart rate, skin response, etc. The ability to recognise these cues and identify the corresponding emotion enables effective communication and navigation of the social world. Impairments have been noted in the ability to attribute the degree of affect to happy or fearful faces (Lulé et al., 2005; Schmolck, Mosnik, & Schulz,

2007); with ALS patients rating faces as happier, more exciting and calmer (Lulé et al., 2005) or less fearful as compared to healthy controls (Schmolck et al., 2007).

Lulé et al., (2005) employed the International Affective Picture System (IAPS) (Lang, Bradley, & Cuthbert, 2008) to investigate emotion perception in 12 ALS patients and 18 healthy controls. The study presented pictures of social situations representing emotional valence, arousal and association of movement (i.e., pictures with potentially movable content) selected from the IAPS. ALS patients indicated increased ratings of arousal such that calm pictures were rated as more positive. This was associated with physiological differences such as greater deceleration of heart rate and increased galvanic skin response in patients. The authors interpreted these findings as potentially indicating increased attention to the pictures and/or greater emotional involvement, perhaps due to neurological changes in emotion perception (e.g. in limbic, fusiform areas of the brain). The positive valence reported in positive ratings and physiological changes were considered to be related to coping mechanisms in which patients may show a more positive outlook in the face of a terminal condition such as ALS. A follow-up study, investigated blood oxygen level dependent (BOLD) signal responses in an fMRI (fMRI) study using pictures from the IAPS (Lulé et al., 2007). ALS patients displayed an increased activation of the BOLD signal for emotional stimuli (irrespective of parameters such as valence, arousal and movement) in the right supramarginal areas and inferior frontal operculum, bilateral parietal lobes and the cerebellum compared to controls. Ten patients returned for another visit (6-months after baseline) and displayed higher BOLD response in the right supramarginal area at the follow-up visit. Longitudinal follow-up also indicated higher activation in the insula, a part of the limbic system associated with arousal. Both studies by Lulé et al., together indicate

alterations in emotion processing which is reflected in autonomic, physiological and fMRI activity. More recent study suggested that such enhanced BOLD activity may also represent reduced inhibitory circuits (Douaud, Filippini, Knight, Talbot, & Turner, 2011). In the studies by Lule et al., there were no alterations indicated in the amygdala, a region associated with emotional stimuli.

Another study (Schmolck et al., 2007) investigated ratings of approachability of emotion faces in 26 ALS patients and 26 healthy controls using Ekman's Faces (Ekman & Friesen, 1976; Schmolck et al., 2007). The test included photos of six basic emotions and participants were required to rate which faces were more approachable in social situations. ALS patients rated faces with negative emotions such as anger and disgust as more approachable suggesting impaired judgements of emotional stimuli, similar to patients with bilateral amygdala lesions (Adolphs, Baron-Cohen, & Tranel, 2002; Adolphs, Tranel, & Damasio, 1998).

Using the Ekman's faces and other tasks for prosody, studies have identified impaired recognition of sad and surprise faces as well as poor recognition of prosody indicating surprise (Zimmerman, Eslinger, Simmons, & Barrett, 2007). A recent study (Andrews, Staios, Howe, Reardon, & Fisher, 2017) indicated multimodal emotion perception deficits using the Comprehensive Affect Testing System (CATS) (Schaffer, Wisniewski, Dahdah, & Froming, 2009). The CATS includes 5 sub-tests assessing simple facial affect recognition (based on Ekman's Faces), complex facial affect recognition (matching emotional expression of two faces, or selecting facial expression to an affect label), affective prosody recognition (identifying emotion expressed via prosody when meaning of the sentence is either congruent, neutral or incongruent with emotion displayed), lexical comprehension

(identifying the meaning represented via the wording of the sentence) and cross-modal emotion recognition (matching emotional prosody to a facial expression or vice-versa). In the study by Andrews et al, (2017), ALS patients (n=33) displayed impairments in complex facial affect recognition, affective prosody recognition and cross-modal emotion recognition. These findings indicate that emotion recognition impairments are present in non-demented ALS patients for more complex processes such as discriminating between emotions or matching emotions across different faces, and recognising emotions expressed via voice prosody and facial expressions. Another study (Savage et al., 2014) noted that emotion recognition was comparable between ALS patients and healthy controls but was worse in patients with ALS-FTD as compared to patients with bvFTD, ALS or healthy controls using The Awareness of Social Inference Test (McDonald et al., 2006). Finally, a recent study (Aho-Ozhan et al., 2016; Crespi et al., 2014) indicated associations between impaired activations in the inferior frontal gyrus during emotion recognition task, which was associated with a reduced number of patients' social contacts in real life, suggesting that neurological changes may translate to important implications in social activities.

2.2.2.2 Theory of Mind (ToM)

ToM is the ability to attribute mental states such as thoughts (cognitive, C-ToM) or emotions (affective, A-ToM). It includes abilities to recognise emotions and infer possible thoughts or mental states of another individual that may not be explicitly expressed in a given social situation. While some studies in ALS reported no specific ToM deficits in ALS (Gibbons et al., 2007), more recent studies have identified impaired ToM including both C-ToM and A-ToM difficulties (Burke, Elamin, et al., 2016; Burke, Pinto-Grau, et al., 2016; Cavallo et al., 2011; Girardi et al., 2011; Meier et al., 2010; van der Hulst, Bak, & Abrahams, 2014).

Impairments have also been noted for recognition of sarcasm and humor (Staios et al., 2013), both of which include complex processes that require an awareness of both latent and actual content of a social scenario and the subsequent inferences made in context (McDonald, 1999).

C-ToM is most often assessed by false belief tasks (Wimmer & Perner, 1983), whereby participants are shown cartoon depictions of situations in which a character in the picture observed placement of an object in location X. In the absence of the character, the object is moved to location Y. The participant is then asked to infer where the character in the picture thinks the object is located, which would be X since they did not witness relocation of the object to Y (first-order belief). In situations increasing with complexity, the character may secretly witness the relocation of the object and when the participant is asked to respond, the correct location would be Y (second-order belief). These tasks have been adapted to include humor and participants were required to accurately identify humor in social situations (Gibbons et al., 2007; Watermeyer et al., 2015b) or imitate social scenarios (Carluer et al., 2014). While one study reported no significant differences between ALS patients and controls on such tasks (Gibbons et al., 2007), another reported significant impairments in higher order beliefs when compared to controls (Carluer et al., 2014). Studies have also employed tests such as the Emotion Evaluation and Test of Social Inference sub-tests from TASIT (McDonald et al., 2006) to detect humor and sarcasm recognition deficits in 30 non-demented ALS patients (Staios et al., 2013b). In this test, participants are shown video vignettes depicting positive or negative emotions (The Emotion Evaluation sub-test) and video vignettes of actors making sincere, sarcastic or paradoxically sarcastic (seemingly true, yet contradictory) statements. In the study by Staios et al., (2013b), ALS patients performed

comparable to controls in recognizing emotions and sincere statements, while significant impairments in performance were reported for sarcastic and paradoxically sarcastic statements, indicating C-ToM deficits.

Studies investigating A-ToM in ALS have employed tests such as Reading the Mind in the Eyes (RME) test (Baron-Cohen, Wheelwright, Hill, Raste, & Plumb, 2001). In the RME, participants are required to infer people's cognitive-emotional states by viewing black and white pictures of men or women cropped to show only the region of the eyes. Studies have indicated no significant differences (Girardi et al., 2011; Jelsone-Swain, Persad, Burkard, & Welsh, 2015; Watermeyer et al., 2015b) or significantly impaired performance in ALS patients as compared to controls (Burke, Elamin, et al., 2016; Burke, Pinto-Grau, et al., 2016; Trojsi, Siciliano, et al., 2016). One study also indicated that the RME test had good sensitivity and specificity in distinguishing ALS patients and controls (Burke, Pinto-Grau, et al., 2016) and revealed poorer performance in patients with bulbar onset as compared to those with limb onset (Burke, Elamin, et al., 2016). Meier et al. (2010) employed the Faux pas test, specifically the ability to detect the presence of faux pas (social mistake) in a short story. The Faux pas test (Stone, Baron-Cohen, & Knight, 1998) includes short stories that either contain a social mistake (faux pas) or no mistake. Each story is followed by a set of questions in which participants identify the presence (or absence) of faux pas, and if faux pas is present, participants are queried further on the nature of the faux pas such as the inappropriateness of the situation, intention of characters in the vignette and are required to make an inference on the feelings of the victim in the story (empathy). In the study by Meier et al. (2010), impairments in faux pas detection were reported in ALS patients (n=18) compared to controls (n=18). These faux pas impairments persisted when co-varied for EF,

suggesting that frontal regions such as the medial orbitofrontal cortex (mOFC) lesions may be involved (Stone et al., 1998).

A-ToM was also investigated the Judgement of Preference (JP) test, in which participants were shown a smiley face and four objects, one in each corner of a box (Girardi et al., 2011; van der Hulst et al., 2014). The eyes of the smiley face are directed towards one of the objects and participants are required to identify which object the face may like the best, or which object the face is looking at. Distractors such as arrows pointing to incorrect or correct objects are also employed. As a control condition, prior to testing, participants would select objects that they liked the best. In Girardi et al. (2011), group comparisons revealed that patients performed worse in the "like" condition as opposed to the "looking at condition", with patients selecting objects they liked (egocentric errors). This response pattern is similar to those with bvFTD supporting the frontotemporal involvement in ToM impairments (Girardi et al., 2011).

Van der Hulst et al., employed an additional condition on the JP test in which participants were instructed to select the picture that the face was "thinking-of" to assess C-ToM (van der Hulst et al., 2014). Impairments were noted in this condition as well, thus suggesting both A-ToM and C-ToM impairments. However, another study reported no significant differences between ALS patients and controls on the JP test (Burke, Elamin, et al., 2016). Only one other study employed tests to assess both C-ToM and A-ToM (Trojsi, Siciliano, et al., 2016) including one C-ToM measure (Advanced Test of ToM, ATT) and two A-ToM measures (Emotion Attribution task, EAT and RME test). The ATT included 13 written stories of social scenarios in which two or more characters interacted with each other. These stories were read to participants and the participants were asked to explain why certain

characters in the story behaved in a certain way, thus tapping into cognitive aspects. In the EAT, participants were read 35 stories describing emotional situations designed to elicit attributions of sadness, fear, embarrassment, disgust, happiness, anger, or envy (5 stories per emotion). The participants were asked to respond how the main character in the story may be feeling. Trojsi, Siciliano, et al., (2016) identified impairments in ATT and RME test in 22 ALS patients compared to 15 controls, and these impairments were also associated with poor mental health, a domain in Short Form-36 (Apolone & Mosconi, 1998), a quality of life measure. This suggests that ToM impairment may negatively affect the mental health aspect of a patients' quality of life.

2.2.2.3 Contributions of EF to Social Cognition

Of interest to the current thesis is the relationship between the presence of social cognition and EF in ALS patients. One recent study indicated that patients with EF dysfunctions performed lower than controls on emotion recognition tasks and complex integration of emotions with prosody (Andrews et al., 2017). Recent studies have suggested that EF contribute to 44% of the variance in social cognition in ALS patients (Watermeyer et al., 2015b) and ALS patients with impaired EF perform worse on social cognition tasks compared to patients with intact EF (Burke, Pinto-Grau, et al., 2016).But it should also be noted that one previous study suggested no significant relationships (Gibbons et al., 2007), and another study reported only a trend towards associations (Girardi et al., 2011) between EF and social cognition in ALS. These latter studies (Gibbons et al., 2007) enrolled smaller groups of participants (n<20)

2.2.3 Language

Language is essential for effective understanding and communication of thoughts, emotions and ideas. Production, comprehension and communication of both spoken and written words involves several aspects of language such as semantics, syntax and grammar. Studies in ALS have emerged indicating deficits in language, with recent meta-analyses suggesting a medium-effect size for language impairments in ALS (Beeldman et al., 2015).

Semantics refers to the ability to identify and name objects. It relies on complex processes requiring the use of a mental lexicon to enable representation of word meaning. Semantics include additional information such as its sound, written form, the roles in a sentence such as a noun, verb, etc. Naming tests such as Boston Naming Test (BNT) and Graded Naming tests (GNT) have been used to test semantic knowledge in ALS patients (Goldstein & Abrahams, 2013). Individual studies have revealed mixed results indicating either a decreased performance on naming tests in ALS (Hanagasi et al., 2002; Taylor et al., 2013) or no significant differences (Abe et al., 1997; Tsermentseli et al., 2016), which could be attributed to differences in sample size.

Syntax refers to the set of rules specifying the combinations of words to form a sentence. Additionally, the set of rules that govern the structure and system of a language including syntax, morphology and phonology of words is referred to as grammar. Studies have suggested changes in syntax and grammar in ALS patients. Yoshizawa et al. (2014) reported impaired syntax comprehension in 72% of ALS patients (n=25). Of these, at least 50% of patients were reported to have normal EF and visuospatial functions, indicating that language may be a separable domain of cognitive impairment in ALS. The authors obtained SPECT images for two patients (one failed three cognitive tests and the second patient was cognitively intact). Analysis of SPECT images of the patient who failed language, EF and

visuospatial tests, revealed moderately lower regional cerebral blood flow (r-CBF) in the frontal lobes.

Tsermentseli et al. (2016) investigated language function at the level of word, sentence and discourse processing in 26 ALS and 26 healthy controls. Language assessments included tests for confrontation naming, semantic access, and single word and syntactic comprehension. The groups in this study did not differ in EF performance, memory or visuospatial functions. The authors reported impairments in language specifically for connected speech measures indicated by a reduced mean number of words, mean duration of narrative, a higher number of distortions (articulatory speech errors without phonemic substitutions) and a greater number of incomplete sentences, all of which were present in the absence of bulbar-involvement and EF impairments. Statistical models including measures of mean length, incomplete sentences and syntax / single-word comprehension revealed above-average sensitivity (81.8%) and specificity (85%) for accurate classification of ALS patients and controls, suggesting that these measures of syntax are potential tools for identifying language impairment in ALS. However, the major limitation of the study was its highly select cohort of ALS patients with minimal dysarthria, mild motor impairment, mean symptom duration of 10 months and 15% bulbar onset. This limits generalisability of the findings to a larger ALS population displaying varying levels of dysarthria, motor disability and cognitive profiles.

Deficits in the above domains of language may translate into impairments in comprehension and may limit effective communication. In fact, studies have indicated impaired action verb comprehension in ALS patients. York et al. (2014) compared performance for action verbs, cognition verbs, concrete nouns and abstract nouns of 36 ALS, 22 Parkinson's disease (PD)

patients and 31 healthy controls. ALS patients were found to perform worse on action verbs as compared to controls. Comparisons within each group revealed equal comprehension of action and concrete words for ALS patients, while healthy controls performed better on action verbs. PD patients were found to perform poorly on concrete words. Furthermore, neuroimaging analysis indicated that poor action verb performance in ALS was associated with atrophy in the left precentral gyrus (motor cortex), left premotor regions and right prefrontal regions, while no such associations were noted for PD patients. This suggested that atrophy of the motor system may be associated with a loss of representation of action knowledge.

Pathological findings have implicated atrophy in Broadman areas 44 and 45 to be associated with language impairments such as verb processing in MND patients (with and without dementia) (Bak, O'Donovan, Xuereb, Boniface, & Hodges, 2001). Jelsone-Swain et al. (2015) investigated action understanding in a group of 19 ALS patients and noted that deficient performance was associated with functional changes in the motor-regions of ALS patients. Bongioanni, Buoiano, and Magoni (2002) presented a brief review highlighting language and syntax errors in patients with MND and MND with FTD. They proposed that degeneration of premotor and motor cortices may influence action understanding that may snowball into errors in action verbs and syntax. Roberts-South, Findlater, Strong, and Orange (2012) analysed discourse (i.e., communication patterns) in a group of 16 non-demented ALS patients and 12 healthy controls. It was noted that there were no differences between the groups at baseline, however, longitudinally patients displayed greater impairments in communicating content, while production of language revealed milder deficits.

Overall, language deficits in ALS reflect impairments in both language production and comprehension. While some studies have indicated that language deficits may be present in the absence of EF deficits (Yoshizawa et al., 2014), others have indicated that language problems may be secondary to EF deficits in ALS (Taylor et al., 2013). Taylor et al., extensively investigated language impairments in a group of 51 non-demented ALS patients and 35 healthy controls matched for age, gender, education and IQ. One of the aims of the study was to investigate if language impairments in ALS presented an aphasia-like pattern (such as impaired language production and comprehension) or they were secondary to EF impairments (impaired access to mental lexicon displayed as impairments in language). In addition to impairments on EF tests such as verbal fluency (letter) and Brixton spatial anticipation test, ALS patients displayed significantly deficient performance for semantic language, grammar, spelling and verb processing. It was reported that 44% of the variance in language functions was contributed by EF suggesting a link between language and EF impairments. The study also noted a higher prevalence of language deficits, on a composite language score (mean of standardised scores for all language tests) in 43% of patients compared to previous studies. Applying Strong et al's., (2009) criteria for staging impairment (performance <2SD below control means on at least two tests of language functions), the authors reported language impairments in 39% of the patients. EF impairments were noted only in 31% of patients using composite scores while 25% were impaired on the Strong criteria. The caveat of this study is the use of greater number of language tests as compared to EF tests and may have increased sensitivity to language impairments. This study by Taylor et al. (2013) suggests that while EF and language may be

linked in ALS, a large proportion of variance contributing to language dysfunctions remains unexplained.

2.2.4 Learning and Memory

Learning and memory domains were historically considered to be relatively spared in ALS. However, studies have provided some evidence of impairment. Massman et al. (1996) performed a population-based study (n=146 ALS) investigating various aspects of cognition, in which about 20% of patients performed below the 5th percentile on the California Verbal Learning Test (CVLT) for immediate recall. Among the impaired patients, performance on delayed recognition was intact suggesting that patients did not have amnesic difficulties but may have retrieval issues associated with inefficient learning strategies, often associated with frontal involvement. Abrahams et al. (1997) reported differences in recognition of words in patients as compared to controls on the Recognition Memory Test (Warrington, 1984). Some studies indicated that there were no differences in performance between patients and controls on the Auditory Verbal Learning Test (AVLT) (Abe et al., 1997; Ludolph et al., 1992). The use of different memory tests could contribute to differences in findings reported by the studies.

Consonni et al. (2017) investigated learning and memory processes in 26 ALS patients, of which 13 were cognitively impaired (ALSci), along with 48 controls, 18 patients with AD and 15 bvFTD patients. The Rey Auditory Verbal Learning Test (RAVLT) was used to assess learning and memory. The RAVLT involves a 15-item list of unrelated words that are recalled immediately over five learning trials, followed by a delayed recall and recognition task. In word-list learning tasks like the RAVLT, recall is easier for words from the

beginning of the list (primacy effect) or from the end of the list (recency effect), while words from the middle of the list are recalled less frequently (Murdock Jr, 1964). With increasing learning trials, recall frequency of mid-list words also increases. In the study by Consonni et al. (2017), ALSci patients were unable to recall mid-list items immediately after presentation even with repeated learning trials, while primacy and recency effects were preserved. On the other hand, pure ALS patients with intact cognition displayed similar learning as controls, with increasing recall of mid-list items with repeated learning. A strong association (r=0.9) was noted in ALSci patients between mid-list recall and verbal fluency measures (corrected for motor impairments). As mentioned previously, verbal fluency deficits in ALS have been attributed to deficient central executive (Abrahams et al., 2000), and thereby suggesting that impaired mid-list effect in ALSci may be a consequence of deficient central executive.

A previous study attempted to explore the influence of selective attention on learning and memory processes (Christidi et al., 2012). A group of 22 ALS patients and 22 controls were compared on the Stroop test and the RAVLT. Performance on the RAVLT was analysed using the 'item specific deficit approach' to differentiate encoding, consolidation and retrieval processes. Selective attention (Stroop performance) was found to moderate encoding and consolidation, but not retrieval processes.

Abdulla et al. (2014) investigated the brain structures associated with memory impairments in 58 ALS patients and 29 age-, education- and gender-matched healthy controls. Patients were impaired on global cognitive functioning (MoCA), EF (phonemic fluency, digit span and cognitive flexibility) and memory (RAVLT). Hippocampal volume was found to be significantly lower in ALS patients in the right hemisphere than in controls. These differences in volume did not reach significance when four patients meeting criteria for

bvFTD were excluded. Performance on memory tasks especially for immediate free recall, delayed free recall and recognition memory were associated with left hippocampal volume, while only recognition memory was associated with right hippocampal volume in ALS patients. There were no associations between hippocampal volumes and other tasks such as EF or visuospatial abilities, suggesting that the associations were specific to the memory domain. Immediate recall was associated with total grey matter volume, while delayed recall was significantly related to hippocampal volume. The broader correlation patterns for immediate recall were interpreted as potentially reflecting additional cognitive processes at immediate recall (e.g. a certain level of attention is required to perform the initial learning) which may require involvement of cortical regions, while delayed recall requires long-term retrieval from medial temporal / hippocampal memory stores.

Machts et al. (2014) investigated if memory changes in ALS were secondary to EF impairments. Participants included 40 ALS, 39 amnestic mild cognitive impairment (aMCI) patients and 40 healthy controls. Using a similar testing structure as Abdulla et al. (2014), the authors noted distinct memory profiles for the patient groups. The ALS group underperformed in recognition memory, while aMCI group showed impaired performance in immediate and delayed recall, compared to controls. Regression analysis revealed that EF contributed to 20% variance in memory performance in ALS patients, while no such relationships were noted for aMCI or healthy controls. This suggested that EF alone does not explain the recognition memory deficits noted in ALS and may be associated with other processes such as insufficient encoding (temporal lobe dysfunction) or from deficient prefrontal cortical function.

In contrast to the findings by Machts et al. (2014), a previous study by Hanagasi et al. (2002) reported immediate recall deficits and intact recognition memory performance on the CVLT in a cohort of 20 non-demented ALS patients and 13 age- and education-matched healthy controls. This suggests issues with retrieval of information which relies on frontal lobe processes, rather than storage, which relies on limbic system. Differences in sample size and the test employed could play a role in the differential results noted in immediate recall vs. recognition memory.

A slightly different approach was taken by another study whereby the authors investigated visual memory in a group of 203 ALS patients and 117 healthy controls using the Rey Osterrieth Complex Figure Test (ROCFT) (Burke et al., 2017). In this test participants are required to copy a complex line-based figure, followed by immediate and delayed recall trials assessing visuo-perceptual, visuo-constructional and visual memory functions, and to a lesser extent EF such as planning and organisation. Burke et al. (2017) also tested EF using separate tests. Patients were categorised based on the presence or absence of EF impairments and a smaller sub-group met criteria for FTD (n=30). It was noted that more severe EF impairment contributed to lower performance on the ROCFT, with most severe impairments (in ALS-FTD) associated with impairment in all ROCFT measures (copy, immediate and delayed recall), while milder EF impairments was associated only with immediate and delayed recall performance.

Thus, while some studies in ALS have reported impaired learning and immediate recall, others report no such differences and instead have indicated impairments in delayed recall or recognition memory. Differences in reports could be attributed to differing sample sizes and memory tests. EF appears to be associated with learning processes such as encoding,

retrieval and memory, although only few studies have explored this link between EF and memory in ALS.

2.2.5 Visuospatial Functions

The impairment of this domain is relatively uncommon in ALS and if deficits are present, it may indicate possible co-morbid conditions. Yet, some authors argue that visuospatial impairments may reflect fronto-striatal changes in ALS. Hanagasi et al. (2002) reported impairments in visuo-perceptual processes using Benton's Judgement of Line Orientation (JLO) test and visuo-constructive processes using the Block Design test. Patients showed intact performance in the Benton's face perception test. While the Block design test relies on visuo-motor integration, it also requires planning, strategy and behavioural persistence (i.e., executive or frontal lobe functions), and the JLO relies on visual processing, relying on pathways responsible for spatial analysis. These pathways from the visual cortices terminate in frontal eye field located in the dorsolateral prefrontal cortices and impairments on the JLO may involve some frontal lobe involvement. On the other hand, face perception relies on ventral stream pathways that terminate in the temporal lobes. The authors argue that their observed dissociation in ALS patients' performance on visual tasks could be attributed to differential visuospatial processing networks, with primarily frontal lobe dysfunction driving impaired visuospatial processing.

Thus, overall, cognitive impairments in ALS have been reported primarily for EF, while recent studies are revealing impairments for social cognition, language and memory processes. While some studies have reported links between EF and social cognition / language / memory, others have not reported such associations. Of particular interest to the

current thesis is the relationship between EF and social cognition in ALS patients and has been elaborated further in Section 2.8.

2.3 Behavioural Impairment in ALS

As mentioned in Chapter 1, behavioural changes in ALS-FTLD spectrum range from mild to severe and include changes such as apathy, disinhibition, poor empathy, loss of sympathy, stereotypic / perseverative behaviours and changes in dietary habits (Phukan et al., 2012). Investigation of behavioural changes in ALS have been influenced by the FTLD criteria, predominantly for bvFTD (Rascovsky et al., 2011) (see Table 1.3 and section 1.4.2.2). One of the striking features reported in the ALS-FTLD spectrum is the loss of insight to behavioural changes in ALS patients with co-morbid FTD (Woolley, York, et al., 2010). This can be determined using Frontal Systems Behavioural Screen (FrSBe) (Grace & Malloy, 2000) which includes 46 items rated on 5-point Likert scales assessing premorbid and current behavioural characteristics. The FrSBe is not specific to ALS patients and includes some items that could confound with motor impairment (e.g. '(I) am slow moving, lack energy, inactive'). Nevertheless, it assesses behavioural domains relevant to frontal lobe dysfunction and has been used in ALS patients. The items on the FrSBe are grouped into apathy, disinhibition and executive dysfunction. The FrSBe includes a self-rating form which is completed by the participant and a family-rating form which is completed by the caregivers. Woolley, York, et al. (2010) reported that ALS-only patients appeared to rate their behavioural changes more severely compared to caregiver responses, while caregivers of ALS-FTD patients reported significantly more severe behavioural changes than the patients themselves. This suggests that ALS-only patients may be more critical about their behavioural changes, which may indicate either coping style or underlying depressive

symptoms that may reflect as behavioural alterations. Conversely, ALS-FTD patients appear to show a loss of insight to their degree of behavioural changes, whereby caregiver responses may be more accurate in such cases.

Understanding behavioural impairments remains crucial in clinic settings as changes in behaviour are considered to increase caregiver burden in ALS (Lillo, Mioshi, & Hodges, 2012; Watermeyer et al., 2015a). Considering that apathy is the most commonly reported behavioural change, it is discussed in further detail below and outlined in Supplementary Table 2.2. Other behavioural changes are outlined in the subsequent section.

2.3.1 Apathy

Apathy is characterised by diminished interest, enthusiasm or concern and is one of the hallmark features of behavioural changes in ALS. Caregivers often report a decreased initiation of conversations, reduced motivation to part-take in activities and decreased agreeableness with reduced frustration tolerance (Grossman, Woolley-Levine, Bradley, & Miller, 2007). In a study of 55 ALS patients, premorbid apathy was identified in 11% of patients while the rates increased to 55.6% after the onset of illness on the FrSBe apathy sub-scale (Grossman et al., 2007). The authors noted that apathy predicted verbal fluency performance in ALS patients and may be associated with frontal atrophy. This is supported in a large-scale study employing the FrSBe scales in 225 ALS patients reported apathy in approximately 31% of their patients (Witgert et al., 2010). While the study noted associations between EF and FrSBe apathy scores, about 16% ALS patients with normal cognition showed behavioural impairments. The authors also report that about 67% of the patients with moderate to severe cognitive dysfunction showed no behavioural impairments, indicating

that behavioural changes may occur independent of cognitive changes. In contrast to these findings, another study (Gibbons, Richardson, Neary, & Snowden, 2008), with 16 ALS patients reported lower frequency of apathy. Gibbons et al., (2008) reported that about 38% (n=5) displayed blunting of primary emotions such as happiness, sadness, fear and anger. Apathy was assessed here with the Manchester FTD behavioural interview (Bathgate, Snowden, Varma, Blackshaw, & Neary, 2001), an interview-based questionnaire that extensively investigates behavioural changes in association with FTD. Apathy reported in this study was lower as compared to the previous study and maybe associated with either small sample size or differences in the behavioural test employed. For instance, FrSBe scales incorporate motor-based items such as low energy to perform tasks which may confound physical disability with motivation.

Recent studies using larger sample sizes have moved towards developing ALS-specific behavioural tools. One study indicated apathy in 28.2% of the sample (n=131 ALS) using the Dimensional Apathy Scale (DAS) (Santangelo et al., 2017). The DAS enables assessment specific aspects of apathy such as impaired planning or attention (Executive subscale), emotional integration (Emotional subscale) and self-generation of behaviours or cognition (Behaviour/Cognitive Initiation subscale). It was previously noted that the Behaviour/Cognitive Initiation subscale was characteristically impaired in ALS (Radakovic et al., 2016), and was also found to be associated with verbal fluency. The Emotional subscale was associated with emotion deficits (Radakovic et al., 2017). It was also noted that depression was strongly associated with scores in the Behaviour/Cognitive Initiation subscale, but not the Emotional subscale, suggesting that emotional aspects of apathy (such

as loss of sympathy or empathy) may not overlap with depression in ALS (Santangelo et al., 2017).

Elamin et al. (2017) developed the Beaumont Behavioural Inventory (BBI) accounting for motor impairments in ALS. This is a 41-item questionnaire completed by the caregivers of the participant, assessing apathy, disinhibition, social cognition deficits, perseverative / stereotypical / obsessive-compulsive behaviours, dietary changes and altered responses to sensory stimuli. The BBI was indicated to show high convergent validity with the FrSBe and was independent from non-behavioural measures.

A few other screening tools specifically developed for ALS-related behavioural impairments are the behavioural screen in ALS-CBS (Woolley, York, et al., 2010), the ECAS (Abrahams et al., 2014) and the MiND-B (Mioshi, Hsieh, et al., 2014). These tools completed by the caregiver of patients enable screening for behavioural impairments and could be followed up with more extensive testing if deemed necessary by the clinician.

2.3.2 Other behavioural domains

In addition to apathy, self- and/or caregiver-observed changes in ALS have also been reported in other behavioural areas such as disinhibition and cognition(e.g. experience in executive dysfunctions). Grossman et al. (2007) reported a non-significant increase in disinhibition in 55 ALS patients from 20% at premorbid levels to about 29% after disease onset. Disinhibition may manifest as lack of social etiquette or inappropriate responses including agitation (anger outbursts or engaging in risky behaviour). Gibbons et al. (2008) reported socially disinhibited behaviour and increased aggression in 2 of 16 ALS patients using the Manchester FTD behavioural interview.

In a population-based study (n=225 ALS), disinhibition was noted in approximately 17% of subjects and was less affected than apathy (31%) and executive dysfunction (20%) (Witgert et al., 2010). Reported executive dysfunctions in the FrSBe includes lack of planning and organisation related behaviours required for daily activities. Higher frequency of behavioural impairments on the FrSBe executive dysfunction scale have been reported, increasing from 31% at premorbid levels to 46.7% after disease onset (Grossman et al., 2007).

Using the Manchester FTD behavioural interview, the most frequent behavioural change was an increase in self-centredness and loss of empathy (reduced concern for the feelings and needs of others) (Gibbons et al., 2008). At least 10 of 16 ALS patients were reported to be more irritable after symptom onset, compared to before illness. Other behavioural changes such as lack of embarrassment and loss of concern for personal hygiene was noted in 3 of 16 ALS patients. Changes in eating behaviour was noted in one patient, while repetitive behaviours and compulsive rituals were present in three patients.

Lillo et al. (2011) reported similar findings using the Cambridge Behavioural Inventory Revised (CBI-R), which is a caregiver-based questionnaire that assesses neuropsychiatric symptoms and changes in everyday function such as memory and orientation, everyday skills, self-care, mood, beliefs, sleep, abnormal behavioural (such as impulsivity, insensitive remarks), eating habits (increased appetite, preference for sweet foods), stereotypic and/motor behaviours and lack of motivation (apathy). Both ALS patients and bvFTD patients had higher scores as compared to controls. Scores were elevated in the domains of abnormal behaviour, eating habits, stereotypic and motor behaviours and lack of motivation. BvFTD patients displayed greater severity as compared to ALS patients, though these differences failed to reach statistical significance. There is a dearth of studies investigating

the neuroimaging correlates of behavioural changes in ALS. Single photon emission computed tomography (SPECT) in eight ALS patients revealed frontal abnormalities in two patients and temporal abnormalities in four patients. Of these, two patients met criteria for behavioural changes associated with FTD (Gibbons et al., 2008).

2.4 Longitudinal changes in cognition

While baseline assessments are useful, considering the frontotemporal spread of the disease (Brettschneider et al., 2013), it is likely that patients may develop cognitive and behavioural changes over time. Robinson et al. (2006) assessed longitudinal neuropsychometric performance in 19 ALS and 8 matched controls at baseline and after six months. There were no between- or within-group changes in performance over time, however, it was noted that approximately 37% of patients developed abnormal neuropsychological performance after 6 months.

Kilani et al. (2004) investigated longitudinal changes at baseline, 6 months and 12 months in 19 ALS patients and 19 healthy controls. Baseline group comparisons indicated lower performance on the MMSE, Geriatric Depression Scale (GDS), TMT, and BNT in ALS patients. Thirteen ALS patients completed follow-up visits at months 6 and 12 and indicated no significant impairments over time. The study did not report any findings in the control population over time. More recent studies have reported a similar lack of decline in ALS patients (n=24) at follow-ups using the ECAS (Burkhardt, Neuwirth, & Weber, 2017). However, this study noted a higher performance on the ECAS for the control group (n=24) at follow-ups. This lack of decline on the same version of test at follow-up in ALS patients, could indicate compromised cognition. Perhaps slower frontotemporal involvement may

prevent practice effects (as noted in the control group), suggesting mild cognitive impairments in the ALS cohort over time. Both these studies had small groups, and further studies with larger sample sizes would enable a better understanding of cognitive preservation or decline in ALS patients. Recent studies have developed alternate versions of ALS-specific tools such as the ECAS to reduce practice effects reported in control participants and would be of interest to use in further investigations (Crockford et al., 2018; Crockford et al., 2018).

2.5 Clinical variables and the ALS-FTLD spectrum

Considering the variable onset, progression of clinical symptoms and cognitive profiles in ALS, studies have worked towards identifying the relationship between clinical factors and cognitive impairment. Initial studies identified that patients with pseudobulbar affect (PBA) performed worse than ALS patients without PBA on verbal fluency tasks corrected for motor impairment (Abrahams et al., 1997). This could imply a differential spread of the disease in the central nervous system that affects cognitive performance in ALS patients with PBA. Initial studies had also indicated that patients with bulbar onset performed poorly on cognitive tasks (Schreiber et al., 2005; Strong, Grace, Orange, & Leeper, 1996) and may show slower saccades as compared to patients with limb onset. However, other studies have indicated that bulbar onset was not predictive of cognitive impairment in ALS (Beeldman et al., 2016; Jelsone-Swain et al., 2012; Lillo, Savage, et al., 2012; Massman et al., 1996; Zalonis et al., 2012).

Studies have indicated that genetic profiles of patients may influence cognitive profiles in ALS. For instance, patients with familial SOD1 mutations may show no cognitive

impairment, while those with no SOD1 mutations but a positive familial history for ALS, may show impaired cognition and behaviour (Wicks et al., 2009). More recent studies have identified C9orf72 mutations, to show higher rates of cognitive and behavioural changes in ALS patients (Byrne et al., 2012; Irwin et al., 2013; Patel & Sampson, 2015).

Demographic factors such as age, education and gender have also been explored in relation to cognitive impairment in ALS. Phukan et al. (2012) noted that ALS patients (n=160) with executive dysfunction had higher age at symptom onset and lower education as compared to the cognitively unimpaired group. In another previous study (Massman et al., 1996), ALS patients (n=146) with cognitive impairment had lower years of education as well as decreased premorbid abilities measured by the American version of the National Adult reading test (ANART) (Grober & Sliwinski, 1991). However, age was not predictive of cognitive impairment in ALS. There appears to be mixed consensus regarding gender, with one study suggesting that gender was not predictive of cognitive impairment in ALS (Massman et al., 1996), while another suggested that female gender may be predictive of greater risk of cognitive impairment in ALS patients (n=145) (Wei et al., 2015).

The impact of presence of cognitive impairment on clinical variables such as physical disability, respiratory capacity and functional decline were also explored by previous studies. One previous study noted that disease progression rate was higher in patients with EF impairments (Phukan et al., 2012). A population-based sample of 186 ALS patients indicated that EF impairment was associated with faster rates of motor decline, particularly in bulbar function, whereas those with normal cognition at baseline tended to remain cognitively intact with slower motor and cognitive decline (Elamin et al., 2013). Other studies have indicated that cognitive deficits do not progress in line with motor decline and may progress more

slowly in ALS patients (Schreiber et al., 2005). In another study, no significant association was noted between cognitive performance and physical disability as measured by the ALSFRS-R (Jelsone-Swain et al., 2012).

The cognitive status of patients at various clinical milestones in ALS was investigated by (Trojsi, Santangelo, et al., 2016). The study used King's Staging system (Roche et al., 2012), that was proposed to identify clinical milestones in ALS identifying specific points in the disease course. The system included the following stages: (1) symptom onset (involvement of first region), (2A) diagnosis, (2B) functional involvement of second region, (3) functional involvement of third region, (4A) need for gastrostomy, (4B) need for respiratory support and non-invasive ventilation. The authors noted that the frequency of cognitive impairment increased at higher stages of the disease course. The percentage of EF impairments increased from 15% at stage 2 to 35% at stage 3 and 38% at stage 4. However, this study used a cross-sectional design (n=74 ALS). Longitudinal follow-ups of patients as they progress through these stages would provide a comprehensive assessment on the nature of cognitive impairment at various stages and clarify the percentage of cognitively intact patients that develop cognitive impairments at later stages.

Depression and mood are two crucial variables that influence performance on cognitive tests and may confound the presence of cognitive impairments in ALS patients. Massman et al. (1996) noted that neuropsychological performance was not associated with depression. Jelsone-Swain et al. (2012) performed extensive cognitive testing including EF, language, memory and visuospatial functions on 22 ALS patients and 23 controls. Depression and mood assessed using Geriatric Depression Inventory (GDI) and Beck's Depression Inventory (BDI). Scores were comparable between patients and controls, but the presence of

depression decreased cognitive performance in both groups. A trend between lower delayed recall performance and depression was reported in ALS patients, while in controls, lower immediate recall performance was associated with depression. Further, increased severity of depression was associated with faster progression and greater limb disability in patients, but not bulbar or respiratory symptoms on the ALSFRS-R.

Survival in cognitively impaired patients is an essential topic in clinical and research studies in ALS. Studies have suggested that cognitively impaired patients, especially those with executive dysfunctions have a poorer prognosis with shorter survival (Elamin et al., 2011; Govaarts et al., 2016; Xu, Alruwaili, Henderson, & McCombe, 2017). Additionally, the association between cognitive impairment and poor survival persists after initiation of non-invasive ventilation (Govaarts et al., 2016). This adds to the previous finding of cognitive impairment in patients at later stages of the disease (Trojsi, Santangelo, et al., 2016), suggesting that cognitive impairment may be pervasive and may not be a consequence of poor respiratory capacity. Conversely, one study had indicated that cognitive impairment may not influence survival in ALS (Mioshi, Caga, et al., 2014). While these studies included relatively large sample sizes, differences in these findings could be attributed to study design, neuropsychometric tools employed and variable clinic profiles of patients.

2.6 Neuroimaging and the ALS-FTLD Spectrum

Neuroimaging techniques including magnetic resonance imaging (MRI), magnetic resonance spectroscopy (MRS), positron emission tomography (PET), single-photon emission computed tomography (SPECT), functional MRI (fMRI) and event-related potentials (ERPs) have provided evidence for frontotemporal changes associated with

cognition in ALS. The current section focuses on imaging findings from MRI studies investigating the structural (grey and white matter) and functional (task-based and resting-state) changes reported in relation to cognitive and behavioural impairment in ALS.

2.6.1 Grey Matter Changes

One of the initial methods of quantifying structural changes in neurological populations was using voxel-based morphometry (VBM). This technique primarily includes comparison of grey matter concentration or volume between two groups of participants (Ashburner & Friston, 2000). A recent meta-analysis on 29 VBM studies in ALS patients (n=638) revealed lower grey matter in right precentral gyrus, left Rolandic operculum, left lenticular nucleus and the right ACC compared to healthy controls (n=622) (Shen et al., 2016). Apart from this, some studies explored grey matter changes along the ALS-FTLD spectrum. Chang et al. (2005) reported frontotemporal reductions in grey matter volume in a group of 10 cognitively and behaviourally normal ALS patients, 10 ALS-FTLD patients and 22 healthy controls. Group comparisons revealed an overlap of motor and frontotemporal degeneration in patients with ALS-only and ALS-FTLD. These regions of atrophy especially in the left inferior frontal gyrus (Brodmann area 44) was associated with reduced verbal fluency (letter) performance, in ALS-FTLD patients.

Another study elaborated the above findings and further provided direct comparisons between patients with ALS (n=10), ALS-FTD (n=10), bvFTD (n=15) and healthy controls (n=18) (Lillo, Mioshi, Burrell, et al., 2012). The authors reported that grey matter reductions in the prefrontal cortex distinguished bvFTD from the ALS-FTLD spectrum, while temporal grey matter atrophy was characteristic of ALS-FTD as compared to ALS. The ALS-only

group indicated changes primarily in the corticospinal tract (CST), which was identified as the marker of atrophy for this group of patients. Additionally, it was noted that grey matter loss in the anterior cingulate cortex (ACC) and the motor cortex overlapped across the ALS and FTD groups, providing neuroimaging evidence of shared atrophy across the ALS-FTLD continuum. The caveat was that the study did not consider ALS patients with mild cognitive and behavioural changes. This was addressed by the same group of researchers in another study published the following year (Mioshi et al., 2013). The authors compared grey matter volume between patients with cognitive impairment (ALS-ci, n=8), normal cognition (ALS-only, n=14) and ALS-FTD groups (n=17). Here, the authors indicated that changes in ALS-FTD extensive and included regions of the motor cortex, somatosensory cortex, prefrontal cortices as well as the temporal lobes, while ALS patients displayed only atrophy in the brainstem. The ALS-ci group had cortical atrophy in the motor and somatosensory cortex compared to ALS-only group, indicating that cortical atrophy may differ between ALS patients with/without cognitive impairment. However, the small sub-groups in the study limit generalisability of the results to a larger population.

Christidi, Karavasilis, Riederer, et al. (2018) investigated grey matter atrophy in 19 ALS-only patients (no cognitive impairment), 31 ALS-ci patients (cognitive impairment present) and 25 healthy controls. Group comparisons revealed atrophy in the left ACC, middle / inferior frontal gyrus, fusiform gyrus and left cerebellum in ALS-only patients as compared to healthy controls. ALS-ci patients showed atrophy in widespread regions including left postcentral gyrus, bilateral inferior orbitofrontal gyrus, posterior insula, right superior orbitofrontal gyrus, bilateral ACC and bilateral amygdala / hippocampus and left caudate and putamen when compared to healthy controls. The study also indicated extra-motor

changes in the ALS-only group as compared to controls. Reduced grey matter was found in the left ACC, middle/inferior frontal gyrus, orbitofrontal gyrus, fusiform gyrus and the cerebellum. Between the ALS sub-groups, ALS-ci patients showed additional atrophy in the left precuneus compared to the ALS-only group. One of the limitations for the study is inclusion of greater number of ALS-ci patients (n=31) as compared to ALS-only patients (n=19), a pattern that is not typically observed in the ALS population.

Another technique to identify cortical degeneration is measuring changes in its cortical thickness, which provides an arbitrary measurement of the cerebral cortex. The thickness of the cerebral cortex varies between 1 and 4.5mm at different regions and overall averages to about 2.5mm (Fischl & Dale, 2000). Automated techniques enable parcellation of different brain regions and provide thickness measures for specific brain regions. In ALS, studies have shown reduced cortical thickness for precentral gyrus as well as frontotemporal regions including superior / middle / inferior frontal gyrus, ACC and temporal gyri (Agosta et al., 2012; Mezzapesa et al., 2013; Roccatagliata, Bonzano, Mancardi, Canepa, & Caponnetto, 2009; Schuster et al., 2013; Verstraete et al., 2012; Walhout et al., 2015). Only a few studies have explored the cortical thickness changes in ALS sub-groups along the ALS-FTD spectrum.

Schuster et al. (2014) investigated cortical thinning in 45 cognitively normal ALS patients, 28 patients with cognitive impairment in at least one domain (ALS-ci), and 8 ALS-FTD patients. Cortical thickness was reduced in the ALS-ci and ALS-FTD groups as compared to cognitively unimpaired patients. Regions of cortical thinning extended to medial prefrontal cortex (mPFC), dlPFC and temporal lobes. ALS-ci and ALS-FTD showed cortical

thinning in similar regions, however ALS-FTD patients displayed greater extent of grey matter loss. The study did not include behavioural impairments in ALS.

Agosta et al. (2016) investigated cortical thickness in a group of MND patients including ALS, UMN and LMN variants of the disease. Participants were divided into cognitive sub-groups based on the presence (MND-plus, n=53) or absence (MND-motor, n=48) of cognitive impairments. Patients in the MND-plus groups revealed more severe atrophy as compared to the MND-motor groups in the precentral gyrus, insular cortices, cingulate cortices and frontotemporal regions.

In non-demented ALS patients, studies have shown associations between grey matter reductions and lower cognitive performance. Menke et al. (2014) showed that reduced grey matter in the Broca's area (Brodmann area 44), and bilateral dlPFC was associated with poor verbal fluency in ALS. Reduced cortical thickness in the dlPFC was associated with lower cognitive flexibility (Evans et al., 2015). Reduced grey matter density in the ACC was associated with reduced emotion processing in ALS patients (Cerami et al., 2014), though no study investigated grey matter associations between EF and social cognition (ToM) in the same ALS cohort.

Thus, overall, in ALS patients, grey matter reductions have been reported primarily in the precentral gyrus (motor cortex), with some studies indicating reduced grey matter in extra-motor regions such as inferior frontal gyri, dlPFC, mPFC, ACC and the insula. A smaller proportion of studies have also identified grey matter loss in sub-cortical structures such as the caudate, putamen, hippocampus and the amygdala. Extra-motor reductions in grey matter is prominent in ALS-ci patients, though there is some evidence of grey matter loss in frontal regions (dlPFC, mPFC and ACC) in ALS-only patients. Reductions in frontal grey matter

have also been associated with reduced EF performance and lower social cognition in ALS patients in separate studies. No study investigated grey matter associations between both EF and ToM in the same ALS cohort.

2.6.2 White Matter Changes

Volume based changes in white matter using VBM were measured by one initial study in ALS (Abrahams, Goldstein, et al., 2005). The study investigated white matter changes in a group of ALS patients with impairments in verbal fluency (ALS-ci, n=11), unimpaired patients (ALS-only, n=12) and healthy controls (n=12). ALS-ci patients showed a significant reduction in white matter volume in the frontotemporal association tracts when compared to controls, while a lesser extent of changes was noted for the ALS-only group. This study provided the first report on white matter changes in ALS cognitive sub-groups and despite the small sample size, the findings indicated the presence of a continuum. With advances in neuroimaging techniques, diffusion tensor imaging (DTI) was subsequently used to investigate white matter changes in ALS. This technique provides indirect measurement of the degree of anisotropy and structural orientation of white matter tracts based on diffusion of water within the boundaries of cellular and tissue barriers (Basser, Mattiello, & LeBihan, 1994; Soares, Marques, Alves, & Sousa, 2013). Diffusion metrics such as fractional anisotropy (FA, indicating the directional preference of water diffusion), molecular diffusion rate (also referred to as mean diffusivity, MD), axial diffusivity (AD, indicating the diffusion rate along the main axis) and radial diffusivity (RD, a measure of diffusion in the transverse direction) provide information regarding specific white matter properties and degeneration in neurological conditions (Soares et al., 2013; Song et al., 2003). Using this technique, studies indicated degeneration in the corticospinal tracts (CST), with DTI emerging as a

potential UMN marker in ALS, specifically indicating reduced FA and RD values in ALS (Agosta et al., 2010; Agosta et al., 2009; Kaufmann et al., 2004; Mitsumoto et al., 2007; Senda et al., 2009). DTI is also considered to be a marker of disease progression in ALS (Kassubek et al., 2018; Menke et al., 2014; Müller et al., 2016). Additionally, studies have identified degeneration in commissural and association tracts such as the corpus callosum, cingulum, superior longitudinal fasciculus (SLF), inferior longitudinal fasciculus (ILF), inferior fronto-occipital fasciculus (IFOF) as well as the uncinate fasciculus (UF) (Agosta et al., 2010; Chaves et al., 2017; Christidi et al., 2014; Dimond et al., 2017; Sarro et al., 2011).

Sarro et al. (2011) investigated the white matter integrity in 16 ALS patients and 15 matched controls. The authors found reduced white matter integrity as indicated by FA in the CST, while other DTI metrics such as MD and RD were altered for the CST as well as extra-motor tracts such as SLF, cingulum and the UF. These extra-motor alterations were associated with poor performances in EF tests such as TMT, Stroop test, WCST, verbal fluency (letter) and memory measured by RAVLT. While 69% of the ALS sample was found to be impaired on at least one EF test, no specific group comparisons were performed between cognitive sub-groups in ALS due to the small sample size (n=16).

Kasper et al. (2014) investigated the differences in DTI metrics of cognitive sub-groups in 49 ALS patients with no cognitive impairment (ALS-only), 23 patients with cognitive impairments (ALS-ci) and 64 healthy controls. Voxel-wise comparisons using Tract-based Spatial Statistics (TBSS) was performed to identify group differences in white matter using DTI metrics. ALS-ci patients showed significant loss of white matter integrity in the CST and frontoparietal association tracts, while ALS-only patients had reduced integrity confined to the CST when compared to the controls. A direct comparison between the groups

indicated a spread of atrophy in ALS-ci patients to the frontal regions, while the CST between the two patient groups revealed minimal changes.

Another study by Dimond et al. (2017) explored the relation of white matter integrity and structural connectomics using TBSS and graph theory to identify differences in cognitively impaired ALS groups (ALS-ci, n=9), cognitively competent groups (ALS-only, n=5) and healthy controls (n=22). ALS-ci patients displayed altered structural integrity and local connectivity in the mPFC and dlPFC compared to controls. These regions that were also associated with verbal fluency performance in this cohort. However, the small sample limits generalisability of the results, especially the classification of ALS-ci patients displayed higher frequency of impairment (>50%) than in ALS population (reported to be around 25-50%).

Additional studies have reported similar degeneration in white matter that also correspond with grey matter loss in ALS, i.e. tracts connecting (Agosta et al., 2016; Christidi, Karavasilis, Riederer, et al., 2018). Christidi, Karavasilis, Riederer, et al. (2018) noted widespread white matter changes as compared to grey matter changes in ALS-ci patients, while Agosta et al. (2016) reported that white matter tracts predicted cognitive performance better than grey matter measures of cortical thickness.

In non-demented ALS patients, reduced white matter integrity (FA) in the frontal white matter of the IFOF, uncinate fasciculus and the corpus callosum were associated with lower cognitive flexibility (Evans et al., 2015). A-ToM impairments on story-based empathy tasks assessing emotion attribution in ALS groups were found to be associated with poor white matter integrity in frontotemporal regions including the forceps minor (genu of corpus

callosum), uncinate fasciculus (UF), superior longitudinal fasciculus (SLF) and inferior fronto-occipital fasciculus (IFOF) (Crespi et al., 2016).

Thus, white matter degeneration in ALS is noted in the CST as well as commisureal tracts (CC) and association tracts (such as the SLF, UF, IFOF, etc.) indicating extra-motor involvement in ALS patients. While studies have indicated reduced FA to be associated with EF and social cognition (Crespi et al., 2016), no study had investigated both grey and white matter changes associated with EF and social cognition in the same cohort of ALS patients.

2.6.3 Functional Changes

Studies in ALS have suggested that functional/metabolic changes precede structural changes and may be a better biomarker of prognosis or disease progression in ALS (Menke et al., 2016). However, studies remain inconsistent with some reporting increased activation (Agosta et al., 2011; Douaud et al., 2011), decreased activation (Mohammadi et al., 2009; Tedeschi et al., 2012; Trojsi et al., 2015; Zhou et al., 2014) or no significant differences in functional networks in ALS (Chenji et al., 2016). Such functional alterations may represent the reorganisation of brain networks.

An initial study in ALS used PET imaging to identify metabolic changes in frontal lobes (Abrahams et al., 1996). The authors compared a small sample of cognitively impaired (ALS-ci, n=6) and cognitively unimpaired groups (ALS-only, n=6). Impaired activation in the mPFC, dlPFC and premotor cortices was noted for intrinsic verbal fluency generation during the PET scan, while the ALS-only group did not display such activations. Using hypometabolic activity in frontal regions to be associated with poor verbal fluency performance (Kew, Goldstein, et al., 1993; Ludolph et al., 1992). Functional activations were

also found to be impaired in these regions in ALS patients while performing verbal fluency tasks (Abrahams et al., 2004). Impaired activations in posterior superior temporal sulcus (pSTS) were found in ALS patients when performing A-ToM task (Judgement of Preference test) (Keller et al., 2017).

Apart from task-based paradigms, studies have identified alterations within the resting state networks (RSNs) of the brain (Douaud et al., 2011); (Chenji et al., 2016; Mohammadi et al., 2009; Tedeschi et al., 2012; Trojsi et al., 2015; Zhou et al., 2014). These networks include temporally associated brain regions that are involved in performing goal-oriented tasks. RSNs include the default mode network (DMN) which is highly active at rest and reduces in activity when to the person performs goal-oriented tasks involving either motor related activities (sensorimotor network, SMN) or cognitive activities (frontoparietal network, FPN; salience network, Sal-N; attentional networks, Att-N, executive network, Exe-N) (Allen et al., 2011).

A few studies have identified alterations in these cognitive RSNs in ALS (Agosta et al., 2013; Trojsi et al., 2017; Trojsi et al., 2015). Only one study performed direct comparisons of 15 ALS patients, 15 bvFTD patients and 15 healthy controls (Trojsi et al., 2015). Both the patient groups displayed decreased connectivity within SMN, FPN, Sal-N and Exe-N. For the DMN, a divergent pattern emerged with bvFTD patients showing increased activations in the posterior cingulate cortex (PCC) and ALS patients displayed decreased activations in the PCC. In the study by Agosta et al. (2013) increased activations at rest in the parietal regions were associated with better EF performance suggesting a possible compensatory mechanism, whereby additional regions are recruited to perform EF tasks which normally rely on the frontal lobes. In another study (Carluer et al., 2014) impaired C-ToM in ALS

patients was associated with reduced resting-state metabolic activity in the dorsolateral prefrontal cortex (dlPFC), supplementary motor area and medial prefrontal cortex (mPFC), regions that are also associated with EF functions (Abrahams et al., 2004).

Thus, task-based and resting-state functional studies in ALS have indicated either increased activity or decreased activity in ALS patients compared to controls. While there is a dearth of research in fMRI alterations in ALS cognitive sub-groups, studies have independently identified regions such as the dlPFC and mPFC to be associated with both EF and ToM and are yet to be investigated in the same ALS cohort.

2.7 Summary

The current literature review provided evidence for cognitive and behavioural changes in patients with ALS. While initial studies focused extensively on executive dysfunctions, more recent studies have provided evidence for language, memory and social cognition deficits in ALS. Along the ALS-FTLD spectrum, ALS patients with co-morbid FTD were found to perform worse than bvFTD and non-demented ALS patients. The role of clinical variables such as disease onset and its impact on cognitive performance remains inconclusive, with some studies indicating worse performance in patients with a bulbar onset while others indicated comparable performance between patients with a bulbar onset and those with a limb onset. Neuroimaging studies have indicated that reduced cognitive performance is associated with grey matter and / or white matter degeneration as well as functional brain changes. More recent studies have indicated that decreased performance in domains such as social cognition may be associated with impairments in EF, with some neuroimaging

evidence suggesting shared structural and functional brain changes though these associations are yet to be established in the same cohort of ALS patients.

2.8 Rationale, and Aims and Hypotheses of the thesis

Studies outlined in this literature review have used extensive neuropsychometric testing to analyse performance on various cognitive domains. Some studies have employed screening tools such as different versions of the Addenbrooke's Cognitive Exam (ACE) (Mathuranath, Nestor, Berrios, Rakowicz, & Hodges, 2000; Mioshi et al., 2006) in which there is a greater emphasis on memory functions compared to EF. It also does not account for motor disability which may confound the presence of cognitive impairment in ALS patients (Chenji et al., 2018). Recently, an ALS-specific cognitive screening tool, the Edinburgh Cognitive and Behavioural Screen (ECAS) was introduced to assess impairments in ALS-specific (language, verbal fluency, executive) as well as ALS non-specific (memory, visuospatial) domains (Abrahams et al., 2014).

The participants included in the thesis were administered the ECAS as well as a neuropsychometric battery assessing verbal fluency (letter F and animals), executive functions (digit span backwards, digit ordering test), semantic naming (BNT short form), learning and memory (HVLT-R) and visuospatial abilities (JLO). While, the neuropsychometric battery was not designed to validate the ECAS, a preliminary in-house analysis revealed moderate associations between the screening tool and the neuropsychometric battery (Supplementary Table 2.3). Furthermore, group comparisons on the neuropsychometric battery did not add to the findings revealed by the ECAS (unpublished work). Thus, considering the preliminary associations and recent studies

indicating high sensitivity and specificity of the ECAS (Abrahams et al., 2014; Niven et al., 2015; Pinto-Grau et al., 2017), a decision was made to include data on the ECAS and omit data on the neuropsychometric battery in the current thesis.

Over the years, studies have reported frontotemporal degeneration with supporting neuroimaging findings in ALS patients with cognitive impairments. As mentioned previously, most studies have employed extensive neuropsychometric testing to identify cognitive sub-groups. However, such extensive cognitive testing may not be feasible given time constraints. Patients may be unable to complete testing at follow-ups given the progressive physical disability in ALS. Thus, employing the ECAS, which can be administered in either verbal or written formats, may be useful in identifying cognitive sub-groups. Currently, while the ECAS is emerging as the preferred screening tool in ALS, there is a lack of evidence on brain changes in cognitive sub-groups identified using the ECAS. Thus, the first aim of the thesis was to investigate the neuroanatomical associations in ALS patients using the ECAS. It was hypothesised that: (1) patients impaired on the ECAS have lower grey matter densities and reduced white matter integrity in the frontotemporal regions compared to controls, and (2) there is an association between the ECAS and neuroimaging metrics of grey matter densities and white matter integrity of frontotemporal regions in ALS patients.

The ECAS also incorporates a sub-test for social cognition (ToM, Judgement of Preference test). The sub-test is included as a part of the executive domain. When the ECAS was initially published, there was some evidence for ToM deficits in ALS, but no specific evidence for the relationship between EF and ToM (Cavallo et al., 2011; Gibbons et al., 2007; Girardi et al., 2011; Lulé et al., 2007; Lulé et al., 2005). More recent studies have supported a link

between EF impairments and lower ToM performance (Burke, Pinto-Grau, et al., 2016; Watermeyer et al., 2015b). Thus, the second aim of the thesis was to investigate ToM in ALS patients using the ECAS as well as additional ToM tests such as the RME test and the Faux pas test. It was hypothesised that (1) ToM and EF impairments are present in ALS patients, and (2) ToM and EF are associated such that patients with EF impairments perform worse than those with intact EF on measures of ToM.

Furthermore, there were some studies reporting associations between lower ToM performance and reduced grey matter density, white matter integrity or reduced functional brain activity (Carluer et al., 2014; Cerami et al., 2014; Crespi et al., 2016). These findings were reported in separate studies, and no study had investigated both structural and functional brain changes associated with ToM in the same cohort of ALS patients. Thus, the final aim of the thesis was to examine both structural and functional brain changes associated with ToM in ALS patients. It was hypothesised that poor ToM in ALS is associated with (1) lower grey matter densities, (2) reduced integrity of white matter tracts, and (3) altered resting state connectivity of regions included in the ToM network.

An outline of the aims and hypotheses are provided below.

Aim 1

To explore neuroimaging associations of the ECAS. Considering the lack of evidence in neuroimaging associations of the ECAS in ALS, the study will attempt to identify brain-related structural differences between cognitive sub-groups identified using the ECAS and to find associations between ECAS performance and neuroimaging metrics.

Hypotheses: (1) Patients impaired on the ECAS have lower grey matter density and reduced white matter integrity in the frontotemporal regions compared to controls, and (2) there is an association between the ECAS and neuroimaging metrics of grey matter density and white matter integrity of frontotemporal regions in ALS patients.

Aim 2

To identify associations between social cognition, specifically, theory of mind (ToM) and executive functions (EF) in ALS. The study will attempt to explore and identify the association between social cognition and executive functions and compare ToM performance among EF-based cognitive sub-groups.

Hypotheses: (1) ToM and EF impairments are present in ALS patients, and (2) ToM and EF are associated such that patients with EF impairments perform worse than those with intact EF on measures of ToM.

Aim 3

To examine the structural and functional associations of ToM in ALS. The study will attempt to find associations between grey matter, white matter and resting state connectivity maps using a ToM network model.

Hypotheses: Poor ToM in ALS is associated with (1) lower grey matter density, (2) reduced integrity of white matter tracts, and (3) altered resting state connectivity of regions included in the ToM network.

2.9 Supplementary Materials

Supplementary Table 2.1. Brief outline of studies highlighting cognitive performance in ALS.

First Author (Year)	Sample size			Cognitive domain	Measure	Main Findings
	ALS	Controls	Others			
Executive functions (EF)						
Talbot (1995)	19	10	8 MND-FTD 29 FTD	Verbal fluency	Verbal fluency (FAS total)	Impaired verbal fluency in MND-FTD and FTD patients. MND-only patients' performance was comparable to controls.
Abrahams (1996)	6	6	-	Verbal fluency	Verbal fluency index	Impaired verbal fluency in ALS.
Abrahams (1997)	28	28	24 ALS-PBA	Verbal fluency	Verbal fluency index	Impaired verbal fluency in ALS, ALS-PBA perform worse than ALS-only patients
Abe (1997)	26	26	-	Verbal fluency	Verbal fluency	Impaired in a smaller sub-group of ALS patients (not corrected for motor function).
Abrahams (2000)	21	25	-	Verbal fluency	Verbal fluency index	Impaired verbal fluency in ALS.
Hanagasi (2002)	20	13	-	Verbal fluency	Verbal fluency (FAS total)	Impaired in ALS (not corrected for motor function).
Stukovnik (2010)	22	21	-	Verbal fluency	Verbal fluency index	Impaired verbal fluency in ALS.
Lepow (2010)	49	25	-	Verbal fluency	Verbal fluency strategies (clusters and switches)	Decreased clusters and switched in ALS. Impaired fluency may be mediated by frontal and temporal lobe.
Phukan (2012)	160	110	-	Verbal fluency	Verbal fluency index	Impaired verbal fluency in ALS.
Abe (1997)	26	26	-	Semantic fluency	Category fluency (animals, fruits, vehicles)	Impaired in a smaller sub-group of ALS patients (not corrected for motor function).
Abrahams (2000)	21	25	-	Semantic fluency	Category fluency index	Impaired category fluency in ALS.
Hanagasi (2002)	20	13	-	Semantic fluency	Category fluency (animals)	Impaired in category fluency in ALS.
Rottig (2006)	15	15	14 NM	Semantic fluency	Category fluency (supermarket)	Impaired in category fluency in ALS.
Lepow (2010)	49	25	-	Semantic fluency	Category fluency strategies (clusters and switches)	Decreased clusters and switched in ALS. Impaired fluency may be mediated by frontal and temporal lobe.
Phukan (2012)	160	110	-	Semantic fluency	Category fluency (animals)	Impaired in category fluency in ALS.

First Author (Year)	Sample size			Cognitive domain	Measure	Main Findings
	ALS	Controls	Others			
Charu (1996)	50	50	23 SpC	Attention Working Memory	Visual working memory (match to sample) Visual Attention (visual search matching to sample)	Impaired simultaneous matching and visual search to matching in ALS. No difference on delayed matching suggesting frontal (not temporal) dysfunction in ALS.
Massman (1996)	146	-	-	Attention	Verbal Series Attention Test	Impaired performance in ALS compared to normative data.
Abe (1997)	26	26	-	Attention	Digit span backwards	Impaired in a smaller sub-group of ALS patients (not corrected for motor function).
Hanagasi (2002)	20	13	-	Attention	Digit span backwards Continuous performance test	Impaired accuracy on continuous performance test indicating attention deficits in ALS.
Christidi (2012)	22	22	-	Attention	Stroop test (interference)	Impaired Stroop interference in ALS (corrected for motor impairments).
Hartikainen (1993)	24	24	22 PD	Cognitive flexibility	Trail Making Test (A & B)	Prolonged processing time indicating reduced cognitive flexibility in ALS.
Abrahams (1997)	28	28	24 ALS-PBA	Set-shifting	WCST	Reduced number of sorting rules identified in ALS compared to controls.
Lulé (2005)	12	18	-	Set-shifting	WCST	No significant differences
Evans (2015)	47	29	9 ALS-FTD	Cognitive flexibility / Set-shifting	Visual-verbal test	Impaired performance in ALS and ALS-FTD patients compared to controls.
Hanagasi (2002)	20	13	-	Inhibition	Stroop test (interference)	Impaired in ALS (longer time and higher errors).
Raaphorst (2010)	30	24	23 PMA	Inhibition	Stroop test (interference)	No significant differences.
Phukan (2012)	160	110	-	Inhibition	Stroop test (interference)	Impaired in ALS (corrected for motor function).
Amato (2013)	32	27	10 PLS	Inhibition	Stroop test (modified)	Impaired in ALS compared to PLS and controls.
Lillo (2012)	23	20	20 bvFTD	Inhibition	Sentence Completion (incongruent response)	Higher number of errors in both ALS and bvFTD groups.
Donaghy (2010)	44	45	-	Inhibition	Anti-sacchades (errors)	Higher errors in ALS. Redued anti-sacchades associated with lower Stroop test performance.

First Author (Year)	Sample size			Cognitive domain	Measure	Main Findings
	ALS	Controls	Others			
Abrahams (1997)	28	28	24 ALS-PBA	Planning	Tower of Hanoi (planning time)	ALS-PBA displayed significantly shorter planning time compared to ALS-only and controls.
Abrahams (1997)	28	28	24 ALS-PBA	Decision making	Random joystick movement	Trend towards less random movements as compared to controls.
Lillo (2012)	23	20	20 bvFTD	Decision making	Iowa Gambling task	No significant difference between ALS and controls. BvFTD performed worse compared to both ALS and controls.
Girardi (2011)	19	20	-	Decision making	Iowa Gambling task	Significantly risky performance reported in ALS patients.
Meier (2010)	18	18	-	Planning	Tower of London	Impaired in ALS (significantly more attempts to complete the task).
Meier (2010)	18	18	-	Decision making	Holiday Apartment task (search strategy)	Impaired search index in ALS patients.
Social Cognition						
Lulé (2005)	12	18	-	Emotion perception	IAPS	Increased ratings of arousal in ALS, which was associated with physiological response.
Lulé (2007)	13	15	-	Emotion perception	IAPS	Increased brain activation noted in frontal and parietal regions for emotional stimuli. No alterations in amygdala.
Schmolck (2007)	26	26	-	Emotion perception	Ekman's faces	Negative emotions (fear, anger & disgust) rated as more approachable (positive), similar to patients with amygdala lesions.
Zimmerman (2007)	13	12	-	Emotion perception	Emotional faces Prosody	Decreased accuracy in recognition of sad and surprise faces, poor recognition of prosody for surprise in ALS.
Andrews (2017)	33	22	-	Emotion perception	CATS	Impaired complex facial affect recognition, affective prosody recognition and cross-modal emotion recognition in ALS. Significantly worse in ALS with EF deficit.
Savage (2014)	13	30	16 ALS-FTD 25 bvFTD	Emotion perception	TASIT	Emotion recognition comparable between ALS and controls, significantly worse in ALS-FTD compared to bvFTD.

First Author (Year)	Sample size			Cognitive domain	Measure	Main Findings
	ALS	Controls	Others			
Aho-Ozhan (2016)	30	29	-	Emotion perception	Ekman's faces	Impaired activations in inferior frontal gyrus during emotion recognition task associated with lower social contacts.
Gibbons (2007)	16	16	-	ToM	Happé's cartoon pairs	No significant differences. Some associations with EF.
Carluer (2014)	23	23	-	ToM	TOM-15 (false belief task)	Impaired second order false belief in ALS. Poor performance associated with reduced metabolism in prefrontal regions (EF covariate).
Watermeyer (2015b)	55	49	-	ToM	Happé's cartoon pairs RME TASIT	Impaired performance for Happé's task in ALS. No significant differences for RME and TASIT. EF contributed to 44% variance on ToM.
Staios (2013b)	35	30	-	ToM	TASIT (sarcasm & humor)	Impaired sarcasm and humor detection in ALS.
Cavallo (2011)	15	21	-	ToM	RME ToM story completion	No significant differences for RME. Impaired ToM story completion in ALS.
Jelsone-Swain (2015)	19	18	-	ToM / Action processing	RME	No significant difference for RME. Patient sub-groups of high and low action understanding differed on RME scores.
Burke (2016a)	59	59	-	ToM	RME	Impaired in ALS, performance was worse in bulbar-onset patients. EF comparable between bulbar and limb-onset
Burke (2016b)	106	50	-	ToM	RME	Impaired in ALS patients with EF deficits compared to controls. No significant difference between EF normal ALS patients and controls.
Meier (2010)	18	18	-	ToM	Faux pas test	Impaired faux pas detection in ALS (EF used as covariate).
Girardi (2011)	14	20	-	ToM Emotion perception	Judgement of Preference (eye gaze directions) RME Facial expressions of emotions	Impaired performance for Preference in ALS. Trend reported for lower RME in ALS. Impaired emotion recognition in ALS.

First Author (Year)	Sample size			Cognitive domain	Measure	Main Findings
	ALS	Controls	Others			
Van der Hulst (2014)	33	26	-	ToM	Judgement of Preference (eye gaze directions - "like" & "thinking of")	Impaired performance in Preference task, indicating a combination of affective and cognitive ToM deficit in ALS.
Trojsi (2016)	22	15	-	ToM	ATT EAT RME	Impaired performance for ATT and EAT in ALS indicating a combination of affective and cognitive deficit. ToM performance associated with poor mental health (domain in QoL measure)
Language						
Abe (1997)	26	26	-	Naming	BNT	No significant differences.
Hanagasi (2002)	20	13	-	Naming	BNT	Impaired naming ability in ALS patients.
Taylor (2013)	51	35	-	Naming	BNT	Significantly higher number of errors in naming ability in ALS. EF contributed to 44% variance in language abilities.
Yoshizawa (2014)	25	-	-	Comprehension	Syntax test for Aphasia	72% ALS patients had impaired comprehension. Of these 50% had normal EF.
Tsermentseli (2016)	26	26	-	Naming Comprehension Syntax & Grammar	GNT Pyramids & Palm Trees Kissing & Dancing tests Test of Reception of Grammar Token test	No significant differences in naming ability. Impaired syntax and grammar in ALS patients.
York (2014)	36	31	22 PD	Action verb comprehension	Associativity judgement task	ALS patients performed equally well of concrete and action verbs, while controls performed better on action verbs. PD patients performed worse on concrete words.

First Author (Year)	Sample size			Cognitive domain	Measure	Main Findings
	ALS	Controls	Others			
Roberts-South (2012)	16	12	-	Naming Action naming Comprehension Discourse (communication patterns)	Peabody Picture Vocabulary Action Naming test ABCD sub-tests Cookie theft picture description (discourse)	No significant differences for naming or comprehension abilities. Longitudinal decline in ALS patients for discourse (communicating content).
Memory						
Ludolph (1992)	17	12	-	Immediate Recall Delayed Recall	AVLT	No significant differences.
Massman (1996)	146	-	-	Immediate Recall	CVLT	20% patients performed below 5th percentile (normative data)
Abrahams (1997)	28	28	24 ALS-PBA	Recognition	Recognition Memory test	Impaired recognition in ALS patients.
Abe (1997)	26	26	-	Immediate Recall Delayed recall	AVLT	No significant impairments.
Hanagasi (2002)	20	13	-	Immediate Recall Delayed Recall Recognition	CVLT	Impaired immediate and delayed recall in ALS. Intact recognition memory.
Consonni (2017)	26	13	15 bvFTD	Immediate Recall Delayed Recall Recognition	RAVLT	ALS patients with cognitive impairments performed worse on mid-list recall. Pure ALS patients performed similar to controls.
Christidi (2012)	22	22	-	Encoding Consolidation Retrieval	Stroop (for attention) RAVLT	Selective attention (Stroop) was found to moderate encoding and consolidation but not retrieval process in ALS.
Abdullah (2014)	58	29	-	Immediate Recall Delayed Recall Recognition	RAVLT	Reduced performance in delayed recall was associated with reduced hippocampal volume.
Machts (2014)	40	40	39 aMCI	Immediate Recall Delayed Recall Recognition	RAVLT	Impaired recognition in ALS patients, while aMCI group displayed impairments in immediate and delayed recall. EF contributed to 20% variance in memory performance in ALS. No relationship between EF and memory in aMCI / controls.

First Author (Year)	Sample size			Cognitive domain	Measure	Main Findings
	ALS	Controls	Others			
Burke (2017)	203	117	-	Visual Memory	ROCFT (copy, immediate and delayed recall)	Patients with ALS-FTD (n=30) performed worse on all ROCFT measures. Milder EF impairments were associated with lower immediate and delayed recall.
Visuospatial abilities (relatively spared in ALS patients)						
Hanagasi (2002)	20	13	-	Visuo-perception Visuo-construction	JLO Block design test	Impaired in ALS.

Note: MND-FTD = Motor neuron disease with frontotemporal dementia; FTD = Frontotemporal dementia; EF = Executive Functions; ALS-PBA = ALS patients with pseudobulbar palsy; NM = Neuromuscular patients, SpC = Patients with Spinal cord injury but no cerebral dysfunction; PD = Parkinson's disease; ECST = Wisconsin Card Sorting Test; PMA = Progressive muscular atrophy; PLS = Primary lateral sclerosis; bvFTD = behavioural variant Frontotemporal dementia; IAPS = International Affective Picture System; Comprehensive Affect Testing System; TASIT = The Awareness of Social Inference test; ToM = Theory of Mind; RME = Reading the Mind in the Eyes test; ATT = Advanced test of Theory of Mind; EAT = Emotion Attribution task; BNT = Boston Naming test; GNT = Graded Naming test; ABCD = Arizona Battery of Communication Disorders for Dementia; AVLT = Auditory Verbal Learning test; CVLT = California Verbal Learning Test; RAVLT = Rey's Auditory Verbal Learning test; aMCI = amnestic Mild Cognitive Impairment; ROCFT = Rey Osterrieth Complex Figure Test; JLO = Judgement of Line Orientation.

Supplementary Table 2.2. Brief outline of studies of apathy in ALS.

First Author (Year)	Sample size			Behavioural domain	Measure	Main Findings
	ALS	Controls	Others			
Grossman (2007)	55	-	-	Apathy Disinhibition Executive Dysfunction	FrSBe (Family)	Premorbid apathy in 11% of patients, while rates increased to 55.6% after ALS onset.
Gibbons (2008)	16	-	-	Affect and Social beh. Others*	Manchester FTD behavioural interview	Emotional blunting reported in 38% (n=5) ALS patients.
Woolley (2010)	23	-	-	Apathy Disinhibition Executive Dysfunction	FrSBe (Self and Family)	Loss of insight in ALS patients with FTD (n=4). ALS-only patients (n=17) rated their behavioural changes more severely compared to caregivers.
Witgert (2010)	225	-	-	Apathy Disinhibition Executive Dysfunction	FrSBe (Family)	Apathy reported in 31% patients. EF was associated with apathy.
Radakovic (2017)	30	29	30 ALS-carers 29 control informants	Apathy	Dimensional Apathy Scale (executive, emotional and initiation subscales)	Significant initiation apathy noted in ALS patients. Initiation apathy associated with verbal fluency, while emotional apathy associated with emotion recognition deficits in ALS.
Santangelo (2017)	131	-	-	Apathy	Dimensional Apathy Scale (executive, emotional and initiation subscales)	Apathy noted in 28% of ALS patients. Depression associated with initiation but not emotional aspects (e.g. loss of empathy, sympathy).

Note: FrSBe = Frontal Systems Behavioural Screen; Others* = (1) sensory behaviours, (2) eating, oral or vegetative behaviours, (3) repetitive compulsive or ritualistic behaviours, (4) environmentally dependent behaviours (e.g. hoarding), and (5) cognitively-based behaviour (e.g. misplaying objects, using wrong words).

Supplementary Table 2.3. ECAS associations with neuropsychometric tests (in-house experiment).

ECAS Domains	BNT[a]	Letter F	Animals	DS_F	DS_B	DS Tot	DOT Tot	HVLT Imm	HVLT Del	JLO
Language[a]	-	0.3**	-	-	-	0.2*	0.2*	0.3**	-	-
Verbal Fluency	0.3**	**0.6****	**0.4****	-	0.2*	0.2*	0.2*	0.3**	0.2*	-
Executive	0.3**	-	-	**0.3****	**0.3****	**0.3****	**0.3****	0.3*	-	-
Memory	0.2*	0.3**	0.3**	-	-	-	-	**0.3****	**0.3****	-
Visuospatial	-	-	0.2*	-	-	-	0.3**	0.2*	0.2*	-
ECAS Mains Scores	-	-	-	-	-	-	-	-	-	-
ALS-Specific Score	**0.4****	**0.5****	**0.3****	**0.2***	**0.3****	**0.4****	**0.3****	0.4**	0.2*	-
ALS Non-Specific Score	0.2*	0.3**	0.3**	-	-	-	-	**0.4****	**0.4****	-
Total Score	0.4**	0.5**	0.4**	0.2*	0.3**	0.3**	0.3**	0.4**	0.3**	-

[a]BNT was found to be associated with Naming sub-test only in the Language domain (Pearson r=0.3, p<0.01). BNT = Boston Naming Test, DS_F = Digit Span Forward, DS_B = Digit Span Backward, DS Tot = Digit Span Total, HVLT Imm = Hopkin's Verbal Learning Test – Revised Immediate Recall, HVLT Del = Hopkin's Verbal Learning Test – Revised Delayed Recall, JLO = Judgement of Line Orientation. Lack of associations between ECAS visuospatial and JLO could be either associated with differences in construct of the task.

3. Neuroanatomical Associations of the Edinburgh Cognitive and Behavioural ALS Screen (ECAS)

This Chapter is a manuscript prepared for submission. It addresses Aim 1 of the thesis: To explore neuroimaging associations of the ECAS. Considering the lack of evidence in neuroimaging associations of the ECAS in ALS, the study will attempt to identify structural differences between cognitive sub-groups identified using the ECAS and find associations between ECAS performance and neuroimaging metrics.

Hypotheses: (1) Patients impaired on the ECAS have lower grey matter densities and reduced white matter integrity in the frontotemporal regions compared to controls, and (2) there is an association between the ECAS and neuroimaging metrics of grey matter densities and white matter integrity of frontotemporal regions in ALS patients.

Neuroanatomical Associations of the Edinburgh Cognitive and Behavioural ALS Screen (ECAS)

Sneha Chenji[1], Abdullah Ishaque[1], Dennell Mah[2], Christian Beaulieu[3], Peter Seres[3], Simon Graham[4], Richard Frayne[5], Wendy Johnston[2], Lorne Zinman[4], Angela Genge[6], Lawrence Korngut[5], Sanjay Kalra[1,2]*

[1]Neuroscience and Mental Health Institute, University of Alberta, Edmonton, Canada

[2]Division of Neurology, Department of Medicine, University of Alberta, Edmonton, Canada

[3]Department of Biomedical Engineering, University of Alberta, Edmonton, Canada

[4]Sunnybrooke Hospital, University of Toronto, Ontario, Canada

[5]Calgary Neuromuscular Clinic, University of Calgary, Calgary, Canada

[6]Montréal Neurological Institute, McGill University, Montréal, Canada

*Corresponding Author. Address: 7-132F Clinical Sciences Building, 11350 83 Ave, Edmonton, AB T6G 2G3. Email: kalra@ualberta.ca

3.1 Abstract

Background: While loss of motor function is the characteristic feature of Amyotrophic Lateral Sclerosis (ALS), cognitive impairment secondary to frontotemporal lobar degeneration (FTLD) is now well recognised. The Edinburgh Cognitive and Behavioural ALS Screen (ECAS) captures this range of cognitive impairment in the clinical setting and is emerging as the preferred screening tool in ALS, testing cognitive domains such as language, verbal fluency, executive, memory and visuospatial abilities.

Objective: To identify the neuroanatomical correlates of the ECAS in ALS. It was hypothesised that: (1) patients impaired on the ECAS have lower grey matter density and reduced white matter integrity in frontotemporal regions compared to controls, and (2) there is an association between the ECAS and neuroimaging metrics of grey matter density and white matter integrity of frontotemporal regions in ALS patients.

Methods: Fifty-three patients and 43 healthy controls were recruited from four sites as a part of the Canadian ALS Neuroimaging Consortium (CALSNIC). All participants were administered the ECAS and underwent a multimodal magnetic resonance imaging (MRI) protocol harmonised across sites. 3D-T1 (1mm isotropic) and DTI sequences (2mm isotropic, b=1000s/mm^2, 30 non-collinear directions) were considered for the current study. Voxel-based morphometry (VBM) and Tract-based Spatial Statistics (TBSS) were employed to compare grey matter and white matter changes respectively. Voxel-wise correlations with verbal fluency and executive function scores were performed to identify neuroanatomical associations.

Results: Group comparisons revealed lower performance for ALS patients compared to controls in the ECAS verbal fluency and executive domains (p<0.01). Language, memory and visuospatial abilities in ALS were comparable to controls (p>0.01). Twenty-two patients (42%) impaired on executive functions (EF, indicated by verbal fluency or executive domains) were classified as ALS-exi, while the remaining 31 (58%) patients with normal EF were classified as ALS-n. Reduced grey matter density and reduced white matter integrity were found in the full cohort of ALS patients in bilateral motor regions, corticospinal tract and prefrontal regions. ALS-n patients showed abnormalities in motor and prefrontal regions in grey and white matter (p<0.001). The ALS-exi group showed a greater distribution of white matter degeneration in the frontal regions. Correlations with verbal fluency and executive scores revealed focal associations with grey matter density and some associations with white matter integrity in all ALS patients.

Conclusions: Executive impairment was detected using the ECAS in our sample of Canadian ALS patients, in line with previous studies. Frontotemporal changes noted in cognitively normal (ALS-n) patients supports that degeneration beyond motor regions is present in patients with intact cognition. A greater spatial extent of degeneration was present in cognitively impaired (ALS-exi) patients. Regions of degeneration align with pTDP-43 spread in ALS. To our best knowledge this is the first study reporting combined grey and white matter associations with the ECAS.

Keywords: ECAS, Cognitive screening, Amyotrophic Lateral Sclerosis, Cognitive impairment

3.2 Introduction

Clinical incidence of degeneration of both upper and lower motor neurons (UMN and LMN respectively) is required for a diagnosis of amyotrophic lateral sclerosis (ALS). This terminal neurodegenerative condition is characterised by clinical signs of hypertonia, hyperreflexia (UMN sings) in combination with muscle atrophy, weakness, fasciculations (LMN signs) either in bulbar or limb regions. Survival is estimated at 2-3 years after diagnosis and LMN driven respiratory failure accounts for death in most patients with ALS (Brooks et al., 2000; Kiernan et al., 2011). Additional features include cognitive or behavioural changes associated with frontotemporal lobar degeneration (FTLD). . The most prevalent presentation of this is mild to moderate executive dysfunctions and / or apathy in 25-50% patients, while 10-15% may meet criteria for frontotemporal dementia (FTD) (Phukan et al., 2012).

Verbal fluency is the hallmark of executive dysfunction in ALS with patients performing lower than controls in tests of letter-based fluency (Abe et al., 1997; Abrahams et al., 1997; Abrahams et al., 2000; Kew, Goldstein, et al., 1993; Phukan et al., 2012). Other aspects of executive functions such as poor planning and decision making, language and more recently, social cognition are also reported impaired in ALS (Goldstein & Abrahams, 2013). Memory deficits have been reported in smaller ALS sub-groups (Abdulla et al., 2014; Machts et al., 2014) with studies suggesting that memory deficits may be driven by executive dysfunction in ALS (Burke et al., 2017). Most of these studies include extensive neuropsychometric testing with administration time ranging from 1-3 hours (Goldstein & Abrahams, 2013). Administration of such extensive tests may be challenging in a clinical setting given the time constraints of a busy clinic. With advancement in ALS research and implication of

involvement of different brain regions at specific stages of the disease course, there is an emerging emphasis on sub-groups that may be present in ALS patients (Braak et al., 2013; Brettschneider et al., 2013; Müller et al., 2016). Identifying these cognitive sub-groups may be crucial for clinical trials and biomarker research as each group may reflect characteristic features that would enable researchers to better understand underlying disease pathology or spread.

The Edinburgh Cognitive and Behavioural ALS Screen (ECAS) was developed to address the above needs and is emerging as an optimal screening tool in ALS (Abrahams et al., 2014). It tests cognitive domains such as language, verbal fluency, executive, memory and visuospatial abilities. The ECAS behavioural screen completed by the caregivers assesses apathy, disinhibition, loss of empathy, stereotypical / perseverative behaviours and changes in dietary habits. The ECAS has been validated (Niven et al., 2015; Pinto-Grau et al., 2017) and adapted to multiple languages (Loose et al., 2016; Lulé et al., 2015; Poletti et al., 2016; Siciliano et al., 2017; Ye et al., 2016). However, few studies have investigated neuroimaging associations using the ECAS (Keller et al., 2017; Lulé et al., 2018) and none have investigated structural brain differences in cognitive sub-groups derived using the ECAS.

The present study aimed at identifying the neuroanatomical associations of the cognitive domains of the ECAS. It was hypothesised that: (1) patients impaired on the ECAS have lower grey matter density and reduced white matter integrity in frontotemporal regions, and (2) there is an association between the ECAS and neuroimaging metrics of grey matter density and white matter integrity of frontotemporal regions in ALS patients.

3.3 Methods

3.3.1 Participants

The participants for the current study were recruited from ALS clinics at Calgary, Edmonton, Montreal and Toronto (referred to as sites 1-4 respectively) as a part of the Canadian ALS Neuroimaging Consortium (CALSNIC). Participants with incorrect ECAS administrations, English as their second language and age > 80 years were excluded from analysis. Ethics approval was obtained from each site and all participants signed a written informed consent for the study.

Fifty-three ALS patients met inclusion criteria for the study. Demographic details of patients are outlined in Table 3.1. All patients were diagnosed as possible, probable, probable lab-supported or definite ALS as per the revised El-Escorial criteria (Brooks et al., 2000). Forty-three patients presented with limb-onset, 8 with bulbar onset, and 2 with both bulbar and limb onset (Table 3.2). Patients had mild-moderate disability as measured by the ALS Functional Rating Scale – Revised (ALSFRS-R) (Cedarbaum et al., 1999). Respiratory status was measured with forced vital capacity (FVC, percent reference). Disease progression rate was computed as: (date of testing – symptom onset) / ALSFRS-R score (Kimura et al., 2006). In addition to this, a scale of UMN burden was derived from data collected in neurological evaluations. The scale includes two lateralized sub-scores for the right and left side of the body, and a sub-score for jaw related UMN symptoms. These scores account for the presence of increase in muscle tone and hyperreflexia in respective limbs, the presence of Babinski's sign or clonus in the lower extremity and the presence of brisk reflex or clonus in the jaw. Clinical characteristics of all patients and at each site are outlined in Table 3.2.

Forty-three healthy volunteers met inclusion and exclusion criteria for the study. All volunteers were required to be above 40 years of age, unless matched to a patient below 40 years of age. Inclusion criteria for volunteers also required that they have no history of neurological or psychiatric illness. The demographics of the overall and per site control group are described in Table 3.1.

3.3.2 ECAS and Cognitive Sub-groups

The ECAS is a screening tool with an average administration time of 20 to 30 minutes (Abrahams et al., 2014). It can be administered in a written or spoken version, thereby accommodating for motor impairments in ALS patients. A North American adaptation of the ECAS (adapted by Dr. Sharon Abrahams) was used for the current study. The ECAS includes assessment of the cognitive domains of language (naming, spelling and comprehension), verbal fluency (letter S, four-letter words beginning with T), executive (digit span backward, alternation, sentence inhibition, social cognition), memory (immediate and delayed recall, recognition) and visuospatial abilities (dot counting, cube counting, dot position). These five domains are summed to obtain the ECAS total score. The individual scores of the domains and the ECAS total score were considered for analysis.

An executively-impaired cognitive sub-group was determined using the verbal fluency or executive domain scores. Patients were regarded as impaired on EF (ALS-exi) if they obtained scores below 2SD from the mean scores of the control group for either ECAS verbal fluency or executive scores. Patients who scored above the cut-off scores were classified as normal on EF (ALS-n).

Beck's Depression Inventory - II (BDI-II) (Beck, Steer, & Brown, 1996) was used to assess mood in the current sample. The behavioural screen of the ECAS was completed by caregivers in a subset of 15 patients.

3.3.3 MRI acquisition

A harmonised MRI protocol was implemented across the CALSNIC sites of which three-dimensional T1 sequences (3D-T1) and diffusion tensor imaging (DTI) were considered for the current study (

Table 3.3). In-house experiments revealed good reliability for T1 and DTI (Lee, Eurich, Mah, Hanstock, & Kalra, 2016). Nevertheless, to reduce confounding effects, site was controlled for as a factor in statistical models.

3.3.4 MRI preprocessing

3.3.4.1 Voxel based morphometry

Voxel-wise analysis of 3D-T1 data was performed using the Computational Anatomy Toolbox (CAT-12) in SPM-12 (http://dbm.neuro.uni-jena.de/cat12/). All T1 images were aligned to the AC-PC axis using Mango toolbox (Research Imaging Institute, UTHSCA). Next, corrections for field intensity inhomogeneities were completed and the images were normalised to the ICBM template for European brains using both linear and non-linear transformations. These images were segmented into grey matter, white matter and cerebrospinal fluid (CSF) tissue maps. The grey matter tissue maps were smoothed using $8mm^3$ full-width half maximum (FWHM) kernel for further analysis. Group comparisons

for patients and controls as well as with patient sub-groups (ALS-exi / ALS-n) were performed using the full-factorial model in SPM-12 with diagnosis and site as factors, and age and total intracranial volume (TIV) as covariates. Frontal and temporal masks from the WFU Pickatlas toolbox (Maldjian, Laurienti, Kraft, & Burdette, 2003) were employed for identifying regions of interest (ROI) based correlations between grey matter densities and ECAS scores.

3.3.4.2 Diffusion Tensor Imaging (DTI)

The DTI images of each participant were preprocessed to correct for temporal signal drifts, Gibbs ringing artifacts, motion, Eddy currents and EPI distortions using Explore DTI (Leemans, Jeurissen, Sijbers, & Jones, 2009). The previous step co-registered DTI images with T1 scans to minimise anatomical distortions. To facilitate optimal registration of white matter, the preprocessed DTI images were subjected to the DTI Toolkit (DTI-TK) processing pipeline (Zhang, Avants, et al., 2007) and were registered to a study-specific template (Zhang, Yushkevich, Rueckert, & Gee, 2007). Registration involved affine and deformable alignments with template refinement to allow for alteration of global size and shape of DTI images to match the study-specific template as well as minimize differences of size and shape in local structures respectively. Individual DTI volumes were warped to the final template using a single interpolation operation that combined affine and deformable alignments. The registered DTI volumes were then subjected to a custom implementation of post-registration step of tract based spatial statistics (TBSS) for further voxel-wise analysis (Bach et al., 2014). Site and diagnosis were used as a factor and age was a covariate in the GLM models.

3.3.5 Statistical Analysis

Demographic factors such as age and education and mood (BDI-II scores) were compared between the patient and control groups using Mann-Whitney U-test. Clinical characteristics of ALS patients between sites were compared using Kruskal-Wallis test. Normality of the ECAS data pooled from all sites was tested using Shapiro-Wilk test (Supplementary Table 3.1). Considering the non-normal distributions, Mann-Whitney U-test was employed to test differences in performance between controls and patients. Kruskal-Wallis test was used to identify differences in the control or patient groups across sites. For cognitive domains that reached significance threshold ($p<0.05$), post-hoc group comparisons of sub-tests in each domain were performed. Multiple comparisons were corrected using false discovery rate (FDR) for ECAS analysis and threshold free cluster enhancement (TFCE) for imaging analyses at $p<0.05$. Lenient significance thresholds at $p<0.001$ (uncorrected) were also explored to identify mild changes.

3.4 Results

3.4.1 Demographic and Clinical characteristics

The sample was matched for gender ($\chi^2 = 1.3$, $p = 0.26$) and age, $U = 1236.5$, $p = 0.36$ (Median-$_{HC}$ = 56.0 years, Median-$_{ALS}$ = 57.0 years; see Table 3.1). Education was significantly higher in controls (Median-$_{HC}$ = 16.0 years) as compared to patients (Median-$_{ALS}$ = 15.0 years), $U = 844.5$, $p = 0.03$. BDI-II total was also significantly higher in patients (Median-$_{ALS}$ = 15.0 years) as compared to controls (Median-$_{HC}$ = 3.0), $U = 1652.0$, $p < 0.01$. Within controls and patients, there were no significant differences across sites for age (p-$_{HC}$ = 0.10, p-$_{ALS}$ = 0.18), education (p-$_{HC}$ = 0.53, p-$_{ALS}$ = 0.45) and BDI-II (p-$_{HC}$ = 0.87, p-$_{ALS}$ =

0.52). Education and BDI-II did not show significant associations with ECAS performance and were not used as covariates in group comparisons.

Clinical characteristics of the patient group are outlined in Table 3.2. The median symptom duration was 24.0 months (range: 7-129 months). Mild-moderate disability, lower disease progression and above-average respiratory capacity was noted as indicated by the ALSFRS-R score (Median = 39.5, range: 22-47), disease progression rate (Median = 0.3, range: 0.01-1.2) and FVC (Median = 93.0, range: 52-144) respectively. Lower UMN burden was noted among patients (Median = 5.0). Symptom duration was significantly different between sites (χ^2 [3] = 15.7, $p < 0.01$) and post-hoc analysis revealed longer duration for Sites 1 and 4 (Supplementary Figure 3.1A). Similarly, ALSFRS-R scores were also significantly different between sites (χ^2 [3] = 10.2, $p = 0.02$), with post-hoc tests revealing lower ALSFRS-R score for Site 1 (Supplementary Figure 3.1B). Both symptom duration and ALSFRS-R were not associated with performance on the ECAS and hence were not considered as covariates in group comparisons. There were no significant differences in the disease progression rates, FVC and UMN burden between sites. Only the disease progression rate was associated with ECAS language score (r_s = -0.3, p = 0.03) while the other clinical variables showed no associations with ECAS performance.

3.4.2 Lower ECAS performance in ALS

Scores were non-normally distributed on the ECAS (Supplementary Table 3.1 and Supplementary Figure 3.2). The ALS patients displayed significantly lower performance on the ECAS total score ($Median_{-ALS}$ = 108.8, range: 73-121) as compared to controls ($Median_{-HC}$ = 115.0, range: 94-128), U = 645.0, p<0.001. Specifically, ALS patients performed poorly

on verbal fluency (U = 747.5, p<0.01) and executive domains of the ECAS (U = 704.0, p<0.01; Figure 3.1). Post-hoc comparisons revealed that within verbal fluency, patients performed poorly for both letter S (U = 794.5, p<0.01) and letter T fluency (U = 767.0, p<0.01), and within the Executive domain, patients performed poorly for reverse digit span (U = 820.5, p=0.02), alternation (U = 930.5, p=0.04) and sentence completion (U = 773.5, p<0.01), but not social cognition (see Figure 3.2). Performance of patients and controls within each site is displayed in Supplementary Figure 3.3.

Twenty-two patients were classified as impaired on EF (ALS-exi) while 31 patients had normal EF (ALS-n). These classifications were used to explore and identify neuroanatomical differences between the sub-groups. Impairments on individual domains of the ECAS are displayed in Supplementary Table 3.2.

3.4.3 Grey matter degeneration as indicated by VBM

Group comparisons revealed lower grey matter densities in the motor cortex, premotor and medial prefrontal cortex (mPFC) in ALS patients when compared to healthy controls (p<0.001, uncorrected; Figure 3.3A). Sub-group analysis revealed grey matter reductions in the precentral gyrus and the mPFC in ALS-n patients when compared to healthy controls (p<0.001, uncorrected; Figure 3.3B). ALS-exi patients displayed grey matter reductions in the bulbar region of the precentral gyrus, inferior frontal gyrus and insula in the right hemisphere when compared to healthy controls (p<0.001, uncorrected; Figure 3.3C). Group comparisons between the two cognitive sub-groups in patients revealed lower grey matter density in the right insula and left precuneus in ALS-exi patients as compared to ALS-n patients (p<0.001, uncorrected; Figure 3.3D).

3.4.4 Widespread loss of white matter integrity in ALS

Lower white matter integrity as indicated by poor FA was noted in the upper segment of the CST and body of the corpus callosum (CC) in ALS patients, when compared to healthy controls (p<0.05, TFCE corrected; Figure 3.4A). At more lenient thresholds, degeneration in the white matter were noted in the internal capsule of the CST and more frontally in the CC (p<0.001, uncorrected; Figure 3.4A). Regarding patient sub-groups, ALS-n patients displayed reduced FA in the CST and corpus callosum when compared to healthy controls (p<0.001, uncorrected; Figure 3.4B). Similar changes were noted for ALS-exi as compared to healthy controls with greater involvement of anterior corpus callosum (p<0.001, uncorrected; Figure 3.4C). This spread is illustrated in comparison of the sub-groups, whereby ALS-exi patients displayed reduced FA in the corpus callosum and the body of the cingulum compared to ALS-n patients (p<0.001, uncorrected; Figure 3.4D). There was no difference in FA within the CST of ALS-exi patients compared to ALS-n patients.

3.4.5 Frontotemporal associations of grey and white matter with ECAS

Lower performance on ECAS verbal fluency was associated with lower grey matter densities in the right precentral gyrus, left premotor cortex and superior frontal gyrus (p<0.001, uncorrected; Figure 3.5A top-panel). Lower verbal fluency was also associated with reduced FA in bilateral SLF (p<0.001, uncorrected; Figure 3.5A bottom-panel).

Reduced executive performance on the ECAS was associated with lower grey matter densities in the left posterior superior temporal cortex (p<0.001, uncorrected; Figure 3.5B top-panel) and reduced FA in the temporal stream of the SLF (p<0.001, uncorrected; Figure 3.5B bottom panel).

3.5 Discussion

The current study aimed at evaluating the neuroanatomical associations of the ECAS in ALS patients. ECAS performance of patients was lower for ECAS total, verbal fluency and executive scores. Grey matter atrophy in frontal regions and widespread loss of white matter integrity was noted for both cognitive sub-groups of patients, with and without executive dysfunctions.

3.5.1 Executive dysfunction detected by ECAS

To our best knowledge, this is the first report on the clinical use of the North American adaptation of the ECAS. The observed cognitive profile in patients reflected executive dysfunction as commonly reported in ALS cohorts. Verbal fluency and executive domains on the ECAS were found to be impaired in ALS patients, as indicated by previous studies (Lulé et al., 2015; Ye et al., 2016). Poor language and memory performance reported previously on the ECAS was not present in the current sample (Lulé et al., 2015; Ye et al., 2016). Ad-hoc investigation was performed to identify language impairments and a proportion of patients (n=6, 11%) scored below 2SD cut-offs for ECAS language. Among these patients, five were classified as ALS-exi, and one patient indicated borderline performance (1.5-2SD) for the executive domain. This proportion of patients with impaired language is lower than in previous reports (Taylor et al., 2013). However, the difference could be attributed to the nature of the tests employed (screening vs. extensive neuropsychometric assessments) and the exclusion of non-native English speakers. There were no differences noted in the visuospatial domain-, although ad-hoc analysis indicated impairments in a very small proportion of patients (Supplementary Table 3.2).

3.5.2 Frontotemporal changes are present in EF normal (ALS-n) patients

Neuroimaging analysis of the patient group showed degeneration in the motor regions as well as frontal regions on grey matter densities, while white matter displayed loss of integrity along the CST and CC. Similar patterns of change in grey matter and white matter were reported previously in overall group comparisons (ALS vs HC) and sub-group analysis of ALS-n patients (Agosta et al., 2016; Chang et al., 2005; Christidi, Karavasilis, Riederer, et al., 2018; Kasper et al., 2014; Menke et al., 2014). Thus, the current study highlights the frontal spread of the disease in ALS. Reduced grey matter density was noted in the mPFC in the ALS-n sub-group. Similarly, loss of white matter integrity in the ALS-n sub-cohort indicated changes along the CC.

Three ALS-n patients presented with borderline performance (1.5 – 2SD) on either verbal fluency or Executive domain scores, of which one ALS-n patient was impaired on the Language domain. Reduced grey matter density in the mPFC persisted even when these borderline performers were excluded from the analysis. Both the ALS-n and ALS-exi groups did not differ on demographic or clinical variables, therefore it is unlikely that clinical factors contribute to the extra-motor changes noted in ALS-n patients. Thus, extra-motor changes may be present in ALS patients with intact cognition and may precede cognitive decline, similar to pre-symptomatic brain changes in ALS reported previously (Menke et al., 2016; Sgobio et al., 2008). However, it may also indicate the limitation of neuropsychometric testing in detection of mild cognitive changes (Christidi, Karavasilis, Riederer, et al., 2018). This latter hypothesis may explain findings of extra-motor changes in ALS patients with normal EF. It is also possible that a sub-set of ALS-n patients may have behavioural changes that were not captured in the study. Furthermore, given the subtle nature of cognitive changes

in ALS, impairments may develop over time and patients may show a greater cognitive / behavioural impairment at a later stage. Considering the cross-sectional nature of the study, it is beyond the scope of the current study to investigate conversion rate of cognitive impairment in ALS-n patients.

3.5.3 Greater extent of atrophy in EF impaired (ALS-exi) patients

In the ALS-exi group, grey matter degeneration was prevalent in the right hemisphere including the bulbar region of the precentral gyrus, inferior frontal gyrus and the insula. There was no evidence of grey matter loss in the mPFC at the set significance threshold. Reduced grey matter density in the precuneus was noted in the ALS-exi group as compared to the ALS-n group, in line with one previous study (Christidi, Karavasilis, Riederer, et al., 2018). Precuneus cortical thinning was also reported in patients with C9orf72 mutations (Bede, Bokde, Elamin, et al., 2013; Bede, Bokde, Byrne, et al., 2013; Westeneng et al., 2016). The precuneus is considered to be involved in cognitive functions such as visuospatial, memory and processing of autobiographical information. It is also a region of high activity in resting state and decreases in activity during goal-oriented behaviour in healthy participants (Utevsky, Smith, & Huettel, 2014) Functional studies in ALS have reported increased activations in the precuneus, suggesting that it may contribute to maintaining cognitive function in the context of degenerating frontotemporal networks (Agosta et al., 2013; Menke et al., 2016).

Degeneration in the white matter was noted in the CST, the CC and the cingulum revealing greater spread in the ALS-exi group (Christidi, Karavasilis, Riederer, et al., 2018; Kasper et al., 2014). The extent of degeneration in ALS-exi patients was greater in the CC, along the

association fibres in the frontal regions, especially the SLF, and no differences were noted in the CST, when compared to the ALS-n group, supporting previous findings (Kasper et al., 2014). One previous study indicated similar but less extensive changes in a smaller sub-group of cognitively impaired (n=9) and competent (n=5) ALS patients (Dimond et al., 2017). Another study indicated extensive cortical thinning and white matter loss in MND patients with cognitive and / or behavioural impairments (MND-plus) when compared to unimpaired patients (MND-motor) and heathy controls, with higher severity in temporal lobes (Agosta et al., 2016). The authors reported a higher number of MND-plus patients and attributed this to the inclusion of patients with ALS as well as both UMN and LMN phenotype variants (primary lateral sclerosis [PLS] and progressive muscular atrophy [PMA] respectively). Cognitive (39% PLS, 36% PMA) and behavioural (13% PLS) impairments were identified in these phenotype variants, thereby providing neuroimaging evidence of FTLD pattern in the MND-plus group. However, the authors did not distinguish changes within these MND-plus phenotypes. Overall, our findings are in line with previous literature suggesting a greater extent of degeneration in ALS-exi patients.

3.5.4 Neuroanatomical implications of ECAS and MRI metrics

The performance on ECAS verbal fluency and executive scores was associated with focal regions in the grey matter and white matter. Lower verbal fluency was associated with reduced grey matter density in the right precentral gyrus, left premotor and left superior frontal gyri. In healthy controls, a laterality in performance during verbal fluency tasks have been reported, such that left hemisphere, especially Broca's area, is dominant during letter fluency and the right hemisphere is dominant during automatic speech (Birn et al., 2010). In the current study, no specific lateralization was noted. However, the association with left

premotor cortex and right precentral gyri suggests involvement of articulatory processes to generate the words. The involvement of superior frontal gyrus may reflect a broader network contributing to executive dysfunction. The association of reduced grey matter density in posterior region and reduced executive performance is typical. It is likely that the ECAS executive domain requires sustained attention, typically associated with the parietal lobe.

The pattern of grey matter atrophy as detected by VBM may suggest loss of neurons in the motor and prefrontal regions (Abe et al., 1997). These regions of atrophy align with regions displaying phosphorylated TAR-DNA binding protein 43 (pTDP-43) inclusions in ALS (Braak et al., 2013; Brettschneider et al., 2013). A recent study indicated a trend in association between pTDP-43 and lower synaptic density in the prefrontal cortex (BA 9 region) of ALS patients (Henstridge et al., 2018). The study proposed an alternative hypothesis that loss of synaptic density (which precedes neuronal loss) and not pTDP-43 pathology per se may be associated with cognitive impairment in ALS. A sub-cohort of 23 ALS patients in Henstridge et al., (2018) were also administered the ECAS of which 7 patients had cognitive impairment, as indicated by ECAS Total or ALS-specific scores, and these patients displayed lower synaptic density as compared to controls. No difference in synaptic density was noted between the cognitively impaired vs. unimpaired ALS groups, which could be attributed to the small sample size. The authors reported that lower ECAS performance was not associated with cortical thickness, pTDP-43 pathology or beta-amyloid burden. Another interesting exploratory finding in Henstridge et al., (2018) revealed that patients with phosphorylated tau (pTau) pathology (CSF marker indicating neuronal injury; (Grossman et al., 2014) were more prevalent in the cognitively impaired group and pTau negative cases were frequent in the unimpaired group (Henstridge et al., 2018). However,

pTau was not associated with synaptic loss and thus remains to be assessed further as a potential biomarker for cognitive impairment in ALS. Together, these findings suggest that atrophy detected in the current study indicate changes in the grey matter. However, its neuroanatomical underpinnings remain to be determined.

Loss of white matter integrity as indicated by DTI is in line with reduced density of white matter reported previously in ALS (Rafalowska & Dziewulska, 1996). Previous studies have indicated that FA changes in ALS indicate a loss of integrity of the axonal walls (Agosta et al., 2010; Christidi, Karavasilis, Riederer, et al., 2018; Kasper et al., 2014). A large scale multi-centre study reported white matter changes in ALS to correspond with pTDP-43 staging in ALS, such that the motor cortex displayed the highest degree of degeneration (stage 1) followed by changes in reticular formation, red nucleus and precerebellar nuclei in the brainstem (stage 2), somatosensory cortex, orbital gyrus and striatum (stage 3), and anteromedial temporal lobe and hippocampal formation (stage 4) (Brettschneider et al., 2013; Müller et al., 2016). This has been computationally validated indicating that these pTDP-43 regions form a densely interconnected sub-network within the brain and may confine pathological spread within this sub-network (Schmidt, de Reus, Scholtens, van den Berg, & van den Heuvel, 2016). Recent studies have proposed cognitive staging using ECAS and white matter profiles, displaying moderate-to-high congruency between executive functions (stage 2), disinhibition (stage 3) and memory (stage 4) performance and the corresponding DTI-based stages (Lulé et al., 2018), thereby suggesting that pTDP-43 spread may indeed explain poor cognition in ALS patients.

Contradictory to this DTI-based and pTDP-43 association along white matter tracts, another study reported that pTDP-43 pathology is confined to the grey matter regions and localised

sub-cortical oligodendroglia adjacent to the cortex (Fatima et al., 2015). The authors reported pTDP-43 pathology in the grey matter regions and sub-cortical oligodendroglial pTDP-43 of the motor cortex, brainstem, sensory motor cortex, striatum and hippocampus, while there was an absence of pTDP-43 aggregates in the posterior limb of the internal capsule (CST), corpus callosum and the cingulum bundle of ALS patients. Thus, the loss of white matter integrity may not be directly related to pTDP-43 aggregates or inclusions within deep white matter tracts, but in fact may be associated with an overall structural change reflecting on-going degeneration in ALS patients. Indeed, verbal fluency was associated with overall structural integrity of tracts such as the SLF suggesting that diffuse structural integrity and not localised connectivity may contribute to executive dysfunction.

3.5.5 Strengths, Limitations and Future Directions

The ECAS is an emerging standard test for assessing cognition in ALS. To our best knowledge, this the first study providing evidence of both grey and white matter associations with ECAS performance using data derived from a multicentre standardised protocol. Previous studies providing neuroimaging evidence have employed general cognitive screening measures such as the Addenbrooke's Cognitive Exam – Revised (ACE-R) (Menke et al., 2014; Mioshi et al., 2006; Mioshi et al., 2013) or have employed extensive neuropsychometric testing for identifying ALS sub-groups (Agosta et al., 2016; Christidi, Karavasilis, Riederer, et al., 2018; Sarro et al., 2011). The cognitive sub-groups in the current study were matched for age, education and other clinical parameters such as ALSFRS-R, FVC, symptom duration and disease progression rate, thus suggesting that neuroanatomical substrates reflect disease-related pathology.

Some limitations in the study include the use of uncorrected p-values, which increases chances of Type-I errors. The use of multiple-comparisons corrections confined atrophy only to the motor regions and thus removed any subtle changes noted in the sample. Increasing the sample size would provide statistical power to deal with such issues and strengthen sub-group analysis. Incorporating longitudinal analysis would enable investigation of both spatial and temporal neuroanatomical correlates, and their predictive potential for the development and progression of cognitive impairment. The current sample did not include a sufficient number of patients with memory impairments (n=1) and thus restricted exploration of this sub-group. Incorporating behavioural assessment would enable a comprehensive assessment of frontal-clinical associations.

3.6 Conclusions

In summary, using a standardised multicentre protocol, the ECAS detected executive dysfunctions in ALS. Frontal structural changes are present in cognitively normal (ALS-n) patients, while greater extent of degeneration was noted for cognitively impaired (ALS-exi) patients. The presence of extra-motor grey matter changes corresponds with pTDP-43 spread (including ALS-n and ALS-exi sub-groups), while white matter changes are widespread. Associations between structural MRI metrics, verbal fluency or executive performance showed that loss of grey and white matter matter may contribute to reduced performance on these tasks.

3.7 References

Abdulla, S., Machts, J., Kaufmann, J., Patrick, K., Kollewe, K., Dengler, R., . . . Nestor, P. J. (2014). Hippocampal degeneration in patients with amyotrophic lateral sclerosis. *Neurobiol Aging, 35*(11), 2639-2645. doi:10.1016/j.neurobiolaging.2014.05.035

Abe, K., Fujimura, H., Toyooka, K., Sakoda, S., Yorifuji, S., & Yanagihara, T. (1997). Cognitive function in amyotrophic lateral sclerosis. *J Neurol Sci, 148*(1), 95-100.

Abrahams, S., Goldstein, L. H., Al-Chalabi, A., Pickering, A., Morris, R. G., Passingham, R. E., . . . Leigh, P. N. (1997). Relation between cognitive dysfunction and pseudobulbar palsy in amyotrophic lateral sclerosis. *Journal of Neurol Neurosurg Psychiatry, 62*(5), 464-472.

Abrahams, S., Leigh, P. N., Harvey, A., Vythelingum, G. N., Grise, D., & Goldstein, L. H. (2000). Verbal fluency and executive dysfunction in amyotrophic lateral sclerosis (ALS). *Neuropsychologia, 38*(6), 734-747.

Abrahams, S., Newton, J., Niven, E., Foley, J., & Bak, T. H. (2014). Screening for cognition and behaviour changes in ALS. *Amyotroph Lateral Scler Frontotemporal Degener, 15*(1-2), 9-14. doi:10.3109/21678421.2013.805784

Agosta, F., Canu, E., Valsasina, P., Riva, N., Prelle, A., Comi, G., & Filippi, M. (2013). Divergent brain network connectivity in amyotrophic lateral sclerosis. *Neurobiol Aging, 34*(2), 419-427.

Agosta, F., Ferraro, P. M., Riva, N., Spinelli, E. G., Chio, A., Canu, E., . . . Filippi, M. (2016). Structural brain correlates of cognitive and behavioral impairment in MND. *Hum Brain Mapp, 37*(4), 1614-1626. doi:10.1002/hbm.23124

Agosta, F., Pagani, E., Petrolini, M., Caputo, D., Perini, M., Prelle, A., . . . Filippi, M. (2010). Assessment of white matter tract damage in patients with amyotrophic lateral sclerosis: a diffusion tensor MR imaging tractography study. *AJNR Am J Neuroradiol, 31*(8), 1457-1461. doi:10.3174/ajnr.A2105

Bach, M., Laun, F. B., Leemans, A., Tax, C. M., Biessels, G. J., Stieltjes, B., & Maier-Hein, K. H. (2014). Methodological considerations on tract-based spatial statistics (TBSS). *Neuroimage, 100*, 358-369. doi:10.1016/j.neuroimage.2014.06.021

Beck, A. T., Steer, R. A., & Brown, G. K. (1996). *Manual for the Beck Depression Inventory-II*. San Antonio, TX: Psychological Corporation.

Bede, P., Bokde, A., Elamin, M., Byrne, S., McLaughlin, R. L., Jordan, N., . . . Hardiman, O. (2013). Grey matter correlates of clinical variables in amyotrophic lateral sclerosis (ALS): a neuroimaging study of ALS motor phenotype heterogeneity and cortical focality. *J Neurol Neurosurg Psychiatry, 84*(7), 766-773. doi:10.1136/jnnp-2012-302674

Bede, P., Bokde, A. L., Byrne, S., Elamin, M., McLaughlin, R. L., Kenna, K., . . . Hardiman, O. (2013). Multiparametric MRI study of ALS stratified for the C9orf72 genotype. *Neurology, 81*(4), 361-369.

Braak, H., Brettschneider, J., Ludolph, A. C., Lee, V. M., Trojanowski, J. Q., & Del Tredici, K. (2013). Amyotrophic lateral sclerosis--a model of corticofugal axonal spread. *Nat Rev Neurol, 9*(12), 708-714. doi:10.1038/nrneurol.2013.221

Brettschneider, J., Del Tredici, K., Toledo, J. B., Robinson, J. L., Irwin, D. J., Grossman, M., . . . Trojanowski, J. Q. (2013). Stages of pTDP-43 pathology in amyotrophic lateral sclerosis. *Ann Neurol, 74*(1), 20-38. doi:10.1002/ana.23937

Brooks, B. R., Miller, R. G., Swash, M., Munsat, T. L., & World Federation of Neurology Research Group on Motor Neuron, D. (2000). El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Other Motor Neuron Disord, 1*(5), 293-299.

Burke, T., Lonergan, K., Pinto-Grau, M., Elamin, M., Bede, P., Madden, C., . . . Pender, N. (2017). Visual encoding, consolidation, and retrieval in amyotrophic lateral sclerosis: executive function as a mediator, and predictor of performance. *Amyotroph Lateral Scler Frontotemporal Degener, 18*(3-4), 193-201. doi:10.1080/21678421.2016.1272615

Cedarbaum, J. M., Stambler, N., Malta, E., Fuller, C., Hilt, D., Thurmond, B., & Nakanishi, A. (1999). The ALSFRS-R: a revised ALS functional rating scale that incorporates assessments of respiratory function. BDNF ALS Study Group (Phase III). *J Neurol Sci, 169*(1-2), 13-21.

Chang, J. L., Lomen-Hoerth, C., Murphy, J., Henry, R. G., Kramer, J. H., Miller, B. L., & Gorno-Tempini, M. L. (2005). A voxel-based morphometry study of patterns of

brain atrophy in ALS and ALS/FTLD. *Neurology, 65*(1), 75-80. doi:10.1212/01.wnl.0000167602.38643.29

Christidi, F., Karavasilis, E., Riederer, F., Zalonis, I., Ferentinos, P., Velonakis, G., . . . Evdokimidis, I. (2018). Gray matter and white matter changes in non-demented amyotrophic lateral sclerosis patients with or without cognitive impairment: A combined voxel-based morphometry and tract-based spatial statistics whole-brain analysis. *Brain Imaging Behav, 12*(2), 547-563. doi:10.1007/s11682-017-9722-y

Dimond, D., Ishaque, A., Chenji, S., Mah, D., Chen, Z., Seres, P., . . . Kalra, S. (2017). White matter structural network abnormalities underlie executive dysfunction in amyotrophic lateral sclerosis. *Hum Brain Mapp, 38*(3), 1249-1268. doi:10.1002/hbm.23452

Fatima, M., Tan, R., Halliday, G. M., & Kril, J. J. (2015). Spread of pathology in amyotrophic lateral sclerosis: assessment of phosphorylated TDP-43 along axonal pathways. *Acta Neuropathol Commun, 3*, 47. doi:10.1186/s40478-015-0226-y

Goldstein, L. H., & Abrahams, S. (2013). Changes in cognition and behaviour in amyotrophic lateral sclerosis: nature of impairment and implications for assessment. *Lancet Neurol, 12*(4), 368-380. doi:10.1016/S1474-4422(13)70026-7

Grossman, M., Elman, L., McCluskey, L., McMillan, C. T., Boller, A., Powers, J., . . . Trojanowski, J. Q. (2014). Phosphorylated tau as a candidate biomarker for amyotrophic lateral sclerosis. *JAMA Neurol, 71*(4), 442-448. doi:10.1001/jamaneurol.2013.6064

Henstridge, C. M., Sideris, D. I., Carroll, E., Rotariu, S., Salomonsson, S., Tzioras, M., . . . Spires-Jones, T. L. (2018). Synapse loss in the prefrontal cortex is associated with cognitive decline in amyotrophic lateral sclerosis. *Acta Neuropathol, 135*(2), 213-226. doi:10.1007/s00401-017-1797-4

Kasper, E., Schuster, C., Machts, J., Kaufmann, J., Bittner, D., Vielhaber, S., . . . Prudlo, J. (2014). Microstructural white matter changes underlying cognitive and behavioural impairment in ALS--an in vivo study using DTI. *PLoS One, 9*(12), e114543. doi:10.1371/journal.pone.0114543

Keller, J., Bohm, S., Aho-Ozhan, H. E. A., Loose, M., Gorges, M., Kassubek, J., . . . Lule, D. (2017). Functional reorganization during cognitive function tasks in patients with amyotrophic lateral sclerosis. *Brain Imaging Behav*. doi:10.1007/s11682-017-9738-3

Kew, J. J., Goldstein, L. H., Leigh, P. N., Abrahams, S., Cosgrave, N., Passingham, R. E., . . . Brooks, D. J. (1993). The relationship between abnormalities of cognitive function and cerebral activation in amyotrophic lateral sclerosis. A neuropsychological and positron emission tomography study. *Brain, 116 (Pt 6)*, 1399-1423.

Kiernan, M. C., Vucic, S., Cheah, B. C., Turner, M. R., Eisen, A., Hardiman, O., . . . Zoing, M. C. (2011). Amyotrophic lateral sclerosis. *Lancet, 377*(9769), 942-955. doi:10.1016/S0140-6736(10)61156-7

Kimura, F., Fujimura, C., Ishida, S., Nakajima, H., Furutama, D., Uehara, H., . . . Hanafusa, T. (2006). Progression rate of ALSFRS-R at time of diagnosis predicts survival time in ALS. *Neurology, 66*(2), 265-267. doi:10.1212/01.wnl.0000194316.91908.8a

Lee, A., Eurich, D., Mah, D., Hanstock, C., & Kalra, S. (2016). *Advanced MRI in a Multicentre Study: Assessing the Reliability of Candidate Biomarkers in ALS*. ALS Canada Forum Toronto.

Leemans, A., Jeurissen, B., Sijbers, J., & Jones, D. K. (2009). *ExploreDTI: a graphical toolbox for processing, analyzing, and visualizing diffusion MR data.* Paper presented at the 17th Annual Meeting of Intl Soc Mag Reson Med, Hawaii, USA.

Loose, M., Burkhardt, C., Aho-Ozhan, H., Keller, J., Abdulla, S., Bohm, S., . . . Lule, D. (2016). Age and education-matched cut-off scores for the revised German/Swiss-German version of ECAS. *Amyotroph Lateral Scler Frontotemporal Degener*, 1-3. doi:10.3109/21678421.2016.1162814

Lulé, D., Bohm, S., Muller, H. P., Aho-Ozhan, H., Keller, J., Gorges, M., . . . Ludolph, A. C. (2018). Cognitive phenotypes of sequential staging in amyotrophic lateral sclerosis. *Cortex, 101*, 163-171. doi:10.1016/j.cortex.2018.01.004

Lulé, D., Burkhardt, C., Abdulla, S., Bohm, S., Kollewe, K., Uttner, I., . . . Ludolph, A. C. (2015). The Edinburgh Cognitive and Behavioural Amyotrophic Lateral Sclerosis Screen: a cross-sectional comparison of established screening tools in a German-

Swiss population. *Amyotroph Lateral Scler Frontotemporal Degener, 16*(1-2), 16-23. doi:10.3109/21678421.2014.959451

Machts, J., Bittner, V., Kasper, E., Schuster, C., Prudlo, J., Abdulla, S., . . . Bittner, D. M. (2014). Memory deficits in amyotrophic lateral sclerosis are not exclusively caused by executive dysfunction: a comparative neuropsychological study of amnestic mild cognitive impairment. *BMC Neuroscience, 15*, 83.

Menke, R. A., Korner, S., Filippini, N., Douaud, G., Knight, S., Talbot, K., & Turner, M. R. (2014). Widespread grey matter pathology dominates the longitudinal cerebral MRI and clinical landscape of amyotrophic lateral sclerosis. *Brain, 137*(Pt 9), 2546-2555.

Menke, R. A., Proudfoot, M., Wuu, J., Andersen, P. M., Talbot, K., Benatar, M., & Turner, M. R. (2016). Increased functional connectivity common to symptomatic amyotrophic lateral sclerosis and those at genetic risk. *J Neurol Neurosurg Psychiatry, 87*(6), 580-588. doi:10.1136/jnnp-2015-311945

Mioshi, E., Dawson, K., Mitchell, J., Arnold, R., & Hodges, J. R. (2006). The Addenbrooke's Cognitive Examination Revised (ACE-R): a brief cognitive test battery for dementia screening. *Int J Geriatr Psychiatry, 21*(11), 1078-1085. doi:10.1002/gps.1610

Mioshi, E., Lillo, P., Yew, B., Hsieh, S., Savage, S., Hodges, J. R., . . . Hornberger, M. (2013). Cortical atrophy in ALS is critically associated with neuropsychiatric and cognitive changes. *Neurology, 80*(12), 1117-1123.

Müller, H. P., Turner, M. R., Grosskreutz, J., Abrahams, S., Bede, P., Govind, V., . . . Neuroimaging Society in, A. L. S. D. T. I. S. G. (2016). A large-scale multicentre cerebral diffusion tensor imaging study in amyotrophic lateral sclerosis. *J Neurol Neurosurg Psychiatry, 87*(6), 570-579. doi:10.1136/jnnp-2015-311952

Niven, E., Newton, J., Foley, J., Colville, S., Swingler, R., Chandran, S., . . . Abrahams, S. (2015). Validation of the Edinburgh Cognitive and Behavioural Amyotrophic Lateral Sclerosis Screen (ECAS): A cognitive tool for motor disorders. *Amyotroph Lateral Scler Frontotemporal Degener, 16*(3-4), 172-179. doi:10.3109/21678421.2015.1030430

Phukan, J., Elamin, M., Bede, P., Jordan, N., Gallagher, L., Byrne, S., . . . Hardiman, O. (2012). The syndrome of cognitive impairment in amyotrophic lateral sclerosis: A population-based study. *Journal of Neurology & Psychiatry, 83*(1), 102-108.

Pinto-Grau, M., Burke, T., Lonergan, K., McHugh, C., Mays, I., Madden, C., . . . Pender, N. (2017). Screening for cognitive dysfunction in ALS: validation of the Edinburgh Cognitive and Behavioural ALS Screen (ECAS) using age and education adjusted normative data. *Amyotroph Lateral Scler Frontotemporal Degener, 18*(1-2), 99-106. doi:10.1080/21678421.2016.1249887

Poletti, B., Solca, F., Carelli, L., Madotto, F., Lafronza, A., Faini, A., . . . Silani, V. (2016). The validation of the Italian Edinburgh Cognitive and Behavioural ALS Screen (ECAS). *Amyotroph Lateral Scler Frontotemporal Degener, 17*(7-8), 489-498. doi:10.1080/21678421.2016.1183679

Rafalowska, J., & Dziewulska, D. (1996). White matter injury in amyotrophic lateral sclerosis (ALS). *Folia Neuropathol, 34*(2), 87-91.

Sarro, L., Agosta, F., Canu, E., Riva, N., Prelle, A., Copetti, M., . . . Filippi, M. (2011). Cognitive functions and white matter tract damage in amyotrophic lateral sclerosis: a diffusion tensor tractography study. *Ajnr: American Journal of Neuroradiology, 32*(10), 1866-1872.

Schmidt, R., de Reus, M. A., Scholtens, L. H., van den Berg, L. H., & van den Heuvel, M. P. (2016). Simulating disease propagation across white matter connectome reveals anatomical substrate for neuropathology staging in amyotrophic lateral sclerosis. *Neuroimage, 124*(Pt A), 762-769. doi:10.1016/j.neuroimage.2015.04.005

Sgobio, C., Trabalza, A., Spalloni, A., Zona, C., Carunchio, I., Longone, P., & Ammassari-Teule, M. (2008). Abnormal medial prefrontal cortex connectivity and defective fear extinction in the presymptomatic G93A SOD1 mouse model of ALS. *Genes, Brain, & Behavior, 7*(4), 427-434.

Siciliano, M., Trojano, L., Trojsi, F., Greco, R., Santoro, M., Basile, G., . . . Santangelo, G. (2017). Edinburgh Cognitive and Behavioural ALS Screen (ECAS)-Italian version: regression based norms and equivalent scores. *Neurol Sci, 38*(6), 1059-1068. doi:10.1007/s10072-017-2919-4

Taylor, L. J., Brown, R. G., Tsermentseli, S., AlChalabi, A., Shaw, C. E., Ellis, C. M., . . . Goldstein, L. H. (2013). Is language impairment more common than executive

dysfunction in amyotrophic lateral sclerosis? *Journal of Neurology & Psychiatry, 84*(5), 494-498.

Utevsky, A. V., Smith, D. V., & Huettel, S. A. (2014). Precuneus is a functional core of the default-mode network. *J Neurosci, 34*(3), 932-940. doi:10.1523/JNEUROSCI.4227-13.2014

Westeneng, H. J., Walhout, R., Straathof, M., Schmidt, R., Hendrikse, J., Veldink, J. H., . . . van den Berg, L. H. (2016). Widespread structural brain involvement in ALS is not limited to the C9orf72 repeat expansion. *J Neurol Neurosurg Psychiatry, 87*(12), 1354-1360. doi:10.1136/jnnp-2016-313959

Ye, S., Ji, Y., Li, C., He, J., Liu, X., & Fan, D. (2016). The Edinburgh Cognitive and Behavioural ALS Screen in a Chinese Amyotrophic Lateral Sclerosis Population. *PLoS One, 11*(5), e0155496. doi:10.1371/journal.pone.0155496

Zhang, H., Avants, B. B., Yushkevich, P. A., Woo, J. H., Wang, S., McCluskey, L. F., . . . Gee, J. C. (2007). High-dimensional spatial normalization of diffusion tensor images improves the detection of white matter differences: an example study using amyotrophic lateral sclerosis. *IEEE Trans Med Imaging, 26*(11), 1585-1597. doi:10.1109/TMI.2007.906784

Zhang, H., Yushkevich, P. A., Rueckert, D., & Gee, J. C. (2007). Unbiased white matter atlas construction using diffusion tensor images. *Med Image Comput Comput Assist Interv, 10*(Pt 2), 211-218.

3.8 Tables and Figures

Table 3.1. Participant characteristics.

Variables	Group	Median (Min-Max)	p^U	Sites				
				Site 1	Site 2	Site 3	Site 4	p^{KW}
Sample (n)	Controls Patients	43 53	-	10 9	18 19	7 11	8 14	-
Gender (M:F)[a]	Controls Patients	21 : 22 32 : 21	0.26	4 : 6 3 : 6	10 : 8 10 : 9	4 : 3 9 : 2	3 : 5 10 : 4	-
Age (years)	Controls Patients	56.0 (37-69) 57.0 (33-78)	0.36	55.0 (40-69) 59.0 (41-73)	60.0 (37-67) 60.0 (37-74)	54.0 (38-66) 57.0 (53-78)	50.0 (40-60) 53.5 (33-68)	0.10 0.18
Education (years)	Controls Patients	16.0 (11-20) 15.0 (11-25)	**0.03**	17.0 (13-20) 14.0 (12-18)	15.5 (12-20) 15.0 (11-22)	14.0 (11-19) 12.0 (11-25)	16.5 (11-20) 15.0 (12-20)	0.53 0.45
BDI-II Total	Controls Patients	3.0 (0-13) 12.0 (0-30)	**<0.01**	4.0 (0-13) 9.0 (5-19)	3.0 (0-11) 14.0 (2-30)	4.0 (0-10) 13.5 (6-26)	1.5 (0-11) 9.5 (2-22)	0.87 0.52

Sample and gender are represented as frequency (count) for controls and patients. All other values are represented as Median (Min-Max). p^U represents significance threshold on Mann-Whitney U-test for pooled analysis comparing patients and controls, irrespective of site. [a]Gender was compared using Pearson Chi-square test. p^{KW} represents significance threshold on Kruskal-Wallis test comparing each group across sites. Values in bold font represent statistical significance.

Table 3.2. Clinical characteristics of the patient group.

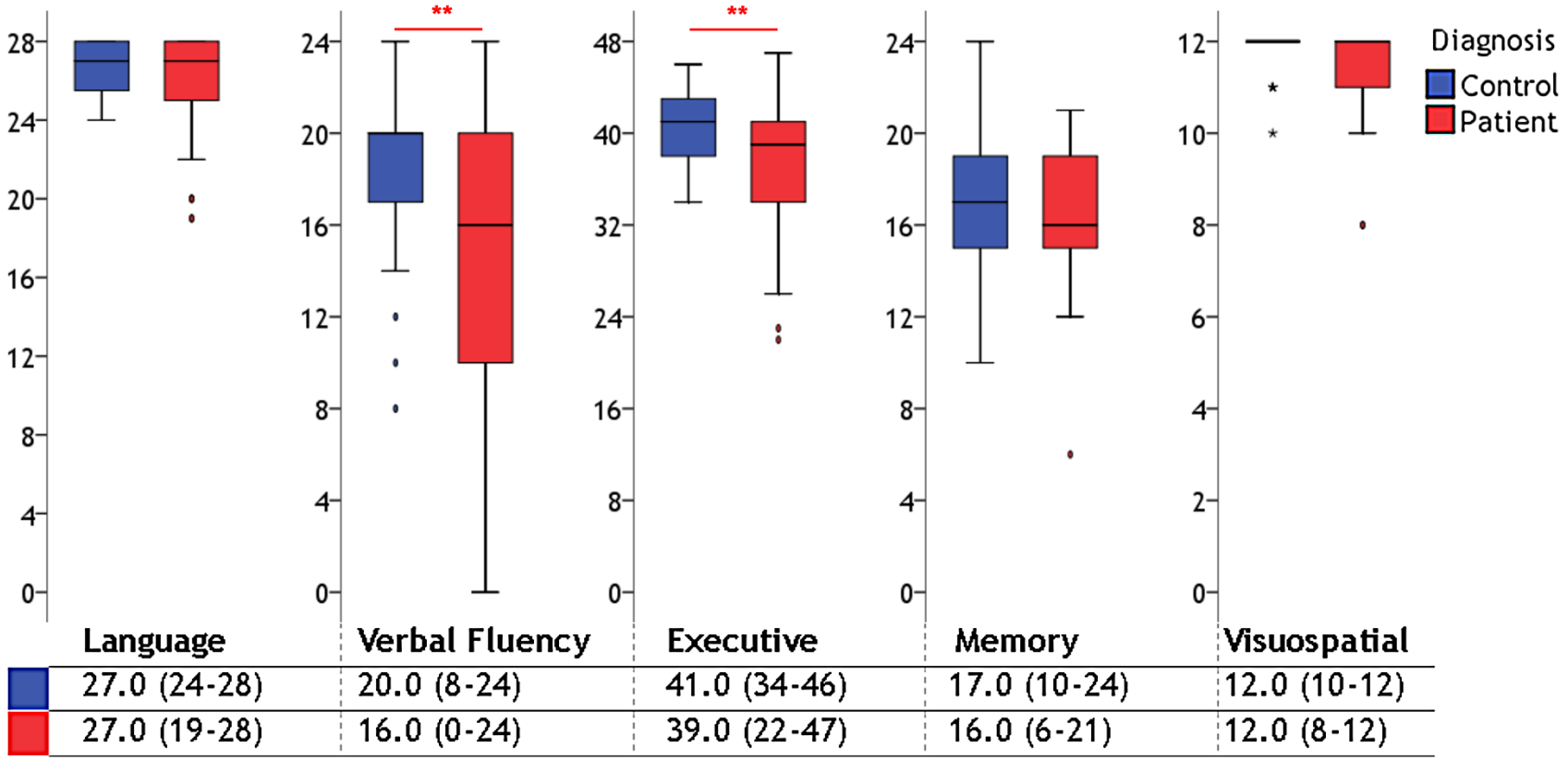
Variables	Overall Patients	Patients per site				
		Site 1	Site 2	Site 3	Site 4	p^{KW}
Site of Onset (n)						
Bulbar	8	-	4	3	1	-
Limb	43	7	15	8	13	-
Both	2	2	-	-	-	-
Symptom Duration (months)	24.0 (7-129)	51.0** (21-117)	18.0 (10-60)	18.0 (7-73)	33.0* (16-129)	**<0.01**
ALSFRS-R	39.5 (22-47)	33.0* (22-45)	41.0 (32-47)	40.0 (33-44)	38.0 (30-47)	**0.02**
Disease Progression Rate	0.3 (0.05-1.2)	0.3 (0.1-0.6)	0.2 (0.1-1.2)	0.4 (0.1-1.0)	0.3 (0.02-0.8)	0.58
FVC (percent reference)	93.0 (52-144)	89.0 (53-107)	94.5 (55-134)	94.5 (86-126)	94.0 (52-144)	0.85
Riluzole intake (n)	22	2	12	4	4	-
Non-invasive ventilation (n)	3	1	-	-	2	-
Percutaneous endoscopic gastronomy (PEG; n)	1	-	-	-	1	-
Total UMN Score (max = 11)	5.0	4.0	5.5	5.0	4.0	0.91
Right UMN Score (max = 5)	2.0	3.0	2.0	2.0	2.0	0.75
Left UMN Score (max = 5)	2.0	2.0	2.5	2.0	2.0	0.91
Jaw Score (max = 1)	0.0	0.0	0.5	0.0	0.0	0.51

Values are represented as Median. Minimum and maximum values are represented in parentheses where applicable. p^{KW} represents significance threshold on Kruskal-Wallis test comparing ALS patients across sites. **p<0.05 on post-hoc Mann-Whitney compared to the other sites. * p<0.05 on post-hoc Mann-Whitney U-test when compared to Edmonton and Montreal.

Table 3.3. MRI protocol

MRI Parameters	GE[a]		Siemens[b]	
	3D T1 (FSPGR)	DTI	3D T1 (MPRAGE)	DTI
Orientation	Axial	Axial	Axial	Axial
Resolution (mm^3)	1 x 1 x 1	2 x 2 x 2	1 x 1 x 1	2 x 2 x 2
Field of view	256 x 256	256 x 256	256 x 256	256 x 256
Acquisition Matrix	256 x 256	128 x 128	256 x 256	128 x 128
Number of Slices	176	70	176	70
Repetition time (TR; ms)	~7.4	9000	2300	10000
Echo time (TE; ms)	3.1	~80	3.4	90
Inversion time (TI; ms)	400	-	900	-
b = 1000 b = 0	- -	30 directions 5 averages	- -	30 directions 5 averages[a]

[a] Scanner at Sites 1 and 4; [b] Scanner at Sites 2 and 3.



	Language	Verbal Fluency	Executive	Memory	Visuospatial
Control	27.0 (24-28)	20.0 (8-24)	41.0 (34-46)	17.0 (10-24)	12.0 (10-12)
Patient	27.0 (19-28)	16.0 (0-24)	39.0 (22-47)	16.0 (6-21)	12.0 (8-12)

Figure 3.1. Box plots displaying ECAS performance.
Controls are indicated in blue and patients in red. Scores of each group for the ECAS domains are represented as Median (range).
**p<0.01.

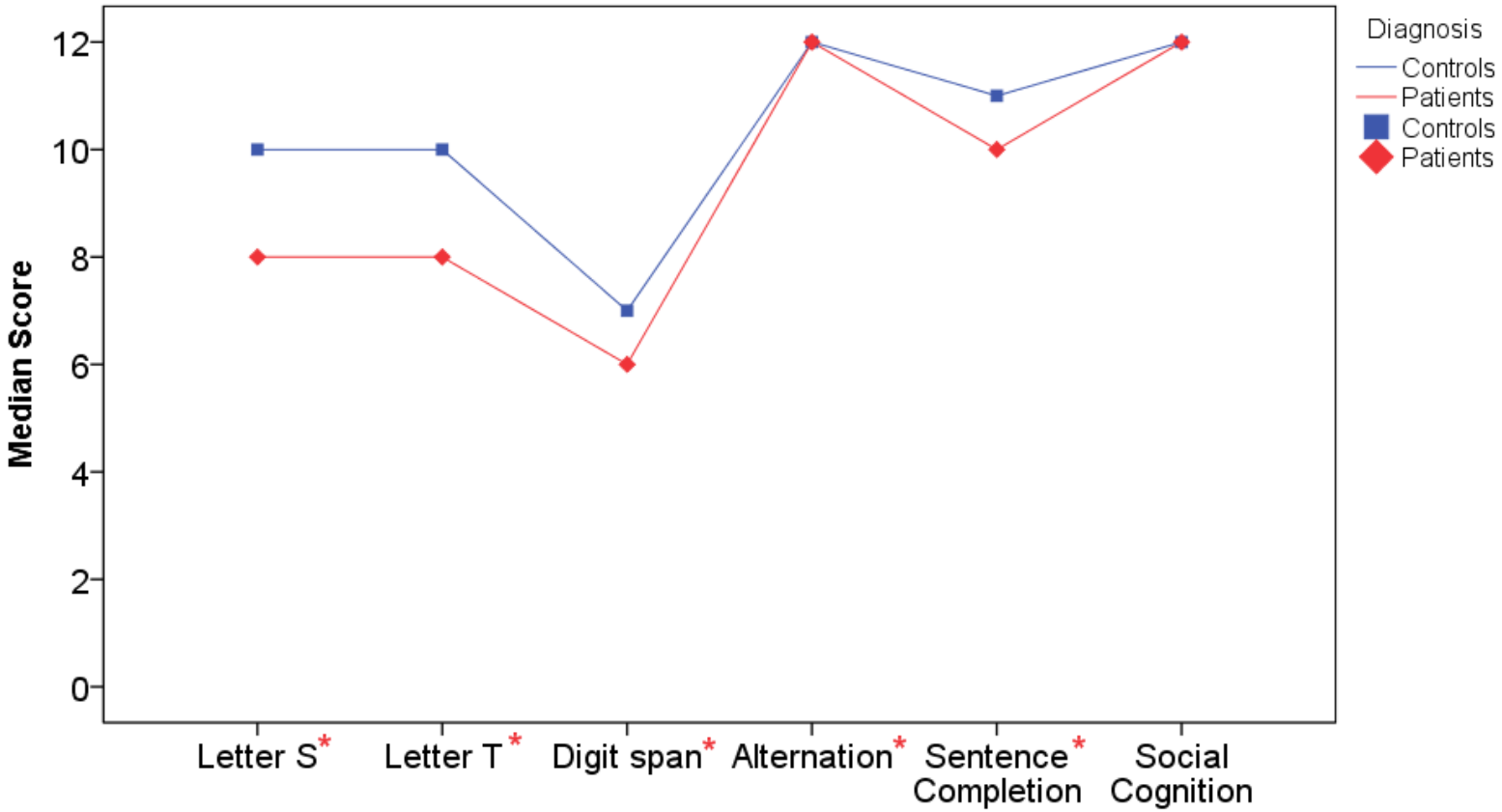


Figure 3.2. Performance on tests of executive function in the ECAS.
Letters S and T are included in verbal fluency and the reverse digit span, alternation, sentence completion and social cognition are included in executive domains. *$p < 0.05$.

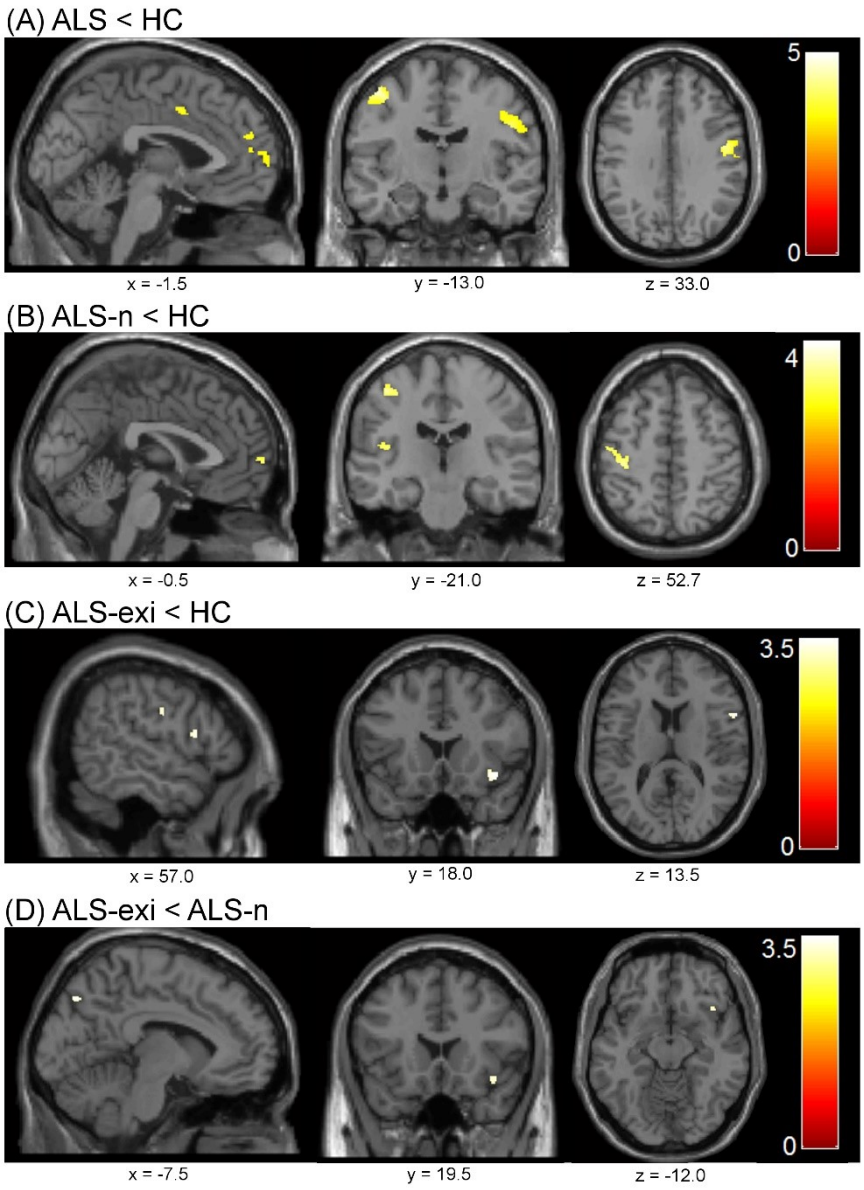


Figure 3.3. Group differences indicating reduced grey matter density in ALS patients. Voxel Based Morphometry (VBM) revealed lower grey matter densities: (A) in all ALS patients compared to controls, (B-C) in ALS cognitive sub-groups compared to controls, and (D) between ALS cognitive sub-groups. Colour bars represent T-values at $p < 0.001$ (uncorrected, cluster size =10). Note: ALS-n = ALS patients with normal EF, ALS-exi = ALS patients with impaired EF. Images are displayed in neurological convention (left is left).

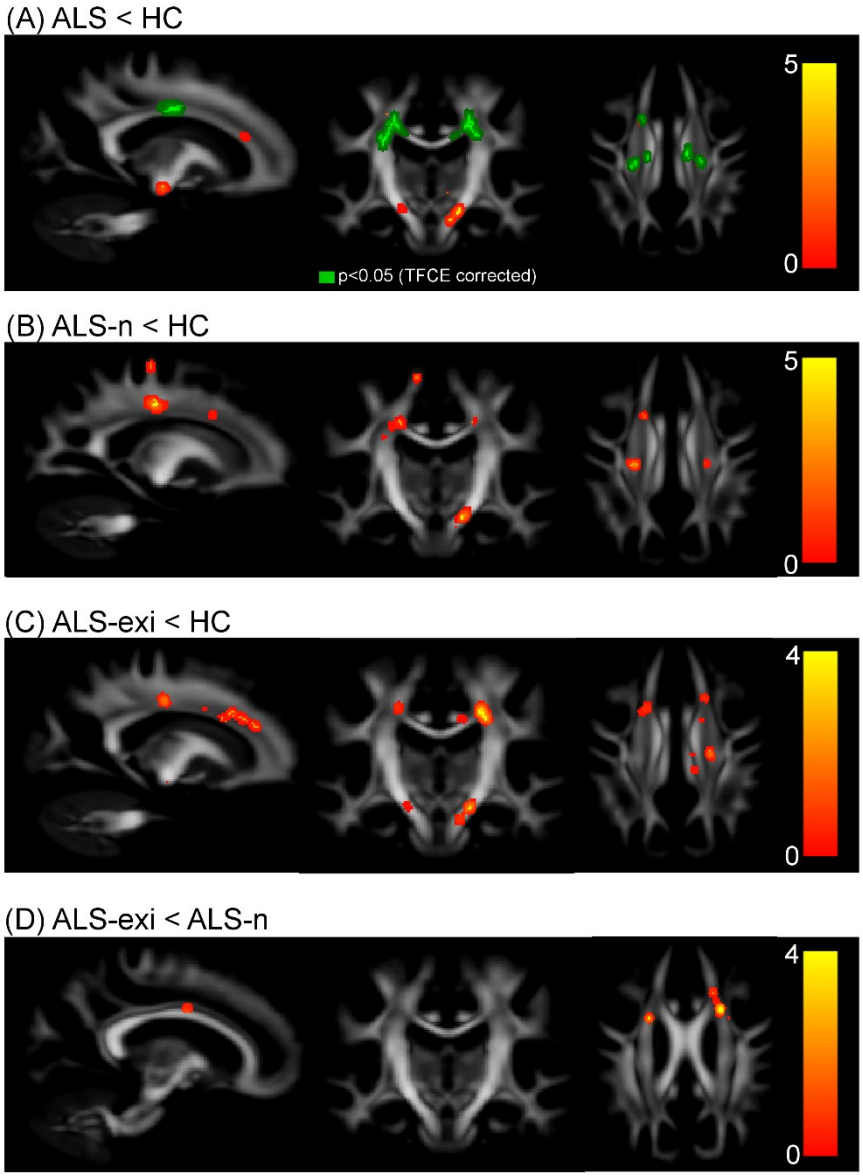


Figure 3.4. Group differences indicating reduced white matter integrity in ALS patients. Tract Based Spatial Statistics (TBSS) revealed reduced fractional anisotropy (FA): (A) in all patients as compared to controls, (B-C) in ALS cognitive sub-groups compared to controls, and (D) between ALS cognitive sub-groups. Colour bars represent T-values at $p < 0.001$ (uncorrected). Clusters corrected for multiple comparisons using threshold free cluster enhancement (TFCE) are represented in green colour. Note: ALS-n = ALS patients with normal EF, ALS-exi = ALS patients with impaired EF. Images are displayed in neurological convention (left is left).

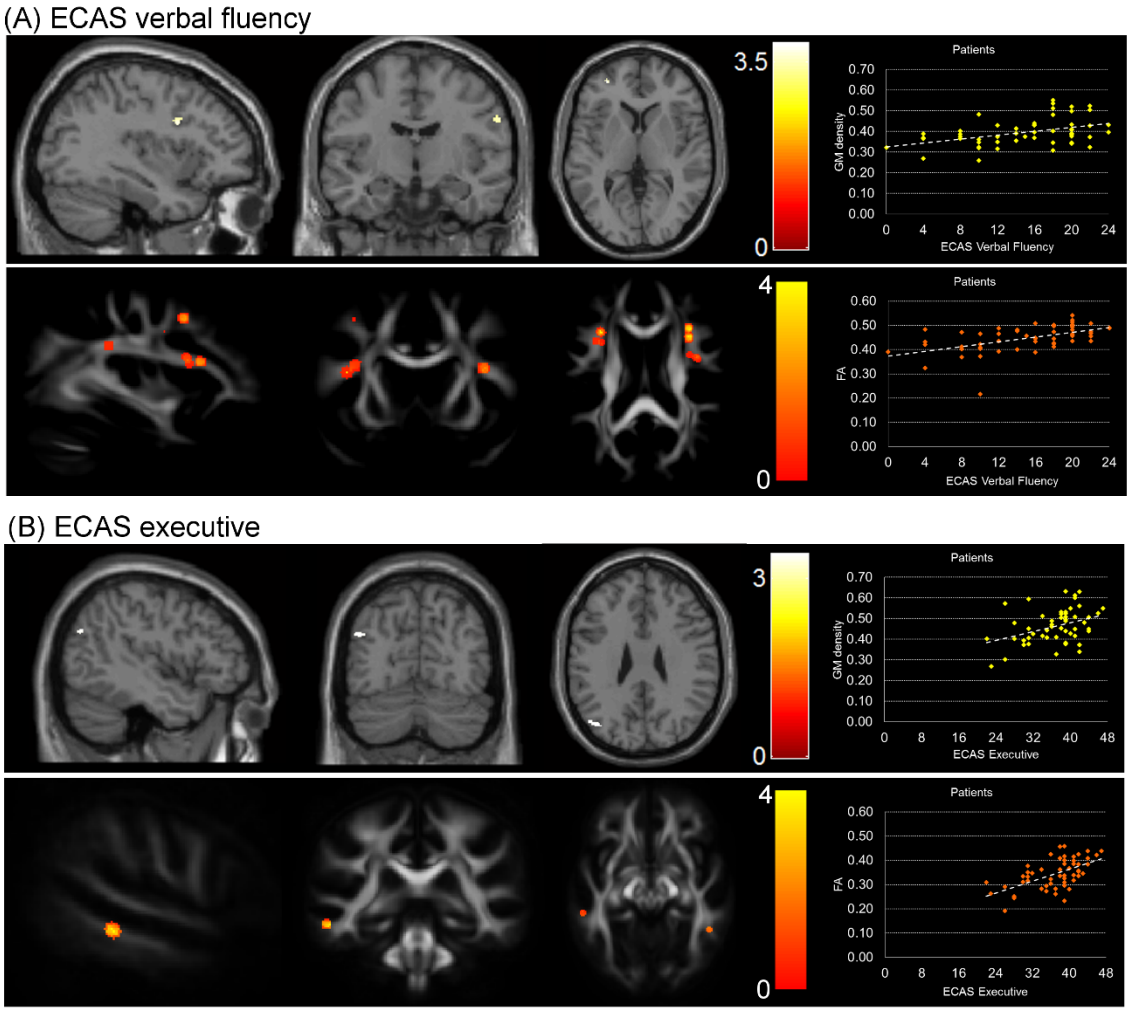
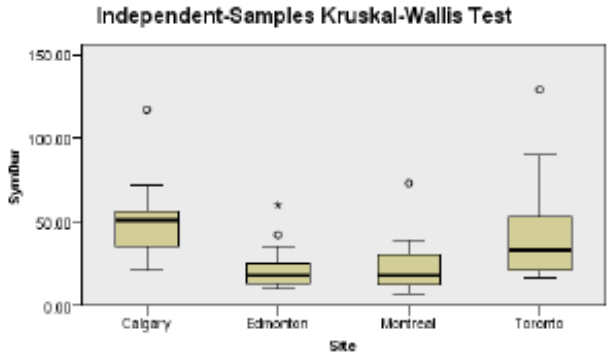


Figure 3.5. Grey matter density (GM) and fractional anisotropy (FA) correlations of ECAS verbal fluency and ECAS executive domains.
N = 53 ALS. Significance threshold at $p<0.001$ (uncorrected). Cluster size threshold was k = 10 for grey matter density

3.9 Supplementary materials

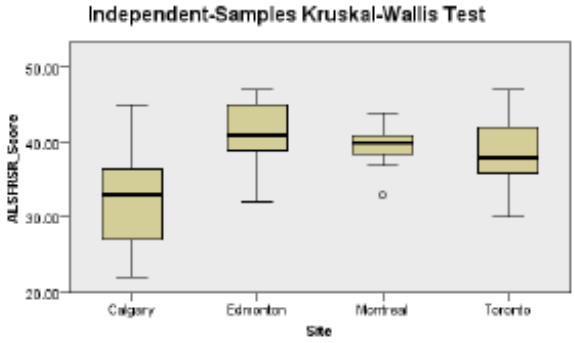
(A)



Total N	53
Test Statistic	15.675
Degrees of Freedom	3
Asymptotic Sig. (2-sided test)	.001

1. The test statistic is adjusted for ties.

(B)



Total N	52
Test Statistic	10.219
Degrees of Freedom	3
Asymptotic Sig. (2-sided test)	.017

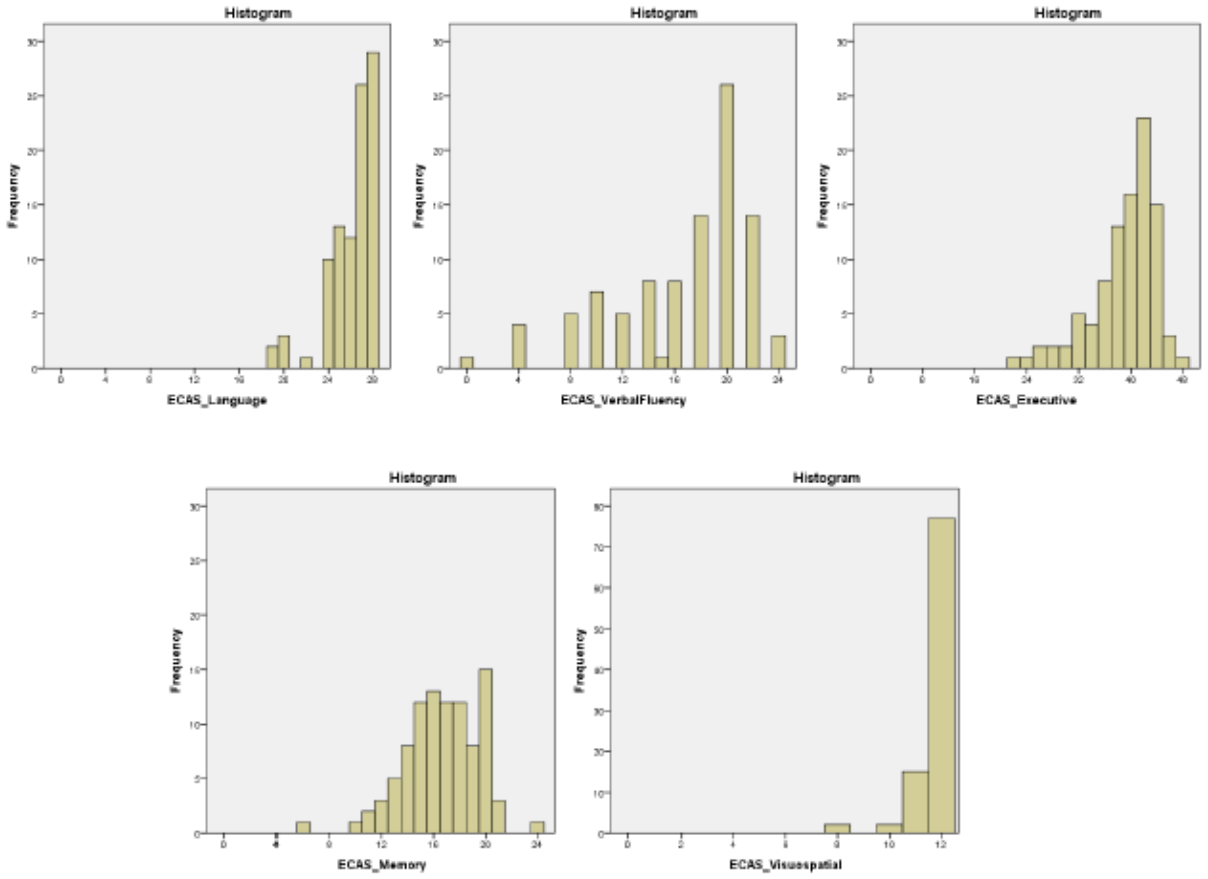
1. The test statistic is adjusted for ties.

Supplementary Figure 3.1. Post-hoc comparisons displaying of clinical variables. (A) significantly longer symptom duration for Sites 1 and 4, and (B) significantly lower ALSFRS-R score for Site 1.

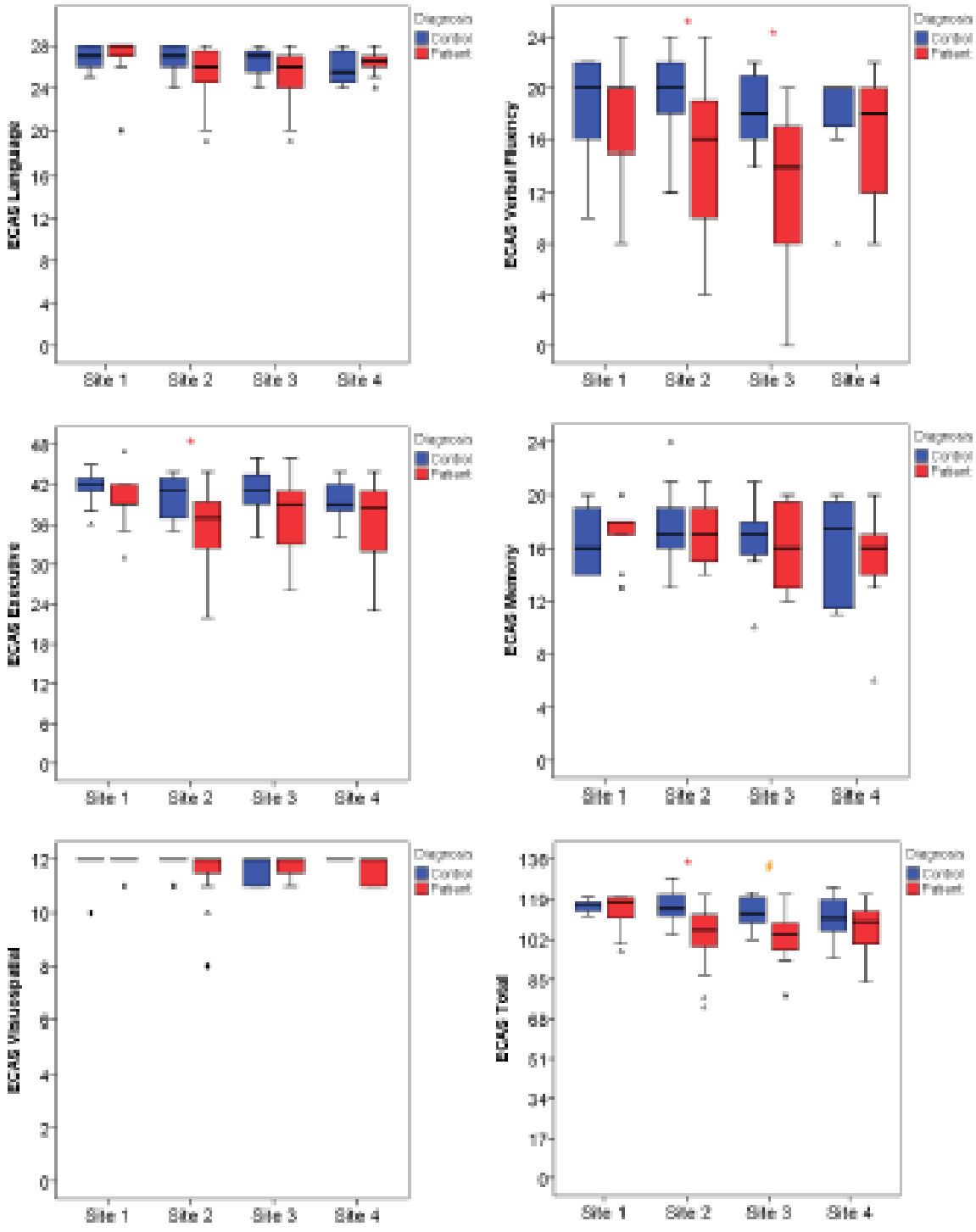
Supplementary Table 3.1. Normality tests for the ECAS.

ECAS Domains	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Language	.230	96	.000	.792	96	.000
Verbal Fluency	.199	96	.000	.894	96	.000
Executive	.148	96	.000	.912	96	.000
Memory	.087	96	.073	.964	96	.009
Visuospatial	.457	96	.000	.446	96	.000

a. Lilliefors Significance Correction



Supplementary Figure 3.2. Histogram indicating frequency of distribution of scores for the ECAS domain.



Supplementary Figure 3.3. Performance on the ECAS per site.

Supplementary Table 3.2. Percentage of ALS patients impaired on individual ECAS domains.

ECAS	Max	2SD Cut-off	Overall ALS patients below cut-offs	
			N	%
Language	28	25	6	11
Verbal Fluency	24	15	15	28
Executive	48	37	15	28
Memory	24	14	1	2
Visuospatial abilities	12	<11	3	6
Total	136	108	14	26

Note: ECAS Behavioural screen was completed by caregivers of 15 patients. Of these, 5 patients showed no behavioural changes, 7 patients displayed mild changes (score range: 1-3) and 3 patients displayed moderate behavioural changes (score range: 5-6). None of the patients displayed severe behavioural changes (score > 6).

4. Executive Functions may Moderate ToM in Amyotrophic Lateral Sclerosis

This Chapter is a part of a manuscript in preparation for submission and would be included as Study A along with Chapter 5 (Study B) in the final manuscript. Forty-one percent of the sample included in this experiment were also included in Chapter 3. This study addresses Aim 2 of the thesis: to identify the association between social cognition, specifically, theory of mind (ToM) and executive functions (EF) in ALS. The study will attempt to explore and identify the association between social cognition and executive functions and compare ToM performance among EF-based cognitive sub-groups.

Hypotheses: (1) ToM and EF impairments are present in ALS patients, and (2) ToM and EF are associated such that patients with EF impairments perform worse than those with intact EF on measures of ToM.

Executive Functions may Moderate ToM in Amyotrophic Lateral Sclerosis

Sneha Chenji[1], Esther Fujiwara[1,2], Dennell Mah[3], Lawrence Korngut[4], Angela Genge[5], Wendy Johnston[3], Sanjay Kalra[1,3*]

[1] Neuroscience and Mental Health Institute, University of Alberta, Edmonton, Canada

[2] Department of Psychiatry, University of Alberta, Edmonton, Canada

[3] Division of Neurology, Department of Medicine, University of Alberta, Edmonton, Canada

[4] Division of Neurology, Department of Medicine, University of Calgary, Calgary, Canada

[5] Clinical Research Unit, Montreal Neurological Institute, Montreal, Canada

*Corresponding Author. 7-132F Clinical Sciences Building, 11385 83 Ave NW, Edmonton, AB, T6G 2G3. Phone: 780-248-1777. Email: kalra@ualberta.ca

4.1 Abstract

Background. The cognitive profile in the neurodegenerative condition of Amyotrophic Lateral Sclerosis (ALS) includes executive function (EF) impairments with recent evidence suggesting changes in social cognition abilities. The current study investigated Theory of Mind (ToM), i.e. the ability to track intentions and beliefs (cognitive ToM, C-ToM) as well as emotions (affective ToM, A-ToM), and its association with EF in ALS patients.

Methods. Forty-five healthy volunteers and 41 ALS patients were administered the Edinburgh Cognitive and Behavioural ALS Screen (ECAS), that assesses cognitive changes specific (language, verbal fluency and executive) and non-specific to ALS (memory and visuospatial abilities). ToM was assessed using ECAS Judgement of Preference (JP) test, the Reading the Mind in the Eyes (RME) test and the Faux pas stories test. Behavioural assessment was completed by 29 caregivers using the Frontal Systems Behavioural Screen (FrSBe) Family form. Group differences were tested using Mann-Whitney U-tests while association between ToM tasks, EF, clinical and behavioural measures were tested using Spearman correlations. Furthermore, sub-group analyses were performed based on onset (Bulbar vs Limb) and cognitive status (EF impaired [ALS-exi] vs. EF normal [ALS-n]).

Results. ALS patients scored significantly lower than controls only on the Faux pas test ($p<0.01$, FDR-corrected), ToM stories and a trend in Control stories. Post-hoc analysis revealed C-ToM deficits for ToM stories while performance on control (non-ToM) stories showed deficits in empathy suggesting A-ToM changes. Performance on the Faux pas test was associated with EF in ALS patients ($p<0.05$, uncorrected), but not controls. ToM was also associated with language and behaviour in ALS patients. Results were comparable

between bulbar and limb-onset patients. Analysis of cognitive sub-groups revealed significantly lower scores on all ToM tests for ALS-exi patients as compared to controls ($p<0.05$, FDR-corrected) and ALS-n patients ($p<0.05$, uncorrected).

Conclusions. Both C-ToM and A-ToM changes are present in ALS and may be moderated by EF impairments in ALS. Underlying pathology that may contribute to these changes is discussed.

Keywords: Amyotrophic lateral sclerosis, Theory of Mind, Social cognition, Executive Function.

4.2 Introduction

Amyotrophic lateral sclerosis (ALS) is a disorder encompassing neurodegeneration of the motor neurons in the brain and spinal cord (upper and lower motor neurons, UMN and LMN respectively). Additional changes in the brain include frontotemporal lobar degeneration (FTLD) which is responsible for a spectrum of cognitive and behavioural impairment in 25-50% patients, of which 10-15% meet criteria for frontotemporal dementia (FTD) (Phukan et al., 2012). Executive function (EF) is the most frequently reported impaired cognitive domain in ALS (Beeldman et al., 2016; Goldstein & Abrahams, 2013). EF is a broad term that encompasses processes that regulate cognitive functions such as working memory, learning, reasoning, cognitive flexibility, inhibition, planning and decision making. Verbal (letter) fluency has been reported as the most sensitive marker of EF impairments in ALS (Goldstein & Abrahams, 2013). Emerging evidence suggests that the cognitive profile in ALS is more variable than previously assumed and includes impairment in language, social cognition and memory (Beeldman et al., 2016; Consonni et al., 2013). Recently revised criteria provide updated guidelines to classify ALS sub-groups (Strong et al., 2017). A noted update was the inclusion of social cognition as one of the diagnostic domains.

Social cognition refers to the ability to perceive, understand and respond to social stimuli (Adolphs, 1999). Some mechanisms involved in social cognition are: (1) learning through observation and interaction with people, (2) association of specific facial cues to mental states (thoughts and emotions), and (3) making inferences of perceptions or intentions of others (Frith & Frith, 2012). The latter process of interpreting mental states and intentions of self or others is widely known as Theory of Mind (ToM). Based on the content, namely

thoughts or emotions, one can further sub-divide this into cognitive (C-ToM) and affective (A-ToM) (Poletti, Enrici, & Adenzato, 2012).

Studies reporting social cognition deficits in ALS, indicate reduced ability to recognize emotions and lower ToM performance. Deficits in emotion perception typically include inaccurate recognition of facial expression for anger, disgust, sadness and fear (Crespi et al., 2014; Girardi et al., 2011; Lulé et al., 2005; Zimmerman et al., 2007). Patients further display impaired judgements and rate unfamiliar faces as approachable while controls do not; this is similar to patients with bilateral amygdala lesions (Schmolck et al., 2007). C-ToM deficits in ALS have been revealed by errors in attributing intentions to characters in cartoon stories (Watermeyer et al., 2015b), poor understanding of social contexts (Cavallo et al., 2011) and impaired detection of humor or sarcasm (Staios et al., 2013). A-ToM deficits were identified as poor understanding of affect and emotion perception using eye gaze directions (Judgement of Preference test) or Reading the Mind in the Eyes (RME) test (Burke, Elamin, et al., 2016; Burke, Pinto-Grau, et al., 2016; Girardi et al., 2011; Trojsi, Siciliano, et al., 2016; van der Hulst et al., 2014). One previous study reported inaccurate identification of faux pas (mistakes in social situations) in patients, even when controlling for EF (Meier et al., 2010). In contrast, a few studies report non-significant differences in emotion recognition (Staios et al., 2013) and ToM (Gibbons et al., 2007).

The prevalence of ToM impairment in ALS cohorts is varied in the above studies. One study reported overall ToM deficits in 24% of ALS patients; 12% had isolated A-ToM deficit, 3% isolated C-ToM deficits, and the remaining had a combination of both (van der Hulst et al., 2014). Another study indicated ToM deficits in a smaller subset of approximately 10% ALS patients (Watermeyer et al., 2015b). The association between ToM deficits and executive

dysfunction in ALS has been of interest in recent years. Some studies support an association between both types of ToM (A-ToM and C-ToM) and executive function (Bora, 2017; Burke, Pinto-Grau, et al., 2016; Watermeyer et al., 2015b), while others report persistent poor C-ToM abilities when controlling for executive dysfunction (Carluer et al., 2014; Meier et al., 2010; Staios et al., 2013). In contrast, some studies do not confirm such a relationship (Schmolck et al., 2007) or report only trend-level associations between ToM and EF in ALS (Girardi et al., 2011).

While ToM deficits are being recognised in ALS patients, there seems to be a lack of consensus on the extent, prevalence and severity of these deficits, and their dependence on EF. The primary objective of the study was to investigate both A-ToM and C-ToM and their association with EF in ALS. As a secondary objective, ToM performance based on symptom onset and its association with other clinical variables was also explored. It was hypothesised that: (1) ToM and EF impairments are present in ALS patients, (2) ToM and EF are associated such that patients with EF impairments perform worse than those with intact EF on measures of ToM.

4.3 Methods

4.3.1 Participants

Participants were recruited prospectively from three ALS programs in Edmonton, Calgary, and Montreal as part the Canadian ALS Neuroimaging Consortium (CALSNIC). Additional subjects were enrolled from Edmonton who were not part of CALSNIC. Inclusion criteria for the study required that: (i) patients met El Escorial criteria of possible, probable or definite ALS (Brooks et al., 2000); (ii) controls be over the age of 40 years, unless matched

with a patient under 40 years, and (iii) participants completed all social cognition tests included in the study. Participants with English as their second language were excluded from analysis. The study was approved by the local Research Ethics board at each CALSNIC site. Written informed consent was obtained from all participants. Caregiver consent was completed to obtain behavioural measures included in the study.

Forty-one patients (24 males and 17 females) and 45 controls met inclusion criteria (Table 3.1). Disability was measured using the ALS Functional Rating Scale – Revised (ALSFRS-R), a self-reported instrument that measures bulbar, limb (fine motor and gross motor skills) and respiratory function (Cedarbaum et al., 1999). It ranges from 0-48 points whereby lower scores indicate higher disability. The disease progression rate was estimated as (48 – ALSFRS-R) / symptom duration (Kimura et al., 2006). Respiratory function was assessed using forced vital capacity (FVC). Two patients were on continuous non-invasive ventilation and none of the patients had percutaneous endoscopic gastrostomy placement. Mood was assessed using Beck's Depression Inventory-II (BDI-II) (Beck et al., 1996).

4.3.2 Tools

4.3.2.1 Edinburgh Cognitive and Behavioural ALS Screen (ECAS)

The North-American version of the ECAS was administered to all participants (Abrahams et al., 2014). This screening tool was developed specifically for ALS-related cognitive changes and can be administered in a spoken or written format. It assesses five cognitive domains: language, verbal fluency, executive, memory and visuospatial abilities. Scoring on the ECAS classifies language, verbal fluency and executive as ALS-specific while

memory and visuospatial abilities are combined into an ALS non-specific domain. The ECAS total score is calculated by summing the ALS-specific and ALS non-specific scores.

The current study considered scores of the five cognitive domains and the ECAS total score. Additionally, the ECAS executive and total scores were modified to test the hypotheses. The ECAS executive section includes a social cognition task (Judgement of Preference test). As the current study is testing the relationship between ToM and EF, the Judgement of Preference score was not included in the ECAS executive and total scores. This ToM test of the ECAS was assessed separately and is further described in the next section.

4.3.2.2 Social Cognition (ToM) Tests

Judgement of Preference Test (ECAS JP): This test is a part of the ECAS (Abrahams et al., 2014). It was based on a paper-pencil version developed by Baron-Cohen, Campbell, Karmiloff-Smith, Grant, and Walker (1995) and Snowden et al. (2003). It includes two sets of six pencil-drawn images, depicting semantically related objects placed in the corners of a box. The first set is a control condition in which the participant is required to choose the image they liked the best in each box. The second set is a ToM condition, including the same objects as the first set and a cartoon face at the centre of each box, with its gaze directed to one of the objects in the box. In the ToM condition, participants are required to select the objects that they think the face likes the best, in each box. As the ToM condition focuses on inferring preference ("like") based on eye-gaze direction (Frith & Frith, 2012), the test was regarded to assess A-ToM ability.

Reading the Mind in the Eyes test (RME): This test was developed by Baron-Cohen et al., (2001) and was obtained from an online resource

(https://www.autismresearchcentre.com/arc_tests). The test includes 36 black and white pictures of both males and females showing the eye region. Each picture includes four words that describe thoughts or emotions. Participants are required to choose the one word that describes best what the person in the picture may think or feel. This test was designed to measure the ability to accurately attribute complex mental states of others based on information from the eye region alone, thereby providing information about A-ToM ability. The underlying assumption is that humans typically match a lexicon of terms associated with mental state and expression in the eyes at an "*unconscious, rapid and automatic level*" (Baron-Cohen et al., 2001).

Faux pas test: This test was developed by Stone et al. (1998) to assess contributions of the frontal lobe to ToM. It is a comprehensive test and evaluates both A-ToM and C-ToM. The test included vignettes that either contain a social mistake (faux pas) or no mistake. The current study adapted the test to include 10 stories, five of which contained a faux pas (ToM condition) and the remaining stories did not include a faux pas (control condition). Each story is followed by a set of eight questions. Participants were required to identify the faux pas, the inappropriateness of the situation, accurately identify intention as a mistake and infer the feelings of the victim in the story (empathy). Questions on the faux pas test required participants to go beyond the text in the story and make inferences that are not directly implied in the text, thereby assessing their ability to infer the state of mind of others. Two final questions tested the comprehension of the story and participants were required to provide details mentioned in the story unrelated to the social context. Participants were provided a copy of the story to follow and refer to, in order to provide responses, thereby

reducing confounding effects of memory. Correct responses were assigned a score of 1 and were summed to obtain total scores for the ToM and the Control conditions.

4.3.3 Statistical Analysis

All statistical analysis was performed on IBM SPSS Statistics for Windows, version 24.0 (IBM Corp., Armonk, N.Y., USA). Statistical significance was set at $p<0.05$ and corrected for multiple comparisons using False Discovery Rate (FDR) to reduce type-I error.

(1) Demographics: Age, education and BDI-II total score of the two groups were compared using independent samples t-test. Gender ratios of the two groups were compared using Pearson Chi-square test. Correlations between BDI-II total score and all cognitive scores was computed for each group to check for associations that may influence performance on cognitive tasks.

(2) Group Comparisons: Shapiro-Wilk test was used to assess normality of the cognitive scores (Supplementary Table 4.1). As most scores displayed non-normal distributions, Mann-Whitney U-tests were used to compare differences between the groups. The ECAS modified scores, ECAS JP score, RME total and Faux pas total scores were compared to identify group differences. Age-, education- and gender-based normative data were unavailable for the ToM measures, so to maintain consistency in the analysis, raw scores were used in all statistical models to compare performance between groups.

(3) ALS sub-group analysis: Considering recent literature in ALS, we explored the differences in ToM scores within patients based on (i) cognitive sub-groups in ALS patients (ALS-exi vs. ALS-n) and (ii) symptom onset (bulbar vs. limb). EF impairment (ALS-exi) was defined at 2SD below control mean for ECAS verbal fluency and / or executive modified

score. Remaining participants who did not meet criteria were classified as competent in EF (ALS-n). Mann-Whitney U tests were applied to compare performance on ToM tests between the ALS sub-groups based on symptom onset. For cognitive sub-groups, Kruskal-Wallis test was applied to check for overall differences between controls, ALS-exi and ALS-n groups. Post-hoc Mann-Whitney U-tests were applied to identify differences between specific cognitive sub-groups. Statistical significance was accepted at $p < 0.05$ (FDR-corrected).

(4) Correlations with EF and clinical variables: Spearman rank-order correlations of the impaired ToM tests, ECAS verbal fluency and executive (modified) scores were performed to identify associations between the two cognitive domains in each group. We also explored associations between ToM and clinical variables such as symptom duration, ALSFRS-R score and UMN signs to assess if clinical burden was associated with poor ToM performance. The UMN score was derived using neurological exams performed on a subset of 24 patients. A summation of the presence of UMN signs such as hyperreflexia, hypertonia, clonus and Babinski's reflex were used to compute the UMN score for each side (right and left) as well as a bilateral (total UMN) score that also included a point for jaw hyperreflexia. Associations between ToM and behavioural measures was also performed in patients. The Frontal Systems Behavioural Scale (FrSBe) (Grace & Malloy, 2000) was completed by caregivers for 29 patients. The current (after illness) FrSBe scores were used to find associations with impaired ToM tests.

4.4 Results

4.4.1 Demographics

Patient and control groups were matched for age and education, t_{age} (84) = -1.7, p = 0.10; t_{edu} (84) = 1.5, p = 0.14. There was a greater proportion of females in the control group, however this did not reach statistical significance ($\chi 2$ = 2.3, p = 0.13). Patients had significantly higher BDI-II scores as compared to controls (t (76) = -4.6, p <0.01). There were no meaningful associations noted between BDI-II and cognitive tasks, and hence mood was not included as a covariate for group comparisons.

4.4.2 Group Comparisons

Edinburgh Cognitive and Behavioural ALS Screen (ECAS). Compared to controls, patients had lower ECAS language (U = 591.5, p <0.01, FDR-corrected), ECAS executive modified (U = 576.5, p < 0.01, FDR-corrected) and ECAS total modified (U = 587.0, p < 0.01, FDR-corrected) scores (Table 4.2).

Social Cognition (ToM) Tests. Patients scored lower on the Faux pas test total score (U = 611.5, p < 0.01, FDR-corrected; Table 4.3). Scores were significantly lower on ToM stories with the faux pas (U = 654.0, p < 0.01, FDR-corrected), and approached significance with reduced scores in the Control stories (U = 733.0, p = 0.06). Further group comparisons of individual domains on the faux pas test revealed significantly lower scores for Faux pas detection, understanding inappropriateness, intentions and beliefs in ToM stories, but not control stories (Supplementary Table 4.2). Patients showed significantly lower empathy scores on the control stories. A qualitative review of responses for empathy indicated that patients frequently reported characters in the story to be “feeling bad”, annoyed, frustrated or embarrassed even in the absence of faux pas. One bulbar patient replied “feeling bad” for the empathy question in four of five stories without faux pas. Another patient was an outlier

for overall performance on the faux pas test (Supplementary Figure 4.1), however, excluding this outlier did not change the overall findings. RME scores were reduced with a trend towards significance ($U = 718.0$, $p = 0.08$). No significant group differences were noted on the ECAS JP test ($U = 845.5$, $p = 0.29$).

4.4.3 ALS Sub-group Analyses

(i) Cognitive sub-groups in ALS: Twelve patients (29%) were impaired on ECAS verbal fluency and / or modified ECAS executive, and were grouped as ALS-exi, with the remaining twenty-nine patients (71%) categorised as ALS-n. Demographics and clinical variables were not significantly different between these sub-groups. Differences in performance were present in all ToM tests. ALS-exi patients had a lower performance on the ALS-exi [ECAS JP test [X^2 (2) = 8.4, $p = 0.02$, FDR-corrected], RME test [X^2 (2) = 7.6, $p = 0.02$, FDR-corrected] and Faux pas test [X^2 (2) = 13.8, $p < 0.01$, FDR-corrected]. Performance on the ToM stories task was lower in the ALS-exi group [X^2 (2) = 15.3, $p < 0.01$, FDR-corrected], but not on the control stories (Figure 4.1).

(ii) Bulbar vs. Limb Onset: Ten bulbar-onset and 30 limb-onset patients were compared on the ToM tests. Mann-Whitney U-test revealed no significant differences on any of the ToM tests based on disease onset ($p > 0.05$, results not shown).

4.4.4 ToM associations with EF and Clinical Variables

In the patient group, the Faux pas test total score was significantly associated with ECAS verbal fluency [r_s (40) = 0.3, $p < 0.05$, uncorrected]. Specifically, ToM stories (with faux pas) were associated with ECAS verbal fluency [r_s (40) = 0.3, $p < 0.05$, uncorrected], but not the control stories (without faux pas). These correlations remained significant even when the

one outlier with poor faux pas scores was excluded from analysis. An exploratory analysis revealed significant associations between RME with both ECAS verbal fluency [r_s (40) = 0.3, p < 0.01, FDR-corrected] and the modified ECAS total score [r_s (40) = 0.3, p < 0.01, FDR-corrected].

Faux pas test total scores correlated with the FrSBe apathy [r_s (28) = -0.5, p < 0.01, uncorrected], FrSBe executive dysfunction [r_s (28) = -0.4, p < 0.05, uncorrected] sub-scores, and with the FrSBe total score [r_s (28) = -0.5, p < 0.01, uncorrected]. Associations between the Faux pas test and clinical variables such as ALSFRS-R, symptom duration, disease progression rate and UMN scores were not significant.

4.5 Discussion

The present study investigated the presence of ToM impairments using three social cognition tests, ECAS-JP, RME and the Faux pas tests. Furthermore, its association with EF deficits was also tested. Both C-ToM and A-ToM deficits were present in the ALS group and were associated with EF. The former is supported by impairments in the Faux pas test, specifically, impaired detection, understanding inappropriateness, beliefs and intentions on the ToM stories and not control stories. Poor empathy for the faux pas control stories in ALS patients implicates an A-ToM deficit. Contrary to previous studies, we did not find impaired judgement of preference which is a test of social cognition in the ECAS (Girardi et al., 2011; van der Hulst et al., 2014). However, it is to be noted that a screening version (ECAS-JP) of this task was used and hence may be limited by ceiling effects.

4.5.1 ToM impairments are present in ALS and moderated by EF

Only one previous study (Meier et al., 2010) used the Faux pas test in ALS and the authors reported poor faux pas detection indicating dissociation based on story type; whereby patients were impaired only on ToM stories. These findings persisted when EF was used as covariate, and the authors concluded that this pattern of deficits was associated with damage to ventromedial prefrontal cortex (vmPFC) (Meier et al., 2010). In the current study, performance on the Faux pas test and the RME was moderately associated with ECAS verbal fluency. Furthermore, differences in ToM were noted for cognitive sub-groups, with ALS-exi patients performing poorly as compared to both the ALS-n and the control groups. This is consistent with recent evidence that EF impairment may influence performance on social cognition tests (Burke, Pinto-Grau, et al., 2016; Watermeyer et al., 2015b). Burke, Pinto-Grau, et al. (2016) noted that patients with impairment on multiple EF tasks perform worse on the RME than patients with impairment on a single EF task. Watermeyer et al. (2015b) reported that 45% of EF variance predicted social cognition in their regression model, while age, education, and behaviour / mood / personality did not predict social cognition performance. However, the authors acknowledged a possibility of other sources of variance such as language that may influence social cognition in ALS. Language is being recognised as one of the primary features of impairment in ALS in addition to executive dysfunction (Taylor et al., 2013). There was a significant association between performance on the Faux pas and ECAS language scores in patients in the current study; higher score on the Faux pas test was associated with higher language scores. The lack of such an association in the control group highlights the possibility that variability of social cognition in ALS may indeed be associated with language. One previous study noted associations between ToM and semantic naming in ALS (Gibbons et al., 2007). Further studies detailing higher order

language processes may help understand or identify specific deficits that contribute to social cognition (Martin & McDonald, 2003).

4.5.2 Neuroanatomical Implications

The association of ToM with both EF and language noted above suggests a broad network-based degeneration associated with FTLD in ALS. Very few studies have investigated neuroimaging associates of ToM in ALS. A recent task-based functional magnetic resonance imaging (fMRI) study reported an association between action understanding and RME test scores and brain activity changes in bilateral superior frontal gyri (Jelsone-Swain et al., 2015). Another recent study revealed higher BOLD signal activity in the posterior superior temporal sulcus (pSTS) in patients with ALS while performing the JP test when compared to controls (Keller et al., 2017). C-ToM deficits as indicated by poor scores on false belief tasks were found to correlate with reduced metabolic rate in the dorsolateral prefrontal cortices (dlPFCs) and the supplementary motor areas of the cortex (Carluer et al., 2014).

Based on developmental and neurodegenerative literature, all the aforementioned regions have been associated with social cognition and are considered a part of a core social cognition network (Adolphs, 1999). Studies have suggested the involvement of different regions for the sub-components of ToM; C-ToM involving the dlPFCs and A-ToM involving the ventromedial prefrontal cortex (vmPFC) (Abu-Akel & Shamay-Tsoory, 2011; Poletti et al., 2012). Furthermore, neuroanatomical and neurochemical pathologies may influence performance on ToM tasks although via different pathways (Abu-Akel & Shamay-Tsoory, 2011). Executive dysfunction in ALS has been associated with TAR DNA-binding protein of 43 kDa (TDP-43) pathology in regions of the dlPFC (middle frontal gyrus), and superior

temporal and middle temporal gyri, thereby reflecting the most common type of frontotemporal lobar degeneration noted in ALS (Brettschneider et al., 2012). Ex-vivo studies have confirmed that the spread of the disease in ALS from motor to pre-motor areas and later to the frontotemporal regions including hippocampus in advanced stages of the disease (Braak et al., 2013; Brettschneider et al., 2013). The prion-like propagation of phosphorylated TDP-43 (pTDP-43) is considered to depend on proximity to the motor cortex and / or the involvement of affected regions in large-scale functional networks (Bak & Chandran, 2012). Structural connectivity analysis using principles of graph theory suggest that regions that are densely interconnected (rich clubs) are often disrupted in neurological conditions (Daianu et al., 2016; McColgan et al., 2015). It is possible that EF moderates ToM in ALS due to shared structural regions required for both processes, particularly the dlPFC and medial PFC. However, the extent of dysfunction would depend on the severity of pathology. It would be of interest to study longitudinal changes in ToM alongside frontotemporal contributions to understand the vulnerability of ToM vs EF over time in ALS.

4.5.3 Clinical associations with ToM

ToM was associated with behaviour in a subset of patients indicating that patients with greater apathy and executive dysfunction may perform worse on ToM. No previous studies have reported associations between ToM and caregiver-reported behavioural changes in ALS. This suggests a potential link between ToM and behaviour, and future studies could investigate if behavioural impairments contribute to reduced ToM performance or if reduced ToM performance could predict behavioural changes in ALS. The present study did not find differences in ToM based on onset (bulbar vs limb). Two previous studies reported lower RME performance in patients with bulbar onset (Burke, Elamin, et al., 2016; Trojsi,

Siciliano, et al., 2016). The relation between site of onset and social cognition is yet to be conclusive, as also indicated in a recent meta-analysis (Bora, 2017).

4.5.4 Strengths and Limitations

The strength of the present study is the use of multiple measures assessing both A-ToM and C-ToM, thus enabling a fair investigation of both sub-components in ALS. Except for two previous studies (Trojsi, Siciliano, et al., 2016; van der Hulst et al., 2014), most studies have either used A-ToM or C-ToM. The current study did not look at gender differences in performance on ToM tasks. Ad-hoc analysis revealed that male ALS patients performed worse than male controls, while no such differences were noted in female ALS and control groups (all groups matched for age and education). Exploring such relationships may provide a further insight into gender differences in cognitive decline and frontotemporal involvement in ALS. Although three independent executive tasks were used from the ECAS, the study may have been strengthened by incorporating separate and more extensive testing. Due to ceiling effects in the scores, the study was limited by the use non-parametric statistics. Including larger and more representative samples for disease onset would provide higher power for statistical analysis.

4.6 Conclusions

The present study reports the presence of both C-ToM and A-ToM deficits in ALS. ToM deficits may be exacerbated by the presence of cognitive impairments in the domains of EF and language. Future studies could consider analysing social cognition in larger cognitive sub-groups to discern the possibility of interaction between these cognitive processes in ALS.

4.7 References

Abrahams, S., Newton, J., Niven, E., Foley, J., & Bak, T. H. (2014). Screening for cognition and behaviour changes in ALS. *Amyotroph Lateral Scler Frontotemporal Degener, 15*(1-2), 9-14. doi:10.3109/21678421.2013.805784

Abu-Akel, A., & Shamay-Tsoory, S. (2011). Neuroanatomical and neurochemical bases of theory of mind. *Neuropsychologia, 49*(11), 2971-2984. doi:10.1016/j.neuropsychologia.2011.07.012

Adolphs, R. (1999). Social cognition and the human brain. *Trends Cogn Sci, 3*(12), 469-479.

Bak, T. H., & Chandran, S. (2012). What wires together dies together: verbs, actions and neurodegeneration in motor neuron disease. *Cortex, 48*(7), 936-944. doi:10.1016/j.cortex.2011.07.008

Baron-Cohen, S., Campbell, R., Karmiloff-Smith, A., Grant, J., & Walker, J. (1995). Are children with autism blind to the mentalistic significance of the eyes? *British Journal of Developmental Psychology, 13*(4), 379-398. doi:10.1111/j.2044-835X.1995.tb00687.x

Baron-Cohen, S., Wheelwright, S., Hill, J., Raste, Y., & Plumb, I. (2001). The "Reading the Mind in the Eyes" Test revised version: a study with normal adults, and adults with Asperger syndrome or high-functioning autism. *J Child Psychol Psychiatry, 42*(2), 241-251.

Beck, A. T., Steer, R. A., & Brown, G. K. (1996). *Manual for the Beck Depression Inventory-II*. San Antonio, TX: Psychological Corporation.

Beeldman, E., Raaphorst, J., Klein Twennaar, M., de Visser, M., Schmand, B. A., & de Haan, R. J. (2016). The cognitive profile of ALS: a systematic review and meta-analysis update. *J Neurol Neurosurg Psychiatry, 87*(6), 611-619. doi:10.1136/jnnp-2015-310734

Bora, E. (2017). Meta-analysis of social cognition in amyotrophic lateral sclerosis. *Cortex, 88*, 1-7. doi:10.1016/j.cortex.2016.11.012

Braak, H., Brettschneider, J., Ludolph, A. C., Lee, V. M., Trojanowski, J. Q., & Del Tredici, K. (2013). Amyotrophic lateral sclerosis--a model of corticofugal axonal spread. *Nat Rev Neurol, 9*(12), 708-714. doi:10.1038/nrneurol.2013.221

Brettschneider, J., Del Tredici, K., Toledo, J. B., Robinson, J. L., Irwin, D. J., Grossman, M., . . . Trojanowski, J. Q. (2013). Stages of pTDP-43 pathology in amyotrophic lateral sclerosis. *Ann Neurol, 74*(1), 20-38. doi:10.1002/ana.23937

Brettschneider, J., Libon, D. J., Toledo, J. B., Xie, S. X., McCluskey, L., Elman, L., . . . Trojanowski, J. Q. (2012). Microglial activation and TDP-43 pathology correlate with executive dysfunction in amyotrophic lateral sclerosis. *Acta Neuropathologica, 123*(3), 395-407.

Burke, T., Elamin, M., Bede, P., Pinto-Grau, M., Lonergan, K., Hardiman, O., & Pender, N. (2016). Discordant performance on the 'Reading the Mind in the Eyes' Test,

based on disease onset in amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Frontotemporal Degener, 17*(7-8), 467-472. doi:10.1080/21678421.2016.1177088

Burke, T., Pinto-Grau, M., Lonergan, K., Elamin, M., Bede, P., Costello, E., . . . Pender, N. (2016). Measurement of Social Cognition in Amyotrophic Lateral Sclerosis: A Population Based Study. *PLoS One, 11*(8), e0160850. doi:10.1371/journal.pone.0160850

Carluer, L., Mondou, A., Buhour, M. S., Laisney, M., Pelerin, A., Eustache, F., . . . Desgranges, B. (2014). Neural substrate of cognitive theory of mind impairment in amyotrophic lateral sclerosis. *Cortex, 65C*, 19-30. doi:10.1016/j.cortex.2014.12.010

Cavallo, M., Adenzato, M., Macpherson, S. E., Karwig, G., Enrici, I., & Abrahams, S. (2011). Evidence of social understanding impairment in patients with amyotrophic lateral sclerosis. *PLoS One, 6*(10), e25948. doi:10.1371/journal.pone.0025948

Cedarbaum, J. M., Stambler, N., Malta, E., Fuller, C., Hilt, D., Thurmond, B., & Nakanishi, A. (1999). The ALSFRS-R: a revised ALS functional rating scale that incorporates assessments of respiratory function. BDNF ALS Study Group (Phase III). *J Neurol Sci, 169*(1-2), 13-21.

Consonni, M., Iannaccone, S., Cerami, C., Frasson, P., Lacerenza, M., Lunetta, C., . . . Cappa, S. F. (2013). The cognitive and behavioural profile of amyotrophic lateral sclerosis: Application of the consensus criteria. *Behavioural Neurology, 27*(2), 143-153.

Crespi, C., Cerami, C., Dodich, A., Canessa, N., Arpone, M., Iannaccone, S., . . . Cappa, S. F. (2014). Microstructural white matter correlates of emotion recognition impairment in Amyotrophic Lateral Sclerosis. *Cortex, 53*, 1-8. doi:10.1016/j.cortex.2014.01.002

Daianu, M., Mezher, A., Mendez, M. F., Jahanshad, N., Jimenez, E. E., & Thompson, P. M. (2016). Disrupted rich club network in behavioral variant frontotemporal dementia and early-onset Alzheimer's disease. *Hum Brain Mapp, 37*(3), 868-883. doi:10.1002/hbm.23069

Frith, C. D., & Frith, U. (2012). Mechanisms of social cognition. *Annu Rev Psychol, 63*, 287-313. doi:10.1146/annurev-psych-120710-100449

Gibbons, Z. C., Snowden, J. S., Thompson, J. C., Happe, F., Richardson, A., & Neary, D. (2007). Inferring thought and action in motor neurone disease. *Neuropsychologia, 45*(6), 1196-1207. doi:10.1016/j.neuropsychologia.2006.10.008

Girardi, A., Macpherson, S. E., & Abrahams, S. (2011). Deficits in emotional and social cognition in amyotrophic lateral sclerosis. *Neuropsychology, 25*(1), 53-65. doi:10.1037/a0020357

Goldstein, L. H., & Abrahams, S. (2013). Changes in cognition and behaviour in amyotrophic lateral sclerosis: nature of impairment and implications for assessment. *Lancet Neurol, 12*(4), 368-380. doi:10.1016/S1474-4422(13)70026-7

Grace, J., & Malloy, P. (2000). *Frontal systems behavior scale: professional manual*: Psychological Assessment Resources, Incorporated.

Jelsone-Swain, L., Persad, C., Burkard, D., & Welsh, R. C. (2015). Action processing and mirror neuron function in patients with amyotrophic lateral sclerosis: an fMRI study. *PLoS One, 10*(4), e0119862. doi:10.1371/journal.pone.0119862

Keller, J., Bohm, S., Aho-Ozhan, H. E. A., Loose, M., Gorges, M., Kassubek, J., . . . Lule, D. (2017). Functional reorganization during cognitive function tasks in patients with amyotrophic lateral sclerosis. *Brain Imaging Behav*. doi:10.1007/s11682-017-9738-3

Kimura, F., Fujimura, C., Ishida, S., Nakajima, H., Furutama, D., Uehara, H., . . . Hanafusa, T. (2006). Progression rate of ALSFRS-R at time of diagnosis predicts survival time in ALS. *Neurology, 66*(2), 265-267. doi:10.1212/01.wnl.0000194316.91908.8a

Lulé, D., Kurt, A., Jurgens, R., Kassubek, J., Diekmann, V., Kraft, E., . . . Anders, S. (2005). Emotional responding in amyotrophic lateral sclerosis. *J Neurol, 252*(12), 1517-1524. doi:10.1007/s00415-005-0907-8

Martin, I., & McDonald, S. (2003). Weak coherence, no theory of mind, or executive dysfunction? Solving the puzzle of pragmatic language disorders. *Brain Lang, 85*(3), 451-466.

McColgan, P., Seunarine, K. K., Razi, A., Cole, J. H., Gregory, S., Durr, A., . . . Track, H. D. I. (2015). Selective vulnerability of Rich Club brain regions is an organizational principle of structural connectivity loss in Huntington's disease. *Brain, 138*(Pt 11), 3327-3344. doi:10.1093/brain/awv259

Meier, S. L., Charleston, A. J., & Tippett, L. J. (2010). Cognitive and behavioural deficits associated with the orbitomedial prefrontal cortex in amyotrophic lateral sclerosis. *Brain: A Journal of Neurology, 133*(11), 3444-3457.

Phukan, J., Elamin, M., Bede, P., Jordan, N., Gallagher, L., Byrne, S., . . . Hardiman, O. (2012). The syndrome of cognitive impairment in amyotrophic lateral sclerosis: A population-based study. *Journal of Neurology & Psychiatry, 83*(1), 102-108.

Poletti, M., Enrici, I., & Adenzato, M. (2012). Cognitive and affective Theory of Mind in neurodegenerative diseases: neuropsychological, neuroanatomical and neurochemical levels. *Neurosci Biobehav Rev, 36*(9), 2147-2164. doi:10.1016/j.neubiorev.2012.07.004

Schmolck, H., Mosnik, D., & Schulz, P. (2007). Rating the approachability of faces in ALS. *Neurology, 69*(24), 2232-2235. doi:10.1212/01.wnl.0000296001.16603.b3

Snowden, J. S., Gibbons, Z. C., Blackshaw, A., Doubleday, E., Thompson, J., Craufurd, D., . . . Neary, D. (2003). Social cognition in frontotemporal dementia and Huntington's disease. *Neuropsychologia, 41*(6), 688-701.

Staios, M., Fisher, F., Lindell, A. K., Ong, B., Howe, J., & Reardon, K. (2013). Exploring sarcasm detection in amyotrophic lateral sclerosis using ecologically valid measures. *Front Hum Neurosci, 7*, 178. doi:10.3389/fnhum.2013.00178

Stone, V. E., Baron-Cohen, S., & Knight, R. T. (1998). Frontal lobe contributions to theory of mind. *J Cogn Neurosci, 10*(5), 640-656.

Strong, M. J., Abrahams, S., Goldstein, L. H., Woolley, S., McLaughlin, P., Snowden, J., . . . Turner, M. R. (2017). Amyotrophic lateral sclerosis - frontotemporal spectrum disorder (ALS-FTSD): Revised diagnostic criteria. *Amyotroph Lateral Scler Frontotemporal Degener, 18*(3-4), 153-174. doi:10.1080/21678421.2016.1267768

Taylor, L. J., Brown, R. G., Tsermentseli, S., AlChalabi, A., Shaw, C. E., Ellis, C. M., . . . Goldstein, L. H. (2013). Is language impairment more common than executive dysfunction in amyotrophic lateral sclerosis? *Journal of Neurology & Psychiatry, 84*(5), 494-498.

Trojsi, F., Siciliano, M., Russo, A., Passaniti, C., Femiano, C., Ferrantino, T., . . . Santangelo, G. (2016). Theory of Mind and Its Neuropsychological and Quality of Life Correlates in the Early Stages of Amyotrophic Lateral Sclerosis. *Front Psychol, 7*, 1934. doi:10.3389/fpsyg.2016.01934

van der Hulst, E. J., Bak, T. H., & Abrahams, S. (2014). Impaired affective and cognitive theory of mind and behavioural change in amyotrophic lateral sclerosis. *J Neurol Neurosurg Psychiatry*. doi:10.1136/jnnp-2014-309290

Watermeyer, T. J., Brown, R. G., Sidle, K. C., Oliver, D. J., Allen, C., Karlsson, J., . . . Goldstein, L. H. (2015). Executive dysfunction predicts social cognition impairment in amyotrophic lateral sclerosis. *J Neurol, 262*(7), 1681-1690. doi:10.1007/s00415-015-7761-0

Zimmerman, E. K., Eslinger, P. J., Simmons, Z., & Barrett, A. M. (2007). Emotional perception deficits in amyotrophic lateral sclerosis. *Cogn Behav Neurol, 20*(2), 79-82. doi:10.1097/WNN.0b013e31804c700b

4.8 Tables and Figures

Table 4.1. Participant characteristics: mean age, education, gender and clinical variables.

Variable	Controls (N = 45)	Patients (N = 41)	p
Age (years) [a]	55.8 (8.1) 38-71	58.8 (8.6) 37-72	.10
Education (years) [a]	15.2 (2.6) 10-21	14.3 (3.3) 8.5-25	.16
Gender (M ; F)[b]	19 M ; 26 F	24 M ; 17 F	.13
Site of Onset			
Bulbar	-	10	
Limb	-	30	
Both Limb and Bulbar	-	1	
Symptom Duration (median months)[c]	-	20.0 (7-101)	
ALSFRS-R	-	35.0 (9.8) 10-47	
Disease Progression Rate	-	0.6 (0.6) 0.1-2.6	
FVC (percent reference)	-	82.7 (29.9) 17-134	
Non-invasive ventilation (count)	-	2	
Riluzole (count)	-	19	
BDI-II Total	6.5 (6.5) 0-25	13.6 (7.0) 0-32	<0.01
Total UMN score (Max = 11)[d]	-	5.3 (2.4) 1-11	
Right UMN score (Max = 5)	-	2.5 (1.1) 0-5	
Left UMN score (Max = 5)	-	2.4 (1.2) 1-5	
Jaw score (Max = 1)	-	0.4 (0.5) 0-1	

[a] Mean age and education between the groups was compared using Student's t-test; [b] Gender distribution compared using Pearson Chi-square test; [c] Symptom duration displayed as Median (Range); [d] Upper motor neuron (UMN) score available for 20 patients.

Table 4.2. Performance on sub-categories of the ECAS.

Tests	Max. Score	Controls			Patients			*U*	*p*
		N	Median	Min-Max	N	Median	Min-Max		
ECAS									
Language	28	45	27.0	24-28	41	26.0	19-28	591.5	**<0.01**
Verbal Fluency	24	45	18.0	10-24	41	16.0	0-24	748.0	0.13
Executive modified (no ToM)[a]	36	45	30.0	17-34	41	27.0	14-35	576.5	**<0.01**
Memory	24	45	17.0	11-22	41	17.0	4-21	828.5	0.41
Visuospatial	12	45	12.0	10-12	41	12.0	8-12	827.0	0.14
ECAS Total modified (no ToM)[b]	124	45	104.0	89-116	41	97.0	63-111	587.0	**<0.01**
FrSBe Family									
FrSBe Total	230	-	-	-	29	93.6 ± 23.9	55-136	-	-
Apathy	70	-	-	-	29	34.4 ± 8.8	20-51	-	-
Disinhibition	75	-	-	-	29	24.7 ± 7.5	15-40	-	-
Dysfunction	85	-	-	-	29	34.5 ± 10.6	17-56	-	-

[a.] The original executive score (max 48) was 42.0 (range: 29-46) for controls and 39.0 (range: 22-47) for patients, $p<0.05$ (FDR-corrected). ECAS executive score was modified to include only Digit span backward, Alternation and Sentence Completion tests. Judgement of Preference test (ToM) that was a part of the original executive score was analysed separately (Table 3). [b.] The original ECAS Total score (max 136) was 115.0 (range: 101-128) for controls and 109.0 (range: 73-123) for patients. FrSBe Family form scores displayed for current behaviour (after illness). p-values in bold font represent $p<0.05$ (FDR-corrected).

Table 4.3. Median scores for social cognition (ToM) tests in controls and patients.

ToM Tests	Max.	Controls (N=45)		Patients (N=41)		*U*	*p*
		Median	Min-Max	Median	Min-Max		
ECAS JP score	12	12.0	2-12	12.0	2-12	845.5	0.29
RME Total	36	28.0	15-33	26.0	14-33	718.0	0.08
Faux pas test Total	80	80.0	71-80	77.0	52-80	611.5	**<0.01**
ToM stories (with faux pas)	40	40.0	34-40	39.0	12-40	654.0	**<0.01**
Control stories (no faux pas)	40	40.0	32-40	40.0	29-40	733.0	0.06

ECAS JP = ECAS Judgement of Preference test; RME = Reading the Mind in the Eyes; *U* = Mann-Whitney U test value. *p*-value in bold font represents significant differences corrected for multiple comparisons (FDR-corrections).

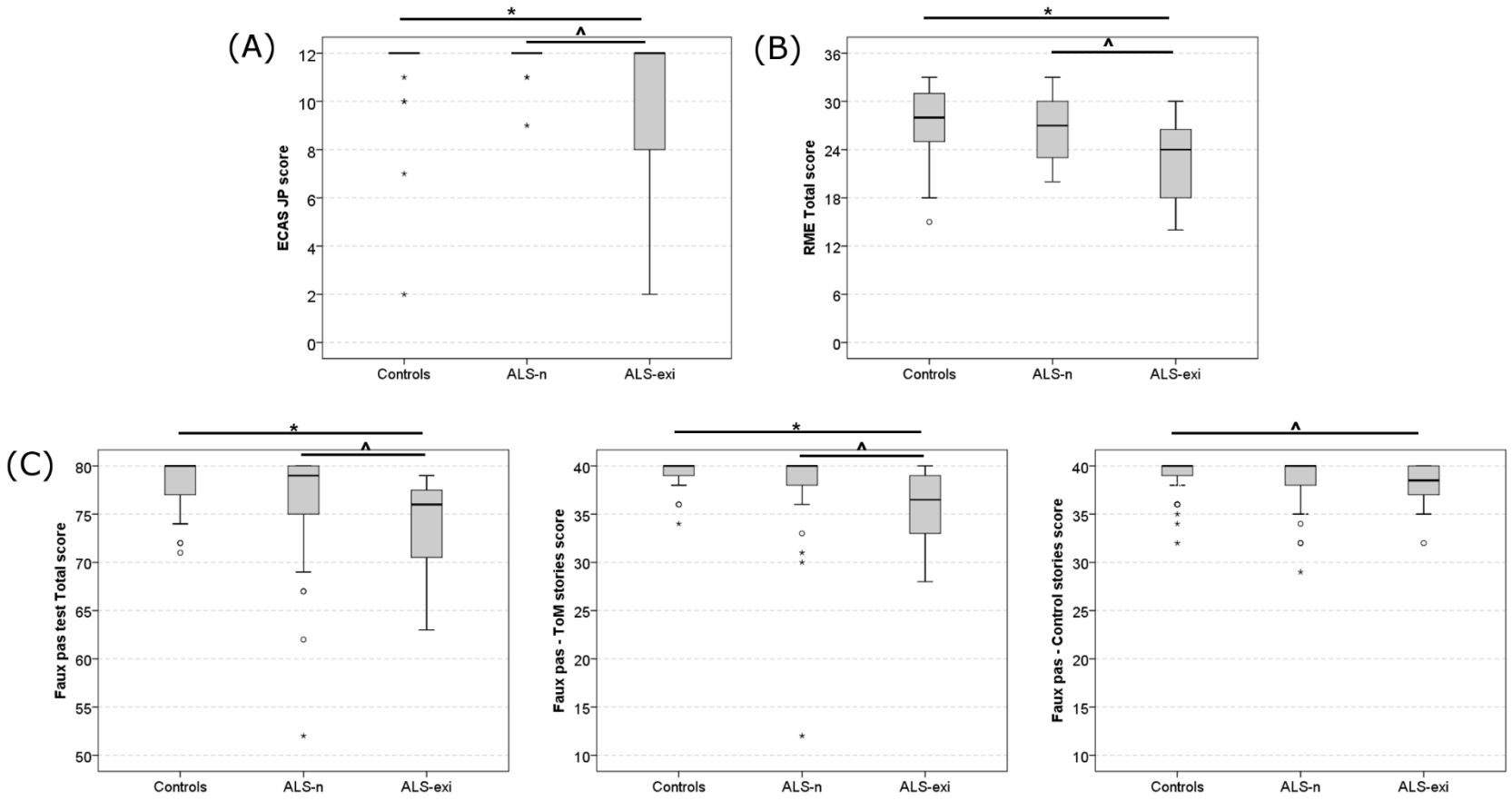


Figure 4.1. Box-plots indicating ToM scores.
(A) ECAS Judgement of Preference (JP) test; (B) Reading the Mind in the Eyes (RME) test and (C) Faux pas test. *p<0.05 (FDR-corrected), ^p<0.05 (uncorrected) on the Mann-Whitney U-tests. ALS-n = EF normal group, ALS-exi = EF impaired group.

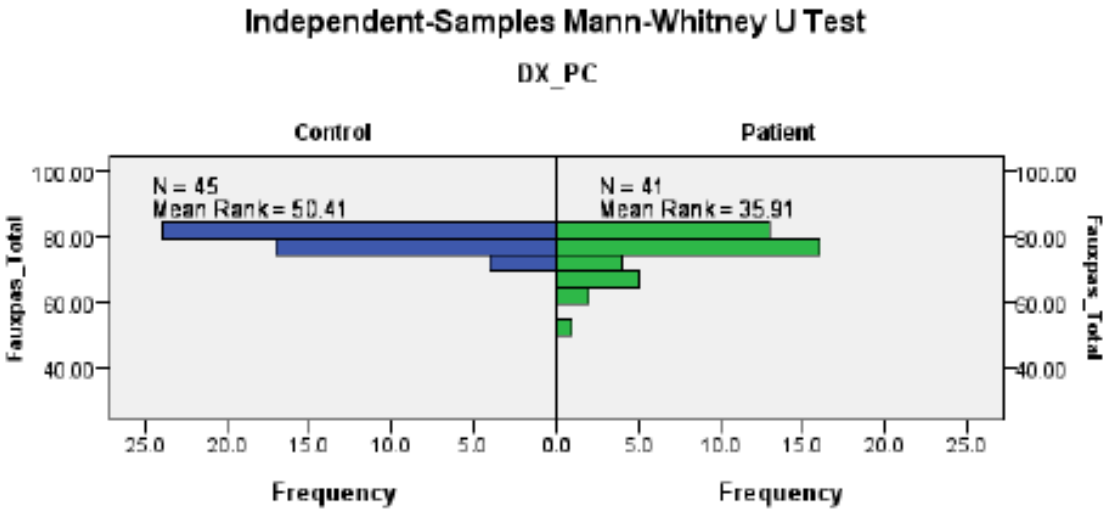
4.9 Supplementary materials

Supplementary Table 4.1. Normality of ECAS and ToM scores.

	Shapiro-Wilk Statistic	df	Sig.
ECAS			
Language	0.791	86	<0.001
Verbal Fluency	0.927	86	<0.001
Executive	0.880	86	<0.001
Executive modified (no ToM)	0.919	86	<0.001
Memory	0.934	86	<0.001
Visuospatial	0.377	86	<0.001
ECAS Total	0.877	86	<0.001
ECAS Total modified (no ToM)	0.900	86	<0.001
ToM			
ECAS Judgement of Preference	0.363	86	<0.001
RME Total	0.943	86	0.001
Faux pas Total	0.678	86	<0.001
ToM stories (with faux pas)	0.484	86	<0.001
Control stories (no faux pas)	0.655	86	<0.001

Supplementary Table 4.2. Post-hoc Mann-Whitney U-test for the Faux pas test.

Faux pas test	Max.	Controls (N=45)		Patients (N=41)		U	p
		Median	Min-Max	Median	Min-Max		
ToM stories (with faux pas)							
Detection	10	10.0	8-10	10.0	2-10	723.0	0.02
Inappropriateness	5	5.0	3-5	5.0	1-5	671.0	<0.01
Intentions	5	5.0	3-5	5.0	1-5	659.5	<0.01
Beliefs	5	5.0	4-5	5.0	1-5	716.0	<0.01
Empathy	5	5.0	3-5	5.0	1-5	820.5	0.17
Comprehension questions	10	10.0	10-10	10.0	6-10	855.5	0.07
Control stories (no faux pas)							
Detection	10	10.0	6-10	10.0	6-10	883.0	0.60
Inappropriateness	5	5.0	3-5	5.0	3-5	861.0	0.43
Intentions	5	5.0	3-5	5.0	3-5	861.0	0.43
Beliefs	5	5.0	3-5	5.0	4-5	852.0	0.25
Empathy	5	5.0	4-5	5.0	1-5	678.0	<0.01
Comprehension questions	10	10.0	10-10	10.0	8-10	877.5	0.14



Total N	86
Mann-Whitney U	611.500
Wilcoxon W	1,472.500
Test Statistic	611.500
Standard Error	110.646
Standardized Test Statistic	-2.811
Asymptotic Sig. (2-sided test)	.005

Supplementary Figure 4.1. Histogram plot and test statistics for the Faux pas test total score.
Outlier detected for patients (score = 60.0).

5. Structural and Functional Associations of ToM in ALS

This Chapter is a part of the manuscript in preparation for submission and would be included as Study B along with Chapter 4 (Study A) in the final manuscript. Seventy-seven percent of the sample included in this experiment were also included in both Chapters 3 and 4, and 23% were included only in Chapter 3. This study addresses Aim 2 of the thesis: to examine the structural and functional associations of ToM in ALS. The study will attempt to find associations between grey matter, white matter and functional connectivity using a ToM network model.

Hypotheses: Poor ToM in ALS is associated with (1) lower grey matter densities, (2) reduced integrity of white matter tracts, and (3) altered resting state connectivity of regions included in the ToM network.

Structural and Functional Associations of ToM in ALS

Sneha Chenji[1], Abdullah Ishaque[1], Dennell Mah[2], Peter Seres[3], Nikolai Malykhin[3], Wendy Johnston[2], Sanjay Kalra[1,2]*

[1]Neuroscience and Mental Health Institute, University of Alberta, Edmonton, Canada

[2]Division of Neurology, Department of Medicine, University of Alberta, Edmonton, Canada

[3]Department of Biomedical Engineering, University of Alberta, Edmonton, Canada

*Corresponding Author. Address: 7-132F Clinical Sciences Building, 11350 83 Ave, Edmonton, AB T6G 2G3. Email: kalra@ualberta.ca

5.1 Abstract

Background. The performance on Theory of Mind (ToM) tasks may be influenced by the presence of executive function (EF) impairments in patients with Amyotrophic Lateral Sclerosis (ALS). Structural imaging had previously indicated either grey matter (GM) or white matter (WM) associations with ToM in separate studies.

Objective. To investigate structural and functional associations of ToM in ALS. It was hypothesised that poor ToM will be associated with (1) lower grey matter densities, (2) reduced integrity of white matter tracts and (3) altered resting state connectivity.

Methods. Fifteen ALS patients and 15 age-education matched healthy controls were compared on the EF and ToM. All participants underwent an MRI scan of which 3D-T1, diffusion tensor imaging (DTI) and resting state functional MRI (rs-fMRI) were considered for the study. A-priori regions-of-interest (ROI) were defined based on Abu-Akel and Shamay-Tsoory (2011) model of ToM network for voxel-based morphometry (VBM), tract based spatial statistics (TBSS) and seed-based connectivity analyses.

Results. Group comparisons revealed significantly lower performance on EF in ALS patients, while a strong trend towards lower ToM performance was noted. Grey matter and white matter atrophy indicated mild changes ($p<0.01$, uncorrected) which failed to survive multiple comparisons correction. Widespread voxel-wise associations were noted for grey matter and white matter regions, with dorsolateral prefrontal cortex (dlPFC) showing associations with both ToM and EF. Analysis of rs-fMRI indicated mild changes for patients that failed to reach statistical significance on group comparisons.

Conclusions. Executive dysfunction and a trend towards ToM impairments were noted. MRI analyses revealed mild atrophy and indicated only mild associations with ToM and EF in the dlPFC, thus supporting the relatively less impaired performance in the current sample.

Keywords: Theory of mind, ToM network, Voxel based morphometry, Tract based spatial statistics, resting state fMRI, Amyotrophic lateral sclerosis

Keywords: Theory of Mind, Theory of Mind Network, Social cognition, Amyotrophic Lateral Sclerosis

5.2 Introduction

The previous chapter highlighted that patients with executive function (EF) impairments perform worse on social cognition tasks, specifically for Theory of Mind (ToM), which is in line with previous studies (Burke, Pinto-Grau, et al., 2016; Watermeyer et al., 2015b). The current chapter focuses on investigating the structural and functional associations of ToM changes in ALS patients.

EF may moderate ToM performance suggesting a widespread involvement of brain regions shared by both cognitive functions. Structural imaging previously indicated either grey matter (GM) or white matter (WM) associations with ToM in separate studies. Lower grey matter density in the insular cortex and medial prefrontal cortex were associated with poor emotion attribution in ALS (Cerami et al., 2014). Low white matter integrity as indicated by reduced fractional anisotropy (FA) in the genu of the corpus callosum, the right inferior fronto-occipital fasciculus (IFOF), the right uncinate fasciculus (UF) were associated with lower emotion attribution in ALS patients (Crespi et al., 2014). Furthermore, functional investigations have revealed associations between poor ToM in ALS and poor resting metabolic activity in the dosrsolateral prefrontal cortex (dlPFC) and supplementary motor area (Carluer et al., 2014). Blood-oxygen-level dependent (BOLD) signals at rest as revealed by functional magnetic resonance imaging (fMRI) in the posterior cingulate cortex (PCC) were significantly associated with affective theory of mind (A-ToM) in ALS patients (Trojsi et al., 2017).

A neuroanatomical model of ToM has been suggested by Abu-Akel and Shamay-Tsoory (2011), including differential involvement of frontal and posterior regions for affective and

cognitive ToM (A-ToM and C-ToM respectively). According to this model, mental states are framed in the temporoparietal junction (TPJ) and then assigned to either ventral or dorsal stream via the superior temporal sulcus (STS) or the precuneus / posterior cingulate cortex (PCC) respectively. The ventral stream including the ventromedial prefrontal cortex (vmPFC), medial orbitofrontal cortices (mOFC), ventral anterior cingulate cortex (vACC), ventral striatum and the amygdala are responsible for A-ToM. Conversely, the dorsal stream including regions such as dlPFC, dorsal anterior cingulate cortex (dACC) and the dorsal striatum are associated with C-ToM. While these regions were individually investigated and reported as deficient in ALS, they have not been collectively investigated to understand the neural basis of ToM changes in ALS.

The current study aimed at investigating ToM and its association with grey matter and white matter changes in the ToM network. A-ToM and C-ToM were investigated using the Judgement of Preference subtest on the Edinburgh Cognitive and Behavioural ALS Screen (ECAS JP) (Abrahams et al., 2014), the Reading the Mind in the Eyes (RME) test (Baron-Cohen et al., 2001) and the Faux pas test (Stone et al., 1998). Select a-priori regions of interest were considered based on neuroanatomical associations of these ToM tests; lower performance on ECAS JP has been associated with reduced BOLD signals in the TPJ (Keller et al., 2017), lower performance on the RME has been associated with reduced grey matter density in the mPFC (Torralva, Roca, Gleichgerrcht, Bekinschtein, & Manes, 2009) and decreased performance on the Faux pas test was associated with mOFC lesions (Gregory et al., 2002; Meier et al., 2010; Stone et al., 1998). The dlPFC and the ACC were also considered as they have been previously implied in EF deficits in ALS (Abrahams et al., 2004). A-priori white matter tracts were also defined based on anatomical connectivity

between these ToM regions and included genu of the corpus callosum (gCC), unicinate fasciculus (UF), the cingulum and the superior longitudinal fasiculus (SLF). Posterior cingulate / precuneus that forms an important part of the default mode network (DMN) which has been found to overlap with ToM tasks (Mars et al., 2012), thereby resting state connectivity using the above a-priori regions as seeds, and the DMN were explored. It was hypothesised that poor ToM will be associated with (1) lower grey matter densities, (2) reduced integrity of white matter tracts and (3) altered resting state connectivity.

5.3 Methods

5.3.1 Participants

Participants for the current Magnetic Resonance Imaging (MRI) study were a sub-cohort of previous experiments in the thesis (77% of participants were included in Chapters 3 and 4; 23% were included only for Chapter 3). All participants underwent neuropsychometric testing and an MRI protocol. The demographic and clinical profile of the sample is outlined in Table 5.1.

Fifteen patients (7 males) meeting the El Escorial criteria for probable, probable lab-supported or definite ALS were recruited (Brooks et al., 2000). The median symptom duration (time elapsed since symptom onset to date of testing) was 21.0 months (range: 10-60). Mean disability was 40.9 (*SD* = 4.8, range: 32-47) as measured by the ALS Functional Rating Scale – Revised scale (ALSFRS-R) (Cedarbaum et al., 1999). They were matched for age, education and gender with a group of 15 healthy controls (9 males; Table 5.1).

5.3.2 Neuropsychometric tests

The Edinburgh Cognitive and Behavioural ALS Screen (ECAS) (Abrahams et al., 2014) was administered to identify overall cognitive profile and sub-groups in ALS. ToM was measured using ECAS Judgement of Preference test (ECAS JP), Reading the Mind in the Eyes test (RME) and the Faux pas stories test. Details of these tests and scoring were described in a previous study (Chapter 4). Group differences on the neuropsychometric tests were calculated on this sub-cohort to review cognitive profile. Correlations of ToM and EF with multimodal MRI images using a region-of-interest (ROI) approach were performed to check for structural and functional associations.

5.3.3 MRI acquisition and analysis

Participants included in the study were from a single site (Site 2 in Chapter 4) as a part of the Canadian ALS Neuroimaging Consortium (CALSNIC). Magnetic resonance imaging (MRI) was performed on a 3T Siemens Prisma scanner with a 20-channel head coil, of which the following scans were considered for the current study:

(1) Three-dimensional T1 MPRAGE images (TR = 2300ms, TE = 3.43ms, TI = 900ms, matrix = 256 x 256, 176 slices, voxel size = 1mm isotropic),

(2) whole-brain diffusion tensor imaging (DTI; TR = 10000ms, TE = 90ms, 70 slices, voxel size = 2mm isotropic, b=1000s/mm^2 for 30 non-collinear directions and b=0s/mm^2 for 5 non-diffusion weighted volumes), and

(3) Resting-state functional MRI (rs-fMRI; TR = 2200ms, TE = 30ms, flip angle = 90°, 40 slices, 192 time-points, voxel size = 3.5mm isotropic).

5.3.3.1 Voxel Based morphometry (VBM)

Computational Anatomy Toolbox (CAT-12) was used to perform VBM analysis in SPM-12 (http://dbm.neuro.uni-jena.de/cat12/). Pre-processing of all T1 images included alignment of the image to the AC-PC axis using Mango toolbox (Research Imaging Institute, UTHSCSA). The AC-PC aligned images were corrected for inhomogeneity in field-intensity and were normalized to ICBM template space (European brains) using linear and non-linear transformations. These were further segmented into grey matter, white matter and CSF tissue maps. Grey matter tissue maps were smoothed using 8mm FWHM kernel and were considered for analysis. Group comparisons were performed using two-sample t-test in the GLM model, correcting for age and Total Intracranial Volume. ROI based approach was used to selectively assess regions involved in ToM.

5.3.3.2 Diffusion Tensor Imaging (DTI)

DTI images were preprocessed in Explore DTI (Leemans et al., 2009). DTI Toolkit (DTI-TK) (Zhang, Avants, et al., 2007) was used to optimise registration of white matter and further voxel-wise comparisons were applied using Tract Based Spatial Statistics (TBSS) in FSL (Bach et al., 2014). Preprocessing of DTI data included correction for temporal signal drifts, Gibbs ringing artifacts, motion, Eddy currents and EPI distortions. The preprocessed images were converted into FSL format to enable integration with DTI-TK and TBSS. Tensor-based DTI-TK registration included four major steps: (1) the integration of DTI volumes to match the input requirements of DTI-TK, (2) creating the initial population specific template by bootstrapping the subject DTI volumes, (3) affine alignment with template refinement, allowing alteration of global size and shape of individual subject DTI volumes to match the initial population specific template, (4) deformable alignment with template refinement, to improve registration of subject DTI volumes by minimizing

differences in size and shape of local structures. Individual subject data were warped to the final population specific template using a single interpolation operation combining both affine and deformable alignments.

A custom implementation of the TBSS post-registration step was implemented as suggested (Zhang, Avants, et al., 2007) to generate spatially normalized DTI data at an isotropic $1mm^3$ resolution. Subsequently a 4D FA map of all subjects and a white matter skeleton (FA threshold > 0.2) were generated to enable voxel-wise comparisons. The randomise module in FSL was used to compare DTI metrics between the patient and control groups. Analysis of covariance (ANCOVAs) with age as covariate were employed using a threshold of 5000 permutations. As an exploratory analysis, other DTI metrics such as axial diffusivity (AD), radial diffusivity (RD) and mean diffusivity (MD) were also compared between the groups to understanding biological basis of FA alterations.

5.3.3.3 Resting-state functional MRI (rs-fMRI)

Rs-fMRI scan was obtained for 13 patients; two patients were administered Ativan prior to their MRI scan and hence were not subjected to rs-fMRI acquisition. The CONN toolbox was used for preprocessing and rs-fMRI analysis (http://www.nitrc.org/projects/conn). For each subject, the initial four time-points of the rs-fMRI scan were discarded to remove spin saturation effects. The remaining 188 time-points were realigned to the first time-point using a rigid body alignment to remove movement related artifacts. They were then unwarped to reduce susceptibility-by-movement interactions noted as distortions in regions of air-tissue interface (e.g. orbitofrontal cortex). The unwarped time-points were co-registered to T1 images and were transformed to MNI space using forward deformations derived from T1 to

MNI transformations. All time-points were smoothed using 8mm FWHM kernel. ART (www.nitrc.org/projects/artifact_detect) scrubbing was performed to remove outliers defined as time-points with more than 2 mm of scan-to-scan frame-wise displacement or with z-normalised signals that were greater than 3SD from mean global BOLD activations. There was one control with four outlier time-points (maximum motion = 3.3 mm) and one patient with 42 outlier time-points (maximum motion = 6.8 mm). For these participants, these outlier time-points were excluded for the rest of the analysis. Overall, there were no significant differences between the number of valid scans between patients and controls (p=0.33) or maximum head movement (p=0.38).

Next, de-noising of the data was performed to remove confounding effects from the BOLD signal. Linear regression of realignment parameters and physiological signals from white matter and CSF derived in the previous steps was performed for each subject. A band-pass filter of 0.01-0.1 Hz was applied. For first-level analysis, regions of interests (ROIs) described in the next section were used as seeds to derive seed-based connectivity maps. These maps represent the strength of connectivity between brain regions and the seeds. Additionally, independent components analysis (ICA) was applied to derive the DMN maps for these subjects. Second-level analysis were performed on these connectivity maps (both seed-based and ICA) to compare functional connectivity between patients and controls. Significance was accepted at p<0.001 with cluster-size threshold (112 voxels) determined using Monte Carlo simulations to correct for multiple comparisons.

5.3.4 ROI selection and Group Comparisons

Considering the a-priori nature of the hypotheses, ROIs were selected as per the ToM network model proposed by Abu-Akel and Shamay-Tsoory (2011). ROIs for VBM and rs-fMRI analysis were created using the WFU PickAtlas software toolbox (version 2.5) in MATLAB (Maldjian et al., 2003). They included: the anterior cingulate cortex (ACC), medial prefrontal cortex (mPFC), medial orbitofrontal cortex (mOFC), dorsolateral prefrontal cortex (dlPFC) and the anterior temporoparietal junction (aTPJ) (see Figure 5.1).

Probabilistic white matter tracts from the IIT Human Brain Atlas (v.4.1) database were used for ROI-based TBSS analysis (https://www.nitrc.org/projects/iit/). These white matter ROIs were selected based on a-priori knowledge of regions connected by these tracts. They included: genu of the corpus callosum (gCC; interhemispheric ACC and mPFC), cingulum (ACC to posterior regions), unicinate fasciculus (UF; mOFC to temporal regions), and superior longitudinal fasciculus (SLF; dlPFC to temporal regions including aTPJ). A 10% probability was determined for each tract and they were binarised to derive corresponding masks. These masks were transformed from standard ICBM space of the IIT atlas to population template generated using the study participants (Figure 5.1).

Furthermore, regions indicating significant differences between the patient and control groups were used to extract the corresponding grey matter volumes, FA or rs-fMRI connectivity for correlations with ToM and EF in IBM SPSS statistics Version 24.0 (IBM Corp., Armonk, NY). Ad-hoc analysis with precentral gyrus (PCG) as seed and whole-brain comparisons were also performed to check for motor-related changes.

5.4 Results

The participant groups were matched for age (U = 127.5, p = 0.62) and education (U = 70.0, p = 0.08). The groups were also matched for gender distribution (χ^2 = 0.5, p = 0.5, see Table 5.1). Correlations between age, education and neuropsychometric scores revealed no significant associations ($p > 0.05$). Patients performed significantly lower on the modified ECAS executive (no ToM; U = 50.0, $p<0.01$, FDR-corrected) and modified ECAS total scores (U = 47.5, $p<0.01$, FDR-corrected) when compared to controls (Table 5.2). At more lenient thresholds, deficient performance in patients was found for ECAS verbal fluency (U = 60.5, p = 0.03, uncorrected), while a trend was noted for poor RME performance (p = 0.06; Supplementary Figure 5.1).

5.4.1 VBM analysis

Significantly lower grey matter density was found in patients in the dorsal region of the ACC (p<0.01, uncorrected), the right superior frontal gyrus of the mPFC (p<0.01, uncorrected), the left middle frontal gyrus and bilateral insula for the dlPFC (p<0.01, uncorrected) and the aTPJ (Figure 5.2). No significant difference was noted for the mOFC. ROI-based analysis of the PCG and whole-brain comparisons revealed only mild changes in the motor cortex that failed to survive at more stringent thresholds (p<0.001, uncorrected). These group comparisons in grey matter densities fail to survive multiple comparisons corrections.

Voxel-wise correlations between ToM and EF with specific ROIs revealed associations at lenient thresholds within the ROIs (p<0.01, uncorrected) while a few survived more stringent thresholds (p<0.001, uncorrected). Both ToM and EF displayed associations with the dlPFC (p<0.01 and p<0.001; Supplementary Table 5.1), such that lower performance was associated with reduced grey matter density in the dlPFC. RME performance was also

associated with mPFC and mOFC. No significant associations were noted between grey matter reductions noted in group comparisons (of the ACC, mPFC, mOFC, dlPFC, aTPJ) and the ToM / EF tests.

5.4.2 DTI analysis

Group comparisons of FA maps revealed lower FA in patients as compared to controls in all the white matter ROIs ($p<0.01$, uncorrected; Figure 5.3). Of these, only lower FA in the cingulum survived multiple comparisons correction ($p<0.05$, TFCE corrected). ToM and EF showed diffuse correlations with specific white matter tracts using voxel-wise ROI associations ($p<0.01$, uncorrected). However, no significant associations were noted when corrected for multiple comparisons. Group comparisons on additional TI metrics indicated reduced AD, increased RD and MD in the white matter ROIs (Supplementary Figure 5.2).

5.4.3 rs-fMRI analysis

One-sample t-tests were used to derive seed-based connectivity maps of the ACC, mPFC, mOFC, dlPFC and aTPJ (Figure 5.4). Each seed-based network displayed an overlap with the DMN, but also displayed involvement of regions unique to each network. The ACC was found to correlate extensively with mPFC, posterior cingulate cortex (PCC), insula and sub-cortical structures such as the thalamus (Figure 5.4-A). The mPFC network showed extensive associations with the ACC, the dlPFC and middle temporal gyrus (Figure 5.4-B). The mOFC seed was associated with mPFC, superior and middle frontal gyri, anterior parts of the superior temporal lobes and the hippocampus (Figure 5.4-C). The dlPFC was associated with the PCC, parietal regions as well as sub-cortical structures such as the caudate (Figure 5.4-D). The aTPJ seed was associated with the parietal regions, precuneus,

bilateral insula, the ACC and superior frontal gyri (Figure 5.4-E). A visual comparison revealed reduced spatial extent in patients that failed to reach statistical significance on the t-test. Similar non-significant reduction in spatial extent was also noted in the DMN generated using ICA analysis (Figure 5.5).

5.5 Discussion

The current study aimed at assessing the structural and functional associations of ToM and EF with regions of the ToM network. Cognitive profile revealed executive dysfunctions and a mild change in ToM, which were not associated with neuroimaging variables such as grey matter densities, white matter integrity or resting state connectivity in ALS.

ToM showed a tendency towards lower performance in patients on the RME test. One previous study had indicated similar findings on a similar sized cohort (Girardi et al., 2011), while another study indicated no significant differences using the RME (Cavallo et al., 2011). On the other hand, a study using a larger sample size suggested significantly lower RME performance (Burke, Pinto-Grau, et al., 2016). A power analysis completed by Trojsi et al. (2017) suggested recruitment of at least 21 patients and 21 controls. The authors compared longitudinal performance on ToM using Strange stories task (Happe, 1994) that required explaining cartoon stories in either ToM or non-ToM conditions. There were no significant differences between the groups at baseline, while mild ToM changes at follow-up were noted (Trojsi et al., 2017). Together these findings suggest that while the small sample size may play a role in a reduced ability to detect impairments, it is likely that ToM changes in ALS are indeed mild at baseline though may progress with advancement of frontotemporal changes.

Mild grey matter degeneration noted in a-priori regions of the ToM network was not associated with ToM / EF performance in ALS. This appears to be in line with the mild ToM change noted in ALS patients, increased severity in ToM impairments such as those noted in FTD may be associated with greater structural and functional decline. Previous studies in ALS have suggested that grey matter atrophy in the ACC and the insular cortex may be associated with emotion impairments (Cerami et al., 2014). However, it was surprising to note that reduced grey matter densities in the dlPFC also failed to correlate with EF. On the other hand, ROI-based voxel-wise correlations displayed associations between both ToM and EF for dlPFC, indicating the possibility of a shared neural substrate. These associations, however, did not survive multiple comparisons corrections.

Reduced white matter integrity was noted in ALS patients in the ROI tracts. Specifically, assessment of AD, RD and MD values indicated that reduced FA aligned with increased RD in ALS patients, suggesting demyelination in ALS patients (Song et al., 2003). This mild degeneration of the white matter from group comparisons was not associated with either ToM / EF. However, ROI-based voxel-wise correlations indicated mild associations at lenient statistical thresholds which failed to survive multiple comparisons. Studies investigating other aspects of social cognition such as emotion recognition and attribution report changes in the inferior fronto-occipital fasciculus (IFOF) (Crespi et al., 2014) and frontotemporal regions including the gCC, SLF, UF and ILF. Furthermore, these changes were associated with poor emotion recognition / attribution abilities in ALS patients. The lack of such an association in the current sample may represent a cohort that displayed only mild ToM deficits and thereby no underlying structural or functional impairments.

Some studies have suggested that functional changes precede structural change (Menke et al., 2016). In the current cohort, seed-based resting state networks of the ToM and model-free DMN networks showed non-significant reductions of connectivity in ALS patients. Considering the lack of statistical differences between the two groups, correlations with ToM / EF were not done with rs-fMRI. A recent study displayed associations between lower activations in the frontoparietal network (FPN, which is associated with cognitive processes such as EF) and ToM in ALS (Trojsi et al., 2017).

In the current study, significant EF impairments and a strong trend towards ToM impairments were noted in ALS patients. These changes in cognition were not associated with degeneration in either structural or functional networks. It would be of interest to explore neuroimaging associations of ToM / EF in cognitive sub-groups in ALS. In the current analysis, about 50% of the patients were impaired on either verbal fluency or modified executive domain scores (ALS-exi) while the remaining were normal on these domains (ALS-n). There were no significant differences between the sub-groups on the ToM tests, except for the RME which showed a trend (p=0.056). It is possible that ToM changes are in-fact mild in ALS patients and may increase in severity if patients meet criteria for FTD. Future studies should consider recruiting a larger sample of ALS patients to investigate and replicate frontotemporal findings of ToM in ALS and also explore these associations within ALS sub-groups (e.g. cognitively impaired vs normal patients).

5.6 Conclusions

There was a lack of significant ToM impairments in the current sample. While regions typically associated with ToM displayed mild atrophy for grey matter and white matter, no

significant differences were noted in functional brain changes at rest in patients compared to controls. However, a larger sample size is necessary to further investigate structural and functional associations with ToM.

5.7 References

Abrahams, S., Goldstein, L. H., Simmons, A., Brammer, M., Williams, S. C., Giampietro, V., & Leigh, P. N. (2004). Word retrieval in amyotrophic lateral sclerosis: a functional magnetic resonance imaging study. *Brain, 127*(Pt 7), 1507-1517.

Abrahams, S., Newton, J., Niven, E., Foley, J., & Bak, T. H. (2014). Screening for cognition and behaviour changes in ALS. *Amyotroph Lateral Scler Frontotemporal Degener, 15*(1-2), 9-14. doi:10.3109/21678421.2013.805784

Abu-Akel, A., & Shamay-Tsoory, S. (2011). Neuroanatomical and neurochemical bases of theory of mind. *Neuropsychologia, 49*(11), 2971-2984. doi:10.1016/j.neuropsychologia.2011.07.012

Bach, M., Laun, F. B., Leemans, A., Tax, C. M., Biessels, G. J., Stieltjes, B., & Maier-Hein, K. H. (2014). Methodological considerations on tract-based spatial statistics (TBSS). *Neuroimage, 100*, 358-369. doi:10.1016/j.neuroimage.2014.06.021

Baron-Cohen, S., Wheelwright, S., Hill, J., Raste, Y., & Plumb, I. (2001). The "Reading the Mind in the Eyes" Test revised version: a study with normal adults, and adults with Asperger syndrome or high-functioning autism. *J Child Psychol Psychiatry, 42*(2), 241-251.

Brooks, B. R., Miller, R. G., Swash, M., Munsat, T. L., & World Federation of Neurology Research Group on Motor Neuron, D. (2000). El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Other Motor Neuron Disord, 1*(5), 293-299.

Burke, T., Pinto-Grau, M., Lonergan, K., Elamin, M., Bede, P., Costello, E., . . . Pender, N. (2016). Measurement of Social Cognition in Amyotrophic Lateral Sclerosis: A Population Based Study. *PLoS One, 11*(8), e0160850. doi:10.1371/journal.pone.0160850

Carluer, L., Mondou, A., Buhour, M. S., Laisney, M., Pelerin, A., Eustache, F., . . . Desgranges, B. (2014). Neural substrate of cognitive theory of mind impairment in amyotrophic lateral sclerosis. *Cortex, 65C*, 19-30. doi:10.1016/j.cortex.2014.12.010

Cavallo, M., Adenzato, M., Macpherson, S. E., Karwig, G., Enrici, I., & Abrahams, S. (2011). Evidence of social understanding impairment in patients with amyotrophic lateral sclerosis. *PLoS One, 6*(10), e25948. doi:10.1371/journal.pone.0025948

Cedarbaum, J. M., Stambler, N., Malta, E., Fuller, C., Hilt, D., Thurmond, B., & Nakanishi, A. (1999). The ALSFRS-R: a revised ALS functional rating scale that incorporates assessments of respiratory function. BDNF ALS Study Group (Phase III). *J Neurol Sci, 169*(1-2), 13-21.

Cerami, C., Dodich, A., Canessa, N., Crespi, C., Iannaccone, S., Corbo, M., . . . Cappa, S. F. (2014). Emotional empathy in amyotrophic lateral sclerosis: a behavioural and voxel-based morphometry study. *Amyotroph Lateral Scler Frontotemporal Degener, 15*(1-2), 21-29. doi:10.3109/21678421.2013.785568

Crespi, C., Cerami, C., Dodich, A., Canessa, N., Arpone, M., Iannaccone, S., . . . Cappa, S. F. (2014). Microstructural white matter correlates of emotion recognition

impairment in Amyotrophic Lateral Sclerosis. *Cortex, 53*, 1-8. doi:10.1016/j.cortex.2014.01.002

Girardi, A., Macpherson, S. E., & Abrahams, S. (2011). Deficits in emotional and social cognition in amyotrophic lateral sclerosis. *Neuropsychology, 25*(1), 53-65. doi:10.1037/a0020357

Gregory, C., Lough, S., Stone, V., Erzinclioglu, S., Martin, L., Baron-Cohen, S., & Hodges, J. R. (2002). Theory of mind in patients with frontal variant frontotemporal dementia and Alzheimer's disease: theoretical and practical implications. *Brain, 125*(Pt 4), 752-764.

Happe, F. G. (1994). An advanced test of theory of mind: understanding of story characters' thoughts and feelings by able autistic, mentally handicapped, and normal children and adults. *J Autism Dev Disord, 24*(2), 129-154.

Keller, J., Bohm, S., Aho-Ozhan, H. E. A., Loose, M., Gorges, M., Kassubek, J., . . . Lule, D. (2017). Functional reorganization during cognitive function tasks in patients with amyotrophic lateral sclerosis. *Brain Imaging Behav*. doi:10.1007/s11682-017-9738-3

Leemans, A., Jeurissen, B., Sijbers, J., & Jones, D. K. (2009). *ExploreDTI: a graphical toolbox for processing, analyzing, and visualizing diffusion MR data.* Paper presented at the 17th Annual Meeting of Intl Soc Mag Reson Med, Hawaii, USA.

Maldjian, J. A., Laurienti, P. J., Kraft, R. A., & Burdette, J. H. (2003). An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. *Neuroimage, 19*(3), 1233-1239.

Mars, R. B., Neubert, F. X., Noonan, M. P., Sallet, J., Toni, I., & Rushworth, M. F. (2012). On the relationship between the "default mode network" and the "social brain". *Front Hum Neurosci, 6*, 189. doi:10.3389/fnhum.2012.00189

Meier, S. L., Charleston, A. J., & Tippett, L. J. (2010). Cognitive and behavioural deficits associated with the orbitomedial prefrontal cortex in amyotrophic lateral sclerosis. *Brain: A Journal of Neurology, 133*(11), 3444-3457.

Menke, R. A., Proudfoot, M., Wuu, J., Andersen, P. M., Talbot, K., Benatar, M., & Turner, M. R. (2016). Increased functional connectivity common to symptomatic amyotrophic lateral sclerosis and those at genetic risk. *J Neurol Neurosurg Psychiatry, 87*(6), 580-588. doi:10.1136/jnnp-2015-311945

Stone, V. E., Baron-Cohen, S., & Knight, R. T. (1998). Frontal lobe contributions to theory of mind. *J Cogn Neurosci, 10*(5), 640-656.

Torralva, T., Roca, M., Gleichgerrcht, E., Bekinschtein, T., & Manes, F. (2009). A neuropsychological battery to detect specific executive and social cognitive impairments in early frontotemporal dementia. *Brain, 132*(Pt 5), 1299-1309. doi:10.1093/brain/awp041

Trojsi, F., Di Nardo, F., Santangelo, G., Siciliano, M., Femiano, C., Passaniti, C., . . . Tedeschi, G. (2017). Resting state fMRI correlates of Theory of Mind impairment in amyotrophic lateral sclerosis. *Cortex, 97*, 1-16. doi:10.1016/j.cortex.2017.09.016

Watermeyer, T. J., Brown, R. G., Sidle, K. C., Oliver, D. J., Allen, C., Karlsson, J., . . . Goldstein, L. H. (2015). Executive dysfunction predicts social cognition impairment in amyotrophic lateral sclerosis. *J Neurol, 262*(7), 1681-1690. doi:10.1007/s00415-015-7761-0

Zhang, H., Avants, B. B., Yushkevich, P. A., Woo, J. H., Wang, S., McCluskey, L. F., . . . Gee, J. C. (2007). High-dimensional spatial normalization of diffusion tensor images improves the detection of white matter differences: an example study using amyotrophic lateral sclerosis. *IEEE Trans Med Imaging, 26*(11), 1585-1597. doi:10.1109/TMI.2007.906784

5.8 Tables and Figures

Table 5.1. Demographic and clinical profile of the participants.

Variables	Controls (N = 15)	Patients (N = 15)	p
Age (years)	58.1 (8.1) 37-66	59.1 (10.5) 37-74	0.62
Education (years)	16.3 (2.1) 13-20	14.7 (2.5) 11-20	0.08
Gender (M : F)	9 : 6	7 : 8	0.60
Onset			
Bulbar	-	3	-
Limb	-	12	-
Symptom Duration (months)[a]	-	19.0 (10-60)	-
ALSFRS-R	-	41.2 (4.8) 32-47	-
Disease Progression Rate	-	0.4 (0.4) 0.1-1.2	-

Values are represented as Mean (SD) range. [a] Symptom Duration represented as Median (range). Group comparisons performed using Mann-Whitney U-test and Pearson Chi-square test used to compare gender ratios.

Table 5.2. Group comparisons on ECAS and ToM tests.

Neuropsychometric tests	Max.	Controls (N=15)		Patients (N=15)		p
		M ± SD	Min-Max	M ± SD	Min-Max	
ECAS						
Language	28	26.6 ± 1.2	24-28	24.6 ± 3.2	19-28	0.10
Verbal Fluency	24	18.7 ± 3.2	12-22	12.9 ± 7.2	4-24	0.03
Executive modified (no ToM)[a]	36	29.1 ± 2.6	25-33	24.5 ± 4.8	15-32	**0.01**
Memory	24	17.8 ± 2.5	15-24	16.8 ± 3.6	7-21	0.65
Visuospatial	12	12.0 ± 0.0	12-12	11.5 ± 1.1	8-12	0.22
ECAS Total modified (no ToM)[b]	124	103.9 ± 6.8	92-116	90.3 ± 15.7	63-109	**<0.01**
ToM						
ECAS JP	12	11.3 ± 2.6	2-12	11.1 ± 1.9	6-12	0.39
RME Total	36	27.8 ± 3.9	22-33	24.0 ± 5.1	14-31	0.06
Faux pas Stories Total	80	77.5 ± 5.0	62-80	77.6 ± 2.6	71-80	0.37
ToM condition	40	39.6 ± 0.8	37-40	38.9 ± 1.7	36-40	0.57
Control condition	40	37.9 ± 4.2	25-40	38.7 ± 2.5	31-40	0.94

ECAS Executive score was 40.3 ± 2.8 (35-44) for controls and 35.6 ± 6.2 (22-44) for patients. ECAS Total score was 115.5 ± 6.4 (104-128) for controls and 101.5 ± 16.5 (73-121). Note: p-value in bold font was FDR-corrected.

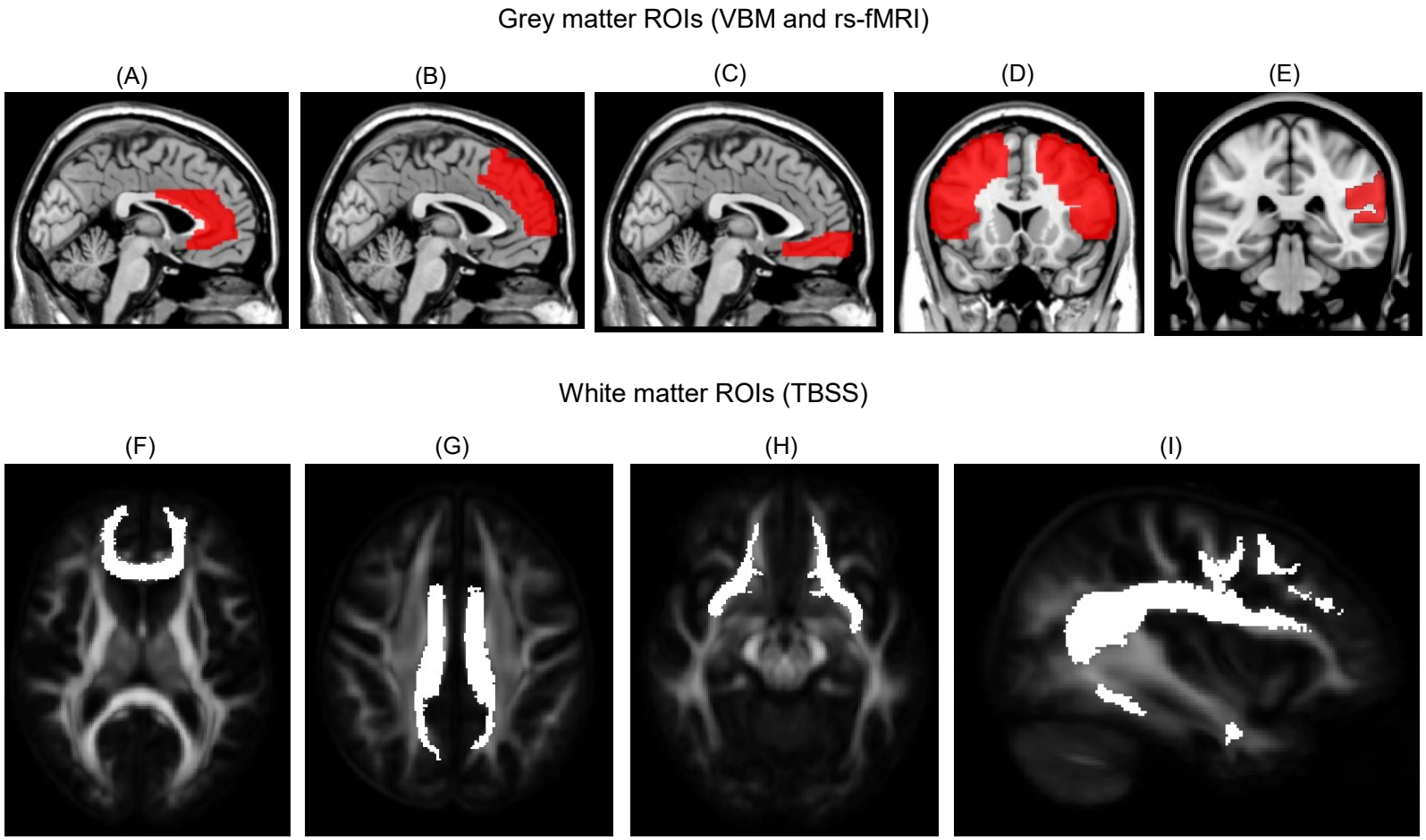


Figure 5.1. Regions of Interest (ROIs) for MRI analysis.
(A) anterior cingulate cortex (ACC), (B) medial prefrontal cortex (mPFC), (C) medial orbitofrontal cortex (mOFC), (D) dorsolateral prefrontal cortex (dlPFC), (E) anterior temporoparietal junction (aTPJ), (F) genu of corpus callosum (gCC), (G) cingulum, (H) uncinate fasciculus (UF) and (I) superior longitudinal fasciculus (SLF). Note: Except for aTPJ, all ROIs are bilateral. Grey matter ROIs were generated using AAL atlas in WFU Pickatlas toolbox. aTPJ was obtained from FSL. White matter ROIs were generated from the IIT atlas.

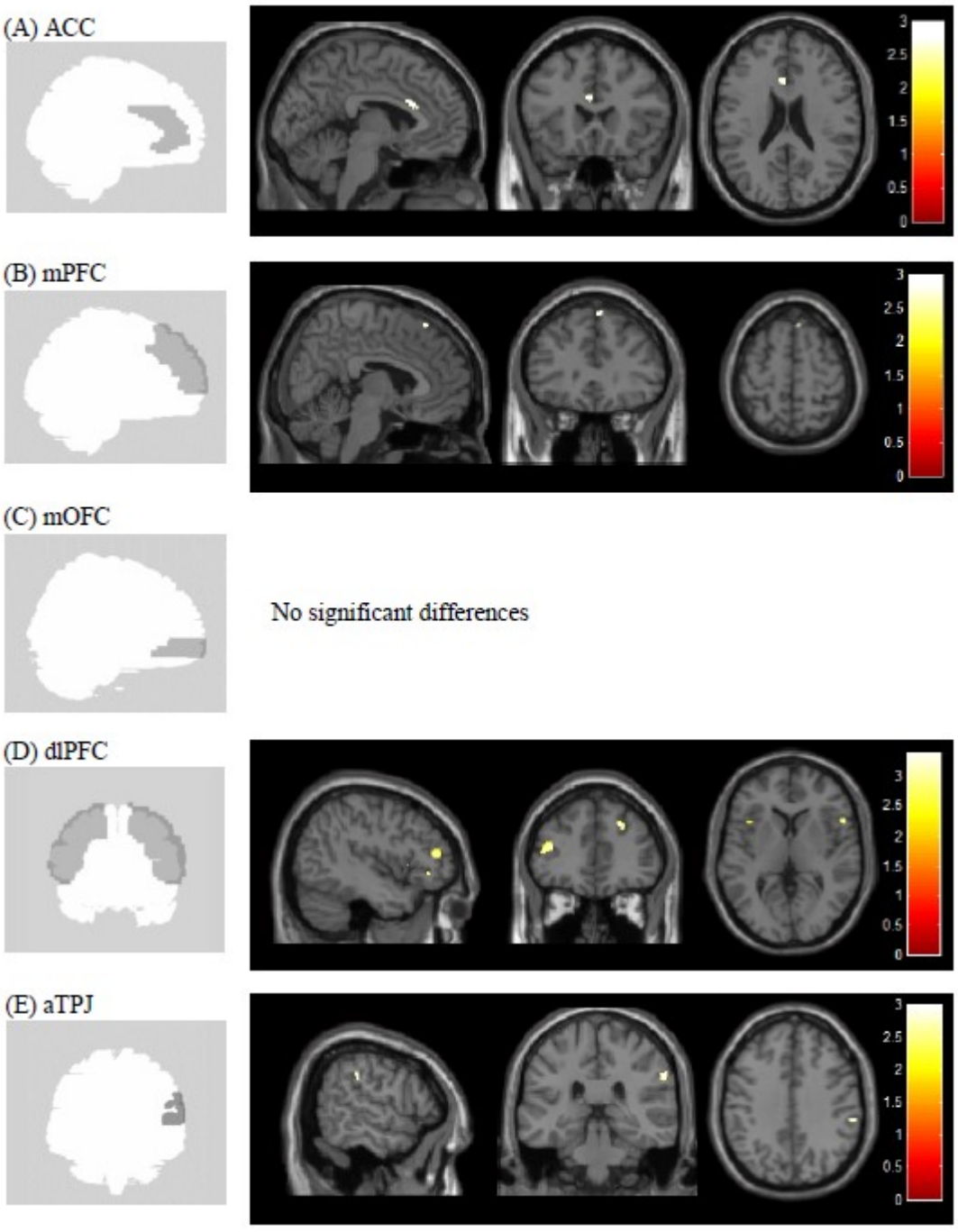


Figure 5.2. ROI-based voxel based morphometry (VBM).
Panels indicating lower grey matter volumes in patients as compared to controls ($p<0.01$, uncorrected). Note: ACC = anterior cingulate cortex, mPFC = medial prefrontal cortex, mOFC = medial orbitofrontal cortex, dlPFC = dorsolateral prefrontal cortex, aTPJ = anterior temporoparietal junction; except for aTPJ, all ROIs were derived using AAL atlas; ROI for aTPJ was obtained from FSL; all images are in neurological convention (left is left).

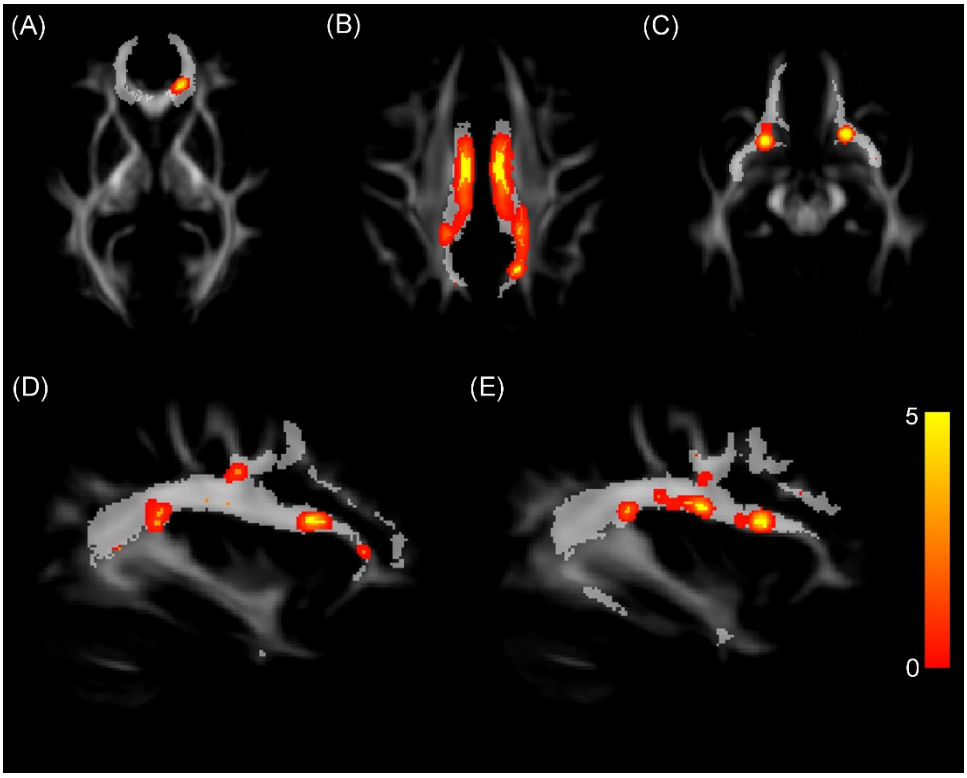


Figure 5.3. ROI-based Tract Based Spatial Statistics (TBSS).
Panels indicate lower fractional anisotropy (FA) in ALS patients as compared to healthy controls for (A) genu of the corpus callosum ($p<0.01$, uncorrected), (B) cingulum ($p<0.05$, TFCE corrected), (C) uncinate fasciculus ($p<0.01$, uncorrected), (D) Left superior longitudinal fasciculus ($p<0.01$, uncorrected), and (E) Right superior longitudinal fasciculus ($p<0.01$ uncorrected). Note: Images are in neurological convention (left is left).

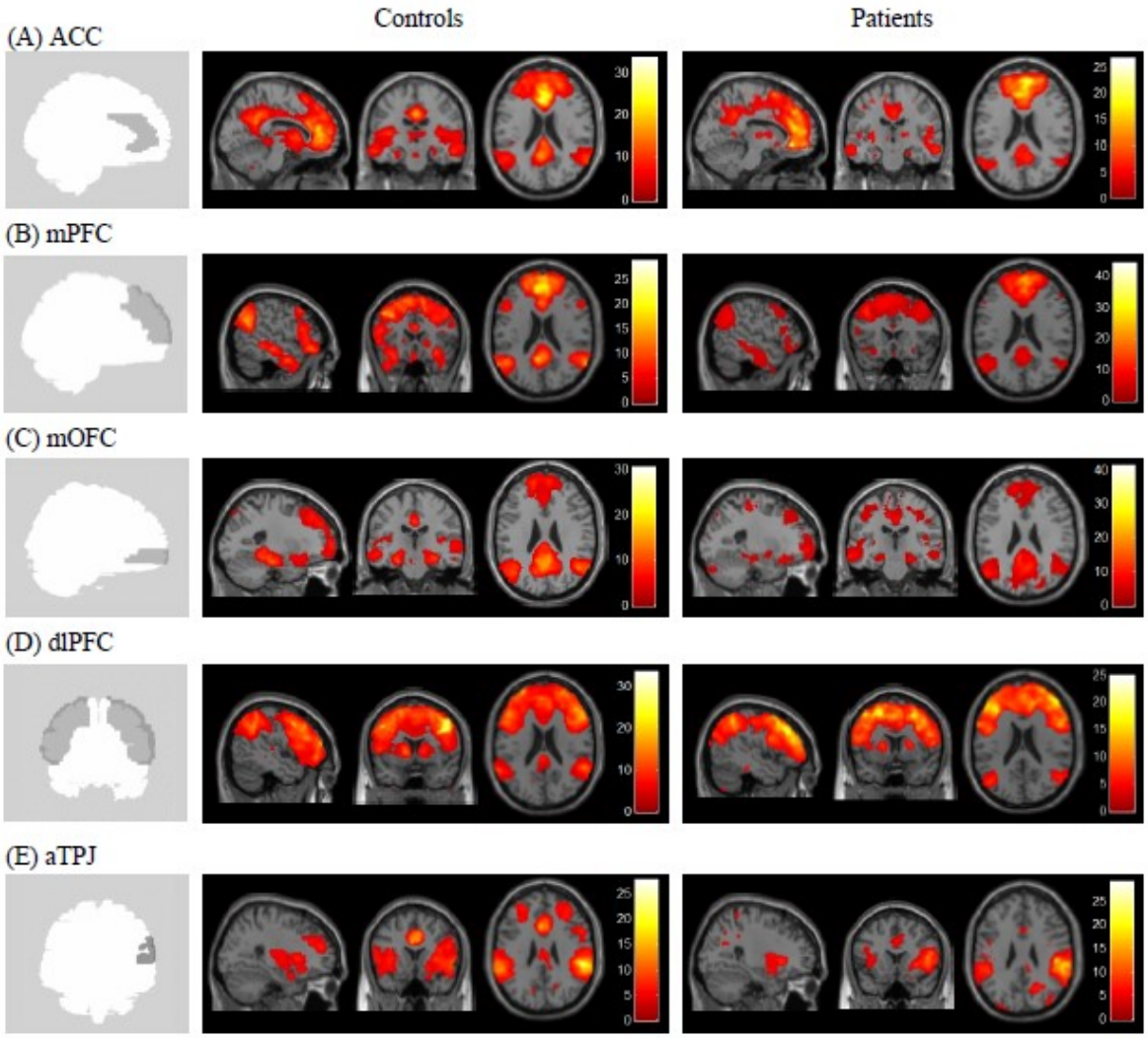


Figure 5.4. One-sample t-test of seed-based analysis.
Panels indicate connectivity maps for controls and patients for (A) anterior cingulate cortex (ACC), (B) medial prefrontal cortex (mPFC), (C) medial orbitofrontal cortex (mOFC), (D) dorsolateral prefrontal cortex (dlPFC), and (E) anterior temporoparietal junction (aTPJ). Threshold for connectivity maps set at $p<0.001$ (cluster-size corrected). Note: Images are displayed in neurological convention (left is left).

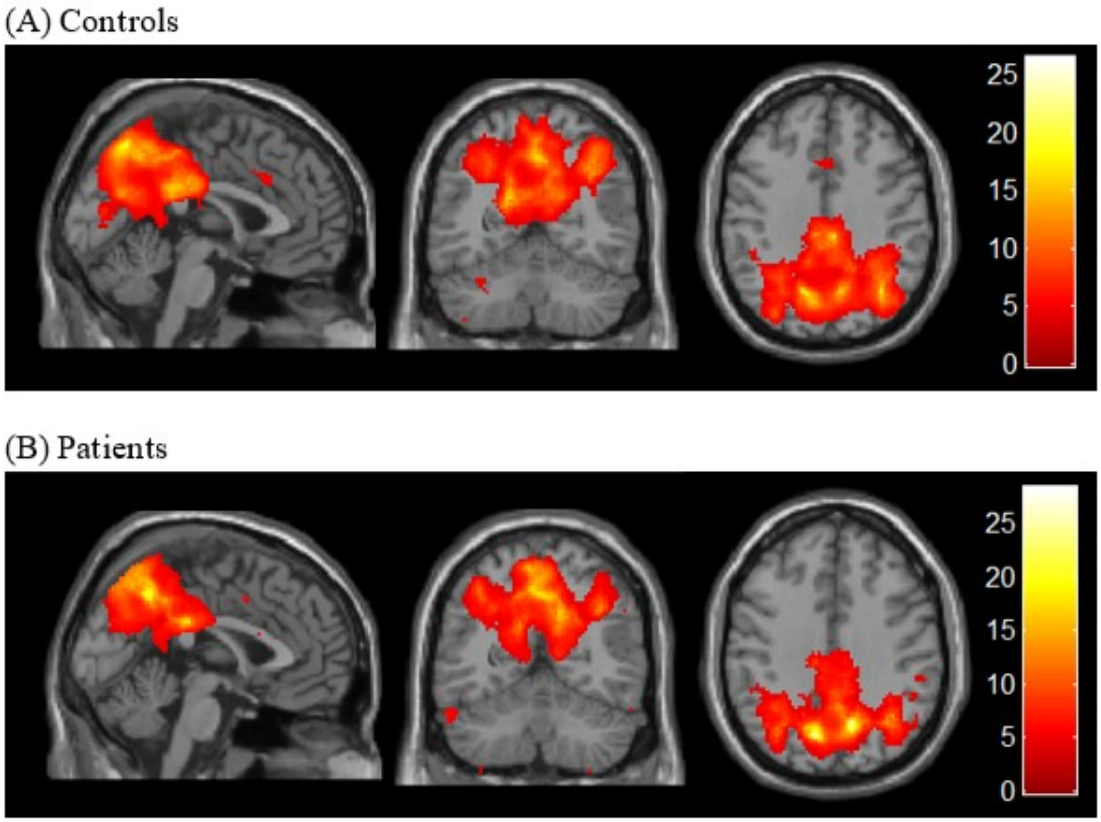
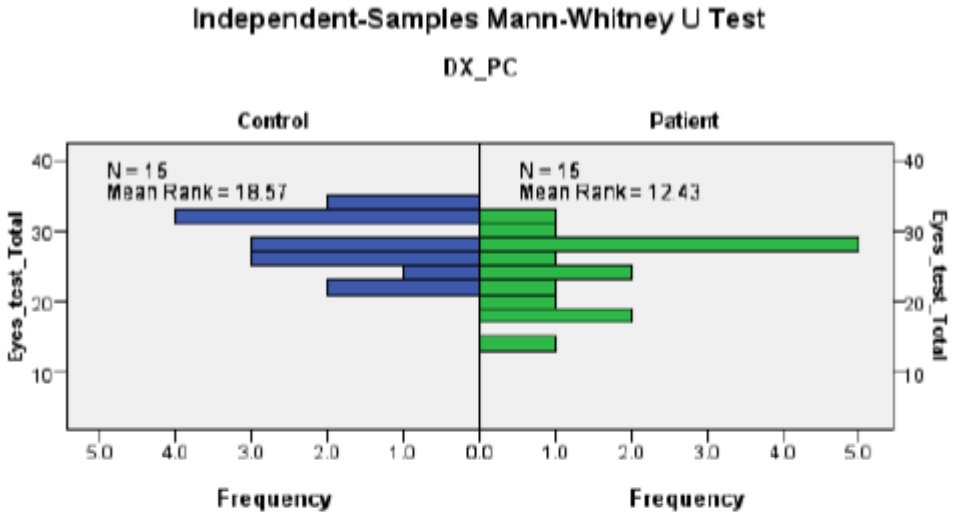


Figure 5.5. One sample t-test indicating the default mode network (DMN).
Panels indicate DMN maps obtained using Independent Components Analysis (ICA) for controls and patients respectively. No significant differences in the connectivity of the DMN between the groups ($p>0.05$)

5.9 Supplementary materials



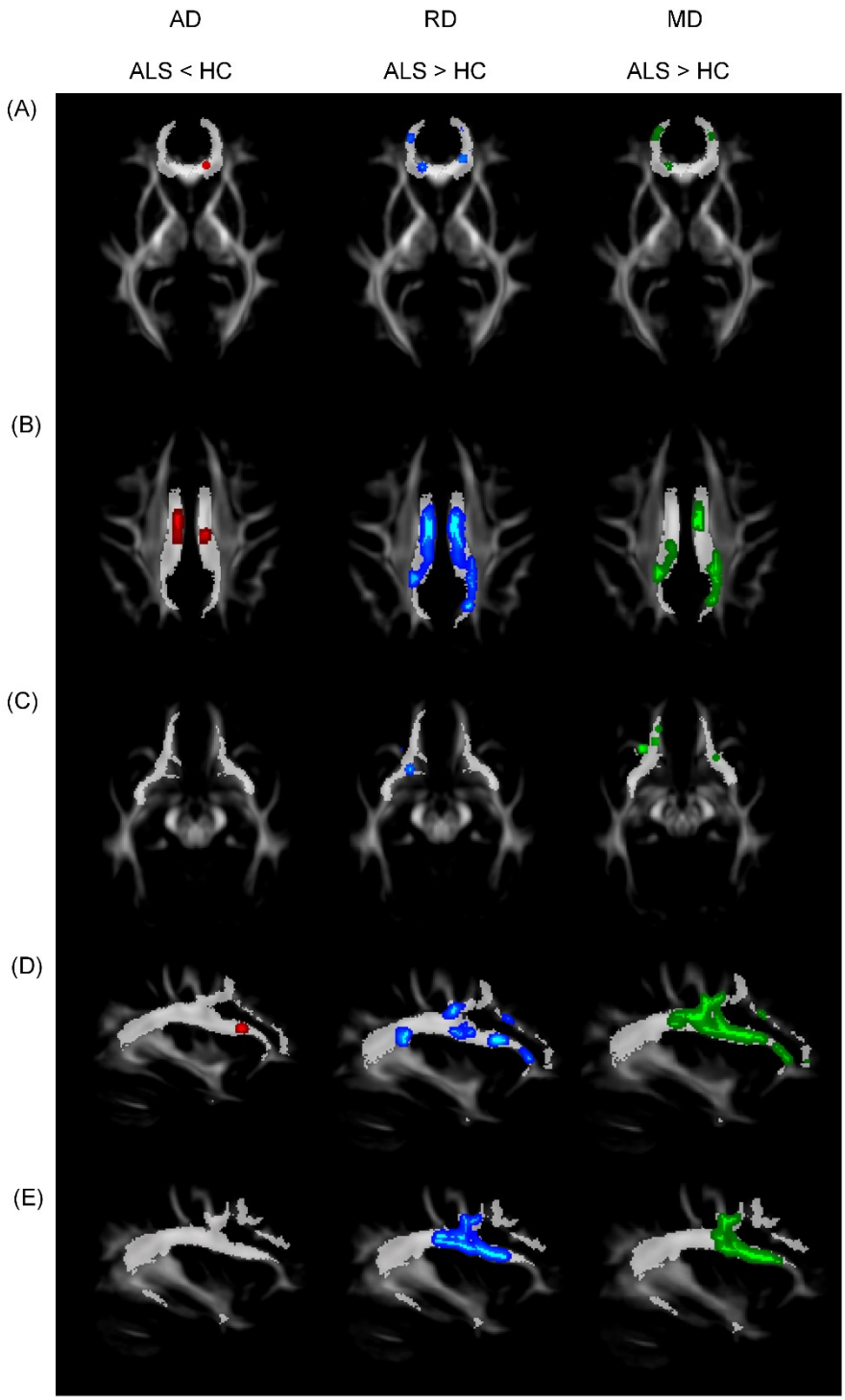
Total N	30
Mann-Whitney U	66.500
Wilcoxon W	186.500
Test Statistic	66.500
Standard Error	23.983
Standardized Test Statistic	-1.918
Asymptotic Sig. (2-sided test)	.055
Exact Sig. (2-sided test)	.056

Supplementary Figure 5.1. Performance on the RME test.
Results indicating a trend towards lower performance between patients and controls.

Supplementary Table 5.1. Summary of voxel-wise correlations between ToM / EF tests and VBM

Tests	ACC	dlPFC	mPFC	mOFC	aTPJ
RME	p<0.01 (k=5)	p<0.001 (k=6-26) p<0.01 (k>100)	p<0.01 (k=12-205)	p<0.001 (k=6) p<0.01 (k=40)	n.s.
Faux pas FP	p<0.01(k=47)	p<0.01 (k=9,37)	n.s.	n.s.	n.s.
ECAS Exe – mod	n.s.	p<0.001 (k = 8) p<0.01 (k=40)	n.s.	n.s.	Not done
ECAS VF	n.s.	p<0.001 (k=8) p<0.01 (k=6-163)	n.s.	Not done	Not done

Note: k = cluster size (number of voxels), RME = Reading the Mind in the Eyes test, Faux pas FP = ToM condition, ECAS Exe – mod = modified ECAS executive, VF = Verbal fluency. All p-values are uncorrected for multiple comparisons.



Supplementary Figure 5.2. ROI-based analysis for AD, RD and MD.
(A) Genu of corpus callosum, (B) cingulum, (C) uncinate fasciculus (UF), (D) Left superior longitudinal fasciculus (L-SLF) and (E) Right superior longitudinal fasciculus (R-SLF). Note: $p<0.01$ (uncorrected) except for (B) AD, RD and MD [$p<0.05$, TFCE corrected], (D) MD [$p<0.05$, TFCE corrected] and (E) RD and MD [$p<0.05$, TFCE corrected]. Images displayed in neurological convention (left is left).

6. General Discussion and Conclusions

The studies completed in this thesis provide neuroimaging support for an ALS-specific screening tool and indicate that social cognition in ALS is moderated by the presence of executive function (EF) impairments. This concluding chapter provides a summary of the research findings and clinical implications. It also outlines the limitations of the studies and discusses scope for future research.

Most neuroimaging studies in ALS have indicated grey matter loss and white matter degeneration in the motor region and corticospinal tracts (CST) of the brain, although studies have also recognised extra-motor changes (Agosta et al., 2012; Mezzapesa et al., 2013; Roccatagliata et al., 2009; Schuster et al., 2013; Verstraete et al., 2012; Walhout et al., 2015). These studies predominantly used extensive neuropsychometric testing, which may not be feasible to administer in a clinical setting. ALS-specific cognitive screening tools such as the ECAS have been found to facilitate cognitive screening and identify cognitive sub-groups, though no study had investigated structural brain changes using the ECAS. This gap was addressed in aim 1 of the thesis. Emerging evidence of ToM impairments and EF (addressed in aim 2) suggested the possibility of shared brain changes for both cognitive functions. Previous studies had looked at either grey matter / white matter / functional changes but not in the same cohort of ALS patients. This was addressed in aim 3 (Chapter 5) of the thesis. A summary of results is provided in Table 6.1.

Table 6.1. Summary of aims, hypotheses and results.

Exp.	Aims	Hypotheses	Results
1	To explore neuroimaging associations of the ECAS	(1) Patients impaired on the ECAS have lower grey matter densities and reduced white matter integrity in the frontotemporal regions compared to controls, and (2) There is an association between the ECAS and neuroimaging metrics of grey matter densities and white matter integrity of frontotemporal regions in ALS patients.	• ALS patients performed significantly worse on ECAS verbal fluency and executive domains. • Patients impaired on EF (ALS-exi) displayed frontal degeneration of grey and white matter. • Patients with intact EF (ALS-n) also displayed mild degeneration in frontal cortex. • Performance on ECAS verbal fluency and executive domains was associated with both grey and white matter in frontotemporal regions.
2	To identify associations between social cognition, specifically, theory of mind (ToM) and executive functions (EF) in ALS.	(1) ToM and EF impairments are present in ALS patients, and (2) ToM and EF are associated such that patients with EF impairments perform worse than those with intact EF in measures of ToM.	• ToM and EF impairments were present in all ALS patients; • ALS-exi patients performed significantly lower on ToM tasks compared to ALS-n and control groups. • Decreased ToM performance was associated with reduced ECAS verbal fluency. • ToM performance was also associated with language and behaviour. To our best knowledge, such associations has not been previously reported in ALS. • ToM performance was comparable in limb and bulbar onset patients.
3	To examine the structural and functional associations of ToM in ALS.	Poor ToM in ALS is associated with (1) lower grey matter densities, (2) reduced integrity of white matter tracts, and (3) altered resting state connectivity of regions included in the ToM network.	• A trend in lower ToM performance was in ALS patients. • Mild grey and white matter degeneration were noted in regions associated with ToM. • Functional alterations in ALS patients did not reach statistical significance. • Both ToM and EF were associated grey matter density in the dlPFC indicating a possible shared neural substrate.

Note: Exp. = Experiment. Aim 1 was addressed in Chapter 3, Aim 2 in Chapter 4 and Aim 3 in Chapter 5 of the thesis. ECAS = Edinburgh Cognitive and Behavioural ALS Screen, EF = Executive functions. Criteria for ALS-exi group: 2SD below control mean on ECAS verbal fluency OR executive domain. dlPFC = dorsolateral prefrontal cortex.

6.1 Cognitive profile in ALS

The cognitive profile of patients in ALS consistently reflected executive dysfunction across all experiments included in the thesis. Executive dysfunction was identified in the aim 1 experiment (Chapter 3), while language performance was significantly lower in experiments for aim 2 (Chapter 4), and not aim 1 (Chapter 3). This suggests subtle differences in cognitive profiles of patients included in the studies overall. This discrepancy may be attributed to the sample included in each study: only 41% of the sample from aim 1 experiments (Chapter 3) were included in aim 2 experiments (Chapter 4), while the remaining participants were recruited locally from Site 2 for aim 2 (Chapter 4). The percentage of patients with impaired executive functions (EF; ALS-exi group) was about 42% for aim 1 (Chapter 3) and 29% for aim 2 (Chapter 4), both of which are rates reported to be higher than previous studies using the Edinburgh Cognitive and Behavioural ALS Screen (ECAS) (Abrahams et al., 2014; Lulé et al., 2015; Ye et al., 2016). This could be attributed to the criteria applied for classifying ALS-exi patients (≤ 2SD below control mean for ECAS verbal fluency or executive scores). This criterion is in line with the recently revised guidelines proposed by Strong et al., (2017), which required impairment on either verbal fluency or two tasks of EF, while the previous studies employed old Strong criteria (Strong et al., 2009) which required impairments on any two tasks of EF (with or without verbal fluency). The former may be more sensitive to ALS-specific deficits as compared to the latter.

Theory of Mind (ToM) impairments in ALS were not prevalent on the ECAS in the experiments for both aims 2 and 3 (Chapters 4 and 5). However impaired ToM was indicated on more detailed testing with significantly poorer performance displayed on the Faux pas test for aim 2 (Chapter 4) and a trend towards poor performance on the Reading the Mind in

the Eyes (RME test) in aims 2 and 3 (Chapters 4 and 5). ALS patients were found to show significantly lower performance on an extended version of the Judgement of Preference test (Girardi et al., 2011; van der Hulst et al., 2014) while a previous study reported no significant differences on the screening version employed in the ECAS, which is used in the current thesis (Keller et al., 2017). This suggests that the Judgement of Preference ToM test included in the ECAS may not have sufficient sensitivity to identify subtle ToM changes. The lower sample size in experiments for aim 3 (Chapter 5) may have contributed to the lack of significant impairments on the Faux pas test. The subtle trend towards poor RME performance was indicated in one previous study (Gibbons et al., 2007) while more recent studies have suggested significant differences using larger samples (Burke, Elamin, et al., 2016; Burke, Pinto-Grau, et al., 2016).

ToM performance was associated with the degree of EF impairments, whereby patients with EF impairments (ALS-exi) performed worse than patients with normal EF (ALS-n), thus supporting some previous studies (Burke, Pinto-Grau, et al., 2016; Watermeyer et al., 2015b). However, associations with language and behaviour were also reported. To our best knowledge, this is the first report on associations between ToM, language and behaviour in ALS. Previous studies have implied the possibility of language dysfunction contributing to ToM impairments in ALS (Watermeyer et al., 2015b). Further studies are required to investigate these associations. It is likely that EF may remain a major factor in moderating ToM, while deficits in language and behavioural changes may further enhance ToM impairments in ALS.

6.2 Neuroimaging Findings

In aim 1 (Chapter 3), cognitive sub-groups for EF impaired (ALS-exi) and EF-normal (ALS-n) performances were identified using the ECAS. A greater extent of white matter degeneration in frontal regions was noted for the ALS-exi group while reduced grey matter density was identified in focal regions of the precentral gyrus, insula, and inferior frontal gyrus. This is in line with one previous study in ALS (Christidi, Karavasilis, Riederer, et al., 2018), while other studies have reported more extensive changes in grey matter for cognitively impaired ALS patients (Agosta et al., 2016). This could be attributed to differences in patients' cohorts included in the studies. For instance, Agosta et al., (2016) had included UMN, LMN and FTLD variants, in which the latter may contribute to greater severity in brain changes.

ALS-n patients identified in the study for aim 1 (Chapter 3) were also noted to show grey matter loss in prefrontal cortex, suggesting that prefrontal changes may be present even in the absence of cognitive changes. Previous studies in ALS have provided some evidence of frontal changes in such as in the dorsolateral prefrontal cortex (dlPFC) or the anterior cingulate cortex (ACC) in non-demented ALS patients (Agosta et al., 2016; Chang et al., 2005; Christidi, Karavasilis, Riederer, et al., 2018; Kasper et al., 2014; Menke et al., 2014). This may indicate that either ALS-n patients have structural changes that precede cognitive decline or may have subtle cognitive deficits that were not identified using a screening tool and may convert to cognitive impairments at follow-up (Christidi, Karavasilis, Velonakis, et al., 2018). Behavioural assessment was completed for a smaller sub-group of patients. It is likely that a small proportion of ALS-n patients may have behavioural impairments that was not identified due to the criteria applied. EF was associated with focal grey matter changes such as in the dlPFC and the precuneus, while associations with the superior longitudinal

fasciculus (SLF) was noted, indicating that white matter degeneration may contribute to poor EF.

In aim 3 (Chapter 5), the possibility of brain changes in regions shared by both ToM and EF were considered. With regard to ToM, very mild associations were noted for the RME test and grey matter and white matter while no specific associations were noted with degeneration (Chapter 5). Previous studies have indicated associations between ToM and grey matter densities in the anterior cingulate cortex (ACC), white matter changes in the longitudinal association tracts such as inferior longitudinal fasciculus (ILF) and inferior fronto-occipital fasciculus (IFOF) (Cerami et al., 2014; Crespi et al., 2014; Crespi et al., 2016) and metabolic decrease in the dlPFC (Carluer et al., 2014). It was noted that dlPFC was associated with both EF and ToM providing some evidence of neuroanatomical associations of both domains (Chapter 5). While these regions are associated with spread of pathology in ALS (Braak et al., 2013; Brettschneider et al., 2013), further studies with larger samples are warranted to increase power in analysis. One previous study found no ToM changes at baseline, despite recruiting their sample (n=23 ALS) after power calculations (Trojsi et al., 2017). The authors noted a trend towards impairment in longitudinal analysis. Further longitudinal studies in ALS are warranted to provide a better understanding of ToM decline in ALS.

6.3 Clinical Implications

The findings in the thesis indicate that the ECAS may be a viable screening tool for identifying cognitive impairments, specifically executive dysfunction in ALS. While language impairments were identified for one of the studies in the thesis (Chapter 4), further

assessment may be warranted to identify specific language dysfunction in ALS patients. More recent studies have emerged providing multiple versions of the ECAS (Crockford et al., 2018; Crockford et al., 2018), thus enabling research studies and clinics to utilise longitudinal follow-ups to identify cognitive impairments at later stages in the disease process. There were no specific associations between demographics, clinical variables and ECAS, thus indicating that cognitive impairment detected by the ECAS may reflect underlying neuropathology and may not be confined to specific factors such as symptom onset (limb vs bulbar).

The impact of ToM on clinical aspects warrants further study in ALS. One previous study noted associations between ToM impairments and inferior quality of life specifically mental health in ALS patients (Trojsi, Siciliano, et al., 2016). The association between ToM and behavioural changes noted in the current thesis (Chapter 4) may also provide insight into some aspects that would enable providing informational support for caregivers. Studies have indicated that behavioural changes increase caregiver burden in ALS (Lillo, Mioshi, & Hodges, 2012; Watermeyer et al., 2015a). Educating caregivers about potential ToM changes that may be associated with behavioural changes would enable care providers to work with caregivers to identify viable coping strategies enabling better and effective communication.

6.4 Strengths, Limitations and Future directions

To our best knowledge this is the first multicentre study in Canada exploring structural associations of the ECAS and investigating executive dysfunction and ToM in ALS. The samples for each study in the thesis had comparable age, education and gender distribution.

Symptom duration was longer in the multi-site ECAS cohort (Chapter 3), while it was marginally shorter (median = 20 months) for the ToM cohort (Chapter 4). Education was different between patients and controls in experiments for aim 1 (Chapter 3), while education was matched between the groups in experiments for aim 2(Chapter 4). Overall, the clinical variables across studies remained comparable.

One of the limitations of the study is the use of a screening battery to identify cognitive sub-groups in ALS. While it is noted that the ECAS is sensitive to cognitive changes in ALS (Abrahams et al., 2014; Niven et al., 2015; Pinto-Grau et al., 2017), a formal / more extensive neuropsychometric battery may identify subtle deficits that could be missed in a screening battery. The ECAS requires relatively good proficiency in the English language which limits its utility for patients who have English as a second language (ESL). While the studies in the thesis excluded ESL participants, further research should bridge this gap to enable inclusion of such patients as they may also show cognitive impairments that may remain undetected due to limitations in testing procedures. In-house modifications of the tools were performed such as deriving modified ECAS executive and total scores to address aims and hypotheses (Chapters 3 and 4). Additional neuropsychometric tests would enable validation of such modifications.

There is a lack of normative data for the North American version of the ECAS, thus cut-off scores derived from the control groups (≤ 2SD below control mean) were used to identify cognitive sub-groups as initially suggested by (Abrahams et al., 2014). Care was taken to ensure that the cut-off scores remained constant across studies. Recent studies have employed age and education based cut-off scores to identify cognitive sub-groups (Loose et al., 2016; Pinto-Grau et al., 2017; Siciliano et al., 2017) which may enable more accurate

classification of cognitive sub-groups. Future studies using the North American version of the ECAS could consider deriving age- and education-based cut-off scores using appropriate control groups. Additionally, given the nature of tests used, ceiling effects were noted suggesting that participants may inherently tend to perform well on the tests. Data transformations attempted for the thesis were not meaningful and hence could not be employed. Future studies could consider increasing sample size to enable using parametric tests and reduce impact of ceiling scores.

While using multicentre data is a strength for the current study, there are some inherent challenges with such a research design. To maintain homogeneity in data collection, training was provided for administration of psychometric tests and a harmonised magnetic resonance imaging (MRI) acquisition protocol was optimised across sites. There were some sites with lower sample sizes in the experiments for aims 1 and 2 (Chapters 3 and 4). Especially for aim 2 (Chapter 4), performance on the tests were weighted from a single centre (Site 2) due to higher sample size. While balancing participants across sites remains a challenge for multicentre studies, performance on tests were comparable across sites within patients and controls. Inclusion of statistical models correcting for site was performed for neuroimaging analysis. Incorporating similar techniques for neuropsychometric data may also enable effective analysis in future studies with larger sample sizes.

Recent evidence has suggested shared patterns of degeneration in ALS, PLS and PMA patients (Müller et al., 2018a; Müller et al., 2018b), whereby patients displayed lower FA in white matter tracts, particularly in the particularly in the CST of non-demented ALS, PLS and PMA patients, while prefrontal changes were noted only in non-demented ALS patients. In these latter studies, ECAS was administered on 50% of the patients and performance was

found to be comparable between the MND groups. Recent studies have explored cognitive staging in ALS using DTI metrics (Lulé et al., 2018), it would be of interest to extend cognitive stages to MND phenotypes.

6.5 Conclusions

The current thesis explored neural substrates of cognitive impairments using a screening test (ECAS) and investigated the association between executive function (EF) impairments and ToM. Cognitive impairment identified using the ECAS reflected executive dysfunction reported in ALS literature. Neuroimaging findings in ALS cognitive sub-groups indicated more widespread changes in patients with executive function (EF) impairments (ALS-exi), implying either a more aggressive form of the disease or a different pathological spread as compared to patients with normal EF (ALS-n). EF may moderate social cognition in ALS, as indicated by poor Theory of Mind (ToM) in ALS-exi patients. However, ToM changes appear to be subtle in the current group of ALS patients. Neuroimaging did not reveal any specific associations with ToM. This is in line with the mild ToM changes noted in this neuroimaging cohort. To our best knowledge this is the first report using the ECAS and exploring relationship between EF and ToM in a Canadian group of ALS patients employing data collected in a prospective multicentre fashion. Limitations of the work and suggestions for future studies were recommended.

Bibliography

Abdulla, S., Machts, J., Kaufmann, J., Patrick, K., Kollewe, K., Dengler, R., . . . Nestor, P. J. (2014). Hippocampal degeneration in patients with amyotrophic lateral sclerosis. *Neurobiol Aging, 35*(11), 2639-2645. doi:10.1016/j.neurobiolaging.2014.05.035

Abe, K., Fujimura, H., Toyooka, K., Sakoda, S., Yorifuji, S., & Yanagihara, T. (1997). Cognitive function in amyotrophic lateral sclerosis. *J Neurol Sci, 148*(1), 95-100.

Abe, K., Itoyama, Y., Sobue, G., Tsuji, S., Aoki, M., Doyu, M., . . . Edaravone, A. L. S. S. G. (2014). Confirmatory double-blind, parallel-group, placebo-controlled study of efficacy and safety of edaravone (MCI-186) in amyotrophic lateral sclerosis patients. *Amyotroph Lateral Scler Frontotemporal Degener, 15*(7-8), 610-617. doi:10.3109/21678421.2014.959024

Abrahams, S., Goldstein, L. H., Al-Chalabi, A., Pickering, A., Morris, R. G., Passingham, R. E., . . . Leigh, P. N. (1997). Relation between cognitive dysfunction and pseudobulbar palsy in amyotrophic lateral sclerosis. *Journal of Neurol Neurosurg Psychiatry, 62*(5), 464-472.

Abrahams, S., Goldstein, L. H., Kew, J. J., Brooks, D. J., Lloyd, C. M., Frith, C. D., & Leigh, P. N. (1996). Frontal lobe dysfunction in amyotrophic lateral sclerosis. A PET study. *Brain, 119 (Pt 6)*, 2105-2120.

Abrahams, S., Goldstein, L. H., Simmons, A., Brammer, M., Williams, S. C., Giampietro, V., & Leigh, P. N. (2004). Word retrieval in amyotrophic lateral sclerosis: a functional magnetic resonance imaging study. *Brain, 127*(Pt 7), 1507-1517.

Abrahams, S., Goldstein, L. H., Suckling, J., Ng, V., Simmons, A., Chitnis, X., . . . Leigh, P. N. (2005). Frontotemporal white matter changes in amyotrophic lateral sclerosis. *J Neurol, 252*(3), 321-331. doi:10.1007/s00415-005-0646-x

Abrahams, S., Leigh, P. N., & Goldstein, L. H. (2005). Cognitive change in ALS: a prospective study. *Neurology, 64*(7), 1222-1226.

Abrahams, S., Leigh, P. N., Harvey, A., Vythelingum, G. N., Grise, D., & Goldstein, L. H. (2000). Verbal fluency and executive dysfunction in amyotrophic lateral sclerosis (ALS). *Neuropsychologia, 38*(6), 734-747.

Abrahams, S., Newton, J., Niven, E., Foley, J., & Bak, T. H. (2014). Screening for cognition and behaviour changes in ALS. *Amyotroph Lateral Scler Frontotemporal Degener, 15*(1-2), 9-14. doi:10.3109/21678421.2013.805784

Abu-Akel, A., & Shamay-Tsoory, S. (2011). Neuroanatomical and neurochemical bases of theory of mind. *Neuropsychologia, 49*(11), 2971-2984. doi:10.1016/j.neuropsychologia.2011.07.012

Adolphs, R. (1999). Social cognition and the human brain. *Trends Cogn Sci, 3*(12), 469-479.

Adolphs, R., Baron-Cohen, S., & Tranel, D. (2002). Impaired recognition of social emotions following amygdala damage. *J Cogn Neurosci, 14*(8), 1264-1274. doi:10.1162/089892902760807258

Adolphs, R., Tranel, D., & Damasio, A. R. (1998). The human amygdala in social judgment. *Nature, 393*(6684), 470-474. doi:10.1038/30982

Agosta, F., Canu, E., Valsasina, P., Riva, N., Prelle, A., Comi, G., & Filippi, M. (2013). Divergent brain network connectivity in amyotrophic lateral sclerosis. *Neurobiol Aging, 34*(2), 419-427.

Agosta, F., Ferraro, P. M., Riva, N., Spinelli, E. G., Chio, A., Canu, E., . . . Filippi, M. (2016). Structural brain correlates of cognitive and behavioral impairment in MND. *Hum Brain Mapp, 37*(4), 1614-1626. doi:10.1002/hbm.23124

Agosta, F., Pagani, E., Petrolini, M., Caputo, D., Perini, M., Prelle, A., . . . Filippi, M. (2010). Assessment of white matter tract damage in patients with amyotrophic lateral sclerosis: a diffusion tensor MR imaging tractography study. *AJNR Am J Neuroradiol, 31*(8), 1457-1461. doi:10.3174/ajnr.A2105

Agosta, F., Rocca, M. A., Valsasina, P., Sala, S., Caputo, D., Perini, M., . . . Filippi, M. (2009). A longitudinal diffusion tensor MRI study of the cervical cord and brain in amyotrophic lateral sclerosis patients. *J Neurol Neurosurg Psychiatry, 80*(1), 53-55. doi:10.1136/jnnp.2008.154252

Agosta, F., Valsasina, P., Absinta, M., Riva, N., Sala, S., Prelle, A., . . . Filippi, M. (2011). Sensorimotor functional connectivity changes in amyotrophic lateral sclerosis. *Cerebral Cortex, 21*(10), 2291-2298.

Agosta, F., Valsasina, P., Riva, N., Copetti, M., Messina, M. J., Prelle, A., . . . Filippi, M. (2012). The cortical signature of amyotrophic lateral sclerosis. *PLoS One, 7*(8), e42816. doi:10.1371/journal.pone.0042816

Aho-Ozhan, H. E., Keller, J., Heimrath, J., Uttner, I., Kassubek, J., Birbaumer, N., . . . Lule, D. (2016). Perception of Emotional Facial Expressions in Amyotrophic Lateral Sclerosis (ALS) at Behavioural and Brain Metabolic Level. *PLoS One, 11*(10), e0164655. doi:10.1371/journal.pone.0164655

Alexander, A. L., Lee, J. E., Lazar, M., & Field, A. S. (2007). Diffusion tensor imaging of the brain. *Neurotherapeutics, 4*(3), 316-329. doi:10.1016/j.nurt.2007.05.011

Allen, E. A., Erhardt, E. B., Damaraju, E., Gruner, W., Segall, J. M., Silva, R. F., . . . Calhoun, V. D. (2011). A baseline for the multivariate comparison of resting-state networks. *Front Syst Neurosci, 5*, 2. doi:10.3389/fnsys.2011.00002

Amato, N., Riva, N., Cursi, M., MartinsSilva, A., Martinelli, V., Comola, M., . . . Leocani, L. (2013). Different frontal involvement in ALS and PLS revealed by Stroop event-related potentials and reaction times. *Frontiers in Aging Neuroscience, 5 Dec*, Art 82-10.

Andersen, P. M., Abrahams, S., Borasio, G. D., de Carvalho, M., Chio, A., Van Damme, P., . . . Weber, M. (2012). EFNS guidelines on the clinical management of amyotrophic lateral sclerosis (MALS)--revised report of an EFNS task force. *Eur J Neurol, 19*(3), 360-375. doi:10.1111/j.1468-1331.2011.03501.x

Anderson, S. W., Damasio, H., Jones, R. D., & Tranel, D. (1991). Wisconsin Card Sorting Test performance as a measure of frontal lobe damage. *J Clin Exp Neuropsychol, 13*(6), 909-922. doi:10.1080/01688639108405107

Andrews, S. C., Staios, M., Howe, J., Reardon, K., & Fisher, F. (2017). Multimodal emotion processing deficits are present in amyotrophic lateral sclerosis. *Neuropsychology, 31*(3), 304-310. doi:10.1037/neu0000323

Apolone, G., & Mosconi, P. (1998). The Italian SF-36 Health Survey: translation, validation and norming. *J Clin Epidemiol, 51*(11), 1025-1036.

Appel, V., Stewart, S. S., Smith, G., & Appel, S. H. (1987). A rating scale for amyotrophic lateral sclerosis: description and preliminary experience. *Ann Neurol, 22*(3), 328-333. doi:10.1002/ana.410220308

Arai, T., Hasegawa, M., Akiyama, H., Ikeda, K., Nonaka, T., Mori, H., . . . Oda, T. (2006). TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Biochem Biophys Res Commun, 351*(3), 602-611. doi:10.1016/j.bbrc.2006.10.093

Armon, C., & Moses, D. (1998). Linear estimates of rates of disease progression as predictors of survival in patients with ALS entering clinical trials. *J Neurol Sci, 160 Suppl 1*, S37-41.

Ashburner, J., & Friston, K. J. (2000). Voxel-based morphometry--the methods. *Neuroimage, 11*(6 Pt 1), 805-821. doi:10.1006/nimg.2000.0582

Bach, M., Laun, F. B., Leemans, A., Tax, C. M., Biessels, G. J., Stieltjes, B., & Maier-Hein, K. H. (2014). Methodological considerations on tract-based spatial statistics (TBSS). *Neuroimage, 100*, 358-369. doi:10.1016/j.neuroimage.2014.06.021

Baddeley, A. (2000). The episodic buffer: a new component of working memory? *Trends Cogn Sci, 4*(11), 417-423.

Baddeley, A., & Della Sala, S. (1996). Working memory and executive control. *Philos Trans R Soc Lond B Biol Sci, 351*(1346), 1397-1403; discussion 1403-1394. doi:10.1098/rstb.1996.0123

Bak, T. H. (2010). Motor neuron disease and frontotemporal dementia: One, two, or three diseases? *Ann Indian Acad Neurol, 13*(Suppl 2), S81-88. doi:10.4103/0972-2327.74250

Bak, T. H., & Chandran, S. (2012). What wires together dies together: verbs, actions and neurodegeneration in motor neuron disease. *Cortex, 48*(7), 936-944. doi:10.1016/j.cortex.2011.07.008

Bak, T. H., O'Donovan, D. G., Xuereb, J. H., Boniface, S., & Hodges, J. R. (2001). Selective impairment of verb processing associated with pathological changes in Brodmann areas 44 and 45 in the motor neurone disease-dementia-aphasia syndrome. *Brain, 124*(Pt 1), 103-120.

Ball, L. J., Beukelman, D. R., & Pattee, G. L. (2004). Communication effectiveness of individuals with amyotrophic lateral sclerosis. *J Commun Disord, 37*(3), 197-215. doi:10.1016/j.jcomdis.2003.09.002

Baloh, R. H., Rakowicz, W., Gardner, R., & Pestronk, A. (2007). Frequent atrophic groups with mixed-type myofibers is distinctive to motor neuron syndromes. *Muscle Nerve, 36*(1), 107-110. doi:10.1002/mus.20755

Baron-Cohen, S., Campbell, R., Karmiloff-Smith, A., Grant, J., & Walker, J. (1995). Are children with autism blind to the mentalistic significance of the eyes? *British Journal of Developmental Psychology, 13*(4), 379-398. doi:10.1111/j.2044-835X.1995.tb00687.x

Baron-Cohen, S., Wheelwright, S., Hill, J., Raste, Y., & Plumb, I. (2001). The "Reading the Mind in the Eyes" Test revised version: a study with normal adults, and adults with Asperger syndrome or high-functioning autism. *J Child Psychol Psychiatry, 42*(2), 241-251.

Basser, P. J., Mattiello, J., & LeBihan, D. (1994). MR diffusion tensor spectroscopy and imaging. *Biophys J, 66*(1), 259-267. doi:10.1016/S0006-3495(94)80775-1

Bathgate, D., Snowden, J. S., Varma, A., Blackshaw, A., & Neary, D. (2001). Behaviour in frontotemporal dementia, Alzheimer's disease and vascular dementia. *Acta Neurol Scand, 103*(6), 367-378.

Beaulieu, C. (2002). The basis of anisotropic water diffusion in the nervous system - a technical review. *NMR Biomed, 15*(7-8), 435-455. doi:10.1002/nbm.782

Bechara, A., Damasio, A. R., Damasio, H., & Anderson, S. W. (1994). Insensitivity to future consequences following damage to human prefrontal cortex. *Cognition, 50*(1-3), 7-15.

Beck, A. T., Steer, R. A., & Brown, G. K. (1996). *Manual for the Beck Depression Inventory-II*. San Antonio, TX: Psychological Corporation.

Beckmann, C. F., DeLuca, M., Devlin, J. T., & Smith, S. M. (2005). Investigations into resting-state connectivity using independent component analysis. *Philos Trans R Soc Lond B Biol Sci, 360*(1457), 1001-1013. doi:10.1098/rstb.2005.1634

Bede, P., Bokde, A., Elamin, M., Byrne, S., McLaughlin, R. L., Jordan, N., . . . Hardiman, O. (2013). Grey matter correlates of clinical variables in amyotrophic lateral sclerosis (ALS): a neuroimaging study of ALS motor phenotype heterogeneity and cortical focality. *J Neurol Neurosurg Psychiatry, 84*(7), 766-773. doi:10.1136/jnnp-2012-302674

Bede, P., Bokde, A. L., Byrne, S., Elamin, M., McLaughlin, R. L., Kenna, K., . . . Hardiman, O. (2013). Multiparametric MRI study of ALS stratified for the C9orf72 genotype. *Neurology, 81*(4), 361-369.

Beeldman, E., Raaphorst, J., Klein Twennaar, M., de Visser, M., Schmand, B. A., & de Haan, R. J. (2016). The cognitive profile of ALS: a systematic review and meta-analysis update. *J Neurol Neurosurg Psychiatry, 87*(6), 611-619. doi:10.1136/jnnp-2015-310734

Bensimon, G., Lacomblez, L., & Meininger, V. (1994). A controlled trial of riluzole in amyotrophic lateral sclerosis. ALS/Riluzole Study Group. *N Engl J Med, 330*(9), 585-591. doi:10.1056/NEJM199403033300901

Bergmann, M., Kuchelmeister, K., Schmid, K. W., Kretzschmar, H. A., & Schroder, R. (1996). Different variants of frontotemporal dementia: a neuropathological and immunohistochemical study. *Acta Neuropathol, 92*(2), 170-179.

Birn, R. M., Kenworthy, L., Case, L., Caravella, R., Jones, T. B., Bandettini, P. A., & Martin, A. (2010). Neural systems supporting lexical search guided by letter and semantic category cues: a self-paced overt response fMRI study of verbal fluency. *Neuroimage, 49*(1), 1099-1107. doi:10.1016/j.neuroimage.2009.07.036

Biswal, B., Yetkin, F. Z., Haughton, V. M., & Hyde, J. S. (1995). Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. *Magn Reson Med, 34*(4), 537-541.

Block, W., Karitzky, J., Traber, F., Pohl, C., Keller, E., Mundegar, R. R., . . . Jerusalem, F. (1998). Proton magnetic resonance spectroscopy of the primary motor cortex in patients with motor neuron disease: subgroup analysis and follow-up measurements. *Arch Neurol, 55*(7), 931-936.

Boillee, S., & Cleveland, D. W. (2008). Revisiting oxidative damage in ALS: microglia, Nox, and mutant SOD1. *J Clin Invest, 118*(2), 474-478. doi:10.1172/JCI34613

Boillee, S., Vande Velde, C., & Cleveland, D. W. (2006). ALS: a disease of motor neurons and their nonneuronal neighbors. *Neuron, 52*(1), 39-59. doi:10.1016/j.neuron.2006.09.018

Bongioanni, P., Buoiano, G., & Magoni, M. (2002). Language impairments in ALS/MND (Amyotrophic Lateral Sclerosis/Motor Neuron Disease).

Bora, E. (2017). Meta-analysis of social cognition in amyotrophic lateral sclerosis. *Cortex, 88*, 1-7. doi:10.1016/j.cortex.2016.11.012

Borenstein, A. R., Mortimer, J. A., Schofield, E., Wu, Y., Salmon, D. P., Gamst, A., . . . Galasko, D. R. (2007). Cycad exposure and risk of dementia, MCI, and PDC in the Chamorro population of Guam. *Neurology, 68*(21), 1764-1771. doi:10.1212/01.wnl.0000262027.31623.b2

Braak, H., Brettschneider, J., Ludolph, A. C., Lee, V. M., Trojanowski, J. Q., & Del Tredici, K. (2013). Amyotrophic lateral sclerosis--a model of corticofugal axonal spread. *Nat Rev Neurol, 9*(12), 708-714. doi:10.1038/nrneurol.2013.221

Braak, H., Ludolph, A., Thal, D. R., & Del Tredici, K. (2010). Amyotrophic lateral sclerosis: dash-like accumulation of phosphorylated TDP-43 in somatodendritic and axonal compartments of somatomotor neurons of the lower brainstem and spinal cord. *Acta Neuropathol, 120*(1), 67-74. doi:10.1007/s00401-010-0683-0

Bradley, W. G., Borenstein, A. R., Nelson, L. M., Codd, G. A., Rosen, B. H., Stommel, E. W., & Cox, P. A. (2013). Is exposure to cyanobacteria an environmental risk factor for amyotrophic lateral sclerosis and other neurodegenerative diseases? *Amyotroph Lateral Scler Frontotemporal Degener, 14*(5-6), 325-333. doi:10.3109/21678421.2012.750364

Brettschneider, J., Del Tredici, K., Toledo, J. B., Robinson, J. L., Irwin, D. J., Grossman, M., . . . Trojanowski, J. Q. (2013). Stages of pTDP-43 pathology in amyotrophic lateral sclerosis. *Ann Neurol, 74*(1), 20-38. doi:10.1002/ana.23937

Brettschneider, J., Libon, D. J., Toledo, J. B., Xie, S. X., McCluskey, L., Elman, L., . . . Trojanowski, J. Q. (2012). Microglial activation and TDP-43 pathology correlate with executive dysfunction in amyotrophic lateral sclerosis. *Acta Neuropathologica, 123*(3), 395-407.

Brettschneider, J., Widl, K., Ehrenreich, H., Riepe, M., & Tumani, H. (2006). Erythropoietin in the cerebrospinal fluid in neurodegenerative diseases. *Neurosci Lett, 404*(3), 347-351. doi:10.1016/j.neulet.2006.06.011

Brooks, B. R., Miller, R. G., Swash, M., & Munsat, T. L. (2000). El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Other Motor Neuron Disord, 1*(5), 293-299.

Bruijn, L. I., Becher, M. W., Lee, M. K., Anderson, K. L., Jenkins, N. A., Copeland, N. G., . . . Cleveland, D. W. (1997). ALS-linked SOD1 mutant G85R mediates damage to astrocytes and promotes rapidly progressive disease with SOD1-containing inclusions. *Neuron, 18*(2), 327-338.

Bruijn, L. I., Houseweart, M. K., Kato, S., Anderson, K. L., Anderson, S. D., Ohama, E., . . . Cleveland, D. W. (1998). Aggregation and motor neuron toxicity of an ALS-linked SOD1 mutant independent from wild-type SOD1. *Science, 281*(5384), 1851-1854.

Bruijn, L. I., Miller, T. M., & Cleveland, D. W. (2004). Unraveling the mechanisms involved in motor neuron degeneration in ALS. *Annu Rev Neurosci, 27*, 723-749. doi:10.1146/annurev.neuro.27.070203.144244

Burke, T., Elamin, M., Bede, P., Pinto-Grau, M., Lonergan, K., Hardiman, O., & Pender, N. (2016). Discordant performance on the 'Reading the Mind in the Eyes' Test, based on disease onset in amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Frontotemporal Degener, 17*(7-8), 467-472. doi:10.1080/21678421.2016.1177088

Burke, T., Lonergan, K., Pinto-Grau, M., Elamin, M., Bede, P., Madden, C., . . . Pender, N. (2017). Visual encoding, consolidation, and retrieval in amyotrophic lateral sclerosis: executive function as a mediator, and predictor of performance. *Amyotroph Lateral Scler Frontotemporal Degener, 18*(3-4), 193-201. doi:10.1080/21678421.2016.1272615

Burke, T., Pinto-Grau, M., Lonergan, K., Elamin, M., Bede, P., Costello, E., . . . Pender, N. (2016). Measurement of Social Cognition in Amyotrophic Lateral Sclerosis: A Population Based Study. *PLoS One, 11*(8), e0160850. doi:10.1371/journal.pone.0160850

Burkhardt, C., Neuwirth, C., & Weber, M. (2017). Longitudinal assessment of the Edinburgh Cognitive and Behavioural Amyotrophic Lateral Sclerosis Screen (ECAS): lack of practice effect in ALS patients? *Amyotroph Lateral Scler Frontotemporal Degener, 18*(3-4), 202-209. doi:10.1080/21678421.2017.1283418

Buxton, R. B., Wong, E. C., & Frank, L. R. (1998). Dynamics of blood flow and oxygenation changes during brain activation: the balloon model. *Magn Reson Med, 39*(6), 855-864.

Byrne, S., Elamin, M., Bede, P., Shatunov, A., Walsh, C., Corr, B., . . . Hardiman, O. (2012). Cognitive and clinical characteristics of patients with amyotrophic lateral sclerosis carrying a C9orf72 repeat expansion: a population-based cohort study. *Lancet Neurol, 11*(3), 232-240. doi:10.1016/S1474-4422(12)70014-5

Byrne, S., Walsh, C., Lynch, C., Bede, P., Elamin, M., Kenna, K., . . . Hardiman, O. (2011). Rate of familial amyotrophic lateral sclerosis: a systematic review and meta-analysis. *J Neurol Neurosurg Psychiatry, 82*(6), 623-627. doi:10.1136/jnnp.2010.224501

Caller, T. A., Doolin, J. W., Haney, J. F., Murby, A. J., West, K. G., Farrar, H. E., . . . Stommel, E. W. (2009). A cluster of amyotrophic lateral sclerosis in New Hampshire: a possible role for toxic cyanobacteria blooms. *Amyotroph Lateral Scler, 10 Suppl 2*, 101-108. doi:10.3109/17482960903278485

Carluer, L., Mondou, A., Buhour, M. S., Laisney, M., Pelerin, A., Eustache, F., . . . Desgranges, B. (2014). Neural substrate of cognitive theory of mind impairment in amyotrophic lateral sclerosis. *Cortex, 65C*, 19-30. doi:10.1016/j.cortex.2014.12.010

Cavallo, M., Adenzato, M., Macpherson, S. E., Karwig, G., Enrici, I., & Abrahams, S. (2011). Evidence of social understanding impairment in patients with amyotrophic lateral sclerosis. *PLoS One, 6*(10), e25948. doi:10.1371/journal.pone.0025948

Cedarbaum, J. M., Stambler, N., Malta, E., Fuller, C., Hilt, D., Thurmond, B., & Nakanishi, A. (1999). The ALSFRS-R: a revised ALS functional rating scale that

incorporates assessments of respiratory function. BDNF ALS Study Group (Phase III). *J Neurol Sci, 169*(1-2), 13-21.

Cerami, C., Dodich, A., Canessa, N., Crespi, C., Iannaccone, S., Corbo, M., . . . Cappa, S. F. (2014). Emotional empathy in amyotrophic lateral sclerosis: a behavioural and voxel-based morphometry study. *Amyotroph Lateral Scler Frontotemporal Degener, 15*(1-2), 21-29. doi:10.3109/21678421.2013.785568

Chang, J. L., Lomen-Hoerth, C., Murphy, J., Henry, R. G., Kramer, J. H., Miller, B. L., & Gorno-Tempini, M. L. (2005). A voxel-based morphometry study of patterns of brain atrophy in ALS and ALS/FTLD. *Neurology, 65*(1), 75-80. doi:10.1212/01.wnl.0000167602.38643.29

Chari, G., Shaw, P. J., & Sahgal, A. (1996). Nonverbal visual attention, but not recognition memory of learning, processes are impaired in motor neurone disease. *Neuropsychologia, 34*(5), 377-385.

Chaves, M., Bettini, M., Fernandez, M. C., Basalo, M. J. G., Rojas, J. I., Besada, C., . . . Rugiero, M. (2017). Usefulness of diffusion tensor imaging in amyotrophic lateral sclerosis: potential biomarker and association with the cognitive profile. *Arq Neuropsiquiatr, 75*(5), 272-276. doi:10.1590/0004-282X20170032

Cheah, B. C., Vucic, S., Krishnan, A. V., & Kiernan, M. C. (2010). Riluzole, neuroprotection and amyotrophic lateral sclerosis. *Curr Med Chem, 17*(18), 1942-1199.

Chen, X., & Shang, H. F. (2015). New developments and future opportunities in biomarkers for amyotrophic lateral sclerosis. *Transl Neurodegener, 4*, 17. doi:10.1186/s40035-015-0040-2

Chenji, S., Jha, S., Lee, D., Brown, M., Seres, P., Mah, D., & Kalra, S. (2016). Investigating Default Mode and Sensorimotor Network Connectivity in Amyotrophic Lateral Sclerosis. *PLoS One, 11*(6), e0157443. doi:10.1371/journal.pone.0157443

Chenji, S., Mah, D., Johnson, W., Camicioli, R., Fisher, N., & Kalra, S. (2018). Utility of the Addenbrooke's Cognitive Examination in Amyotrophic Lateral Sclerosis. *Can J Neurol Sci*.

Chio, A., Benzi, G., Dossena, M., Mutani, R., & Mora, G. (2005). Severely increased risk of amyotrophic lateral sclerosis among Italian professional football players. *Brain, 128*(Pt 3), 472-476. doi:10.1093/brain/awh373

Chio, A., Calvo, A., Bovio, G., Canosa, A., Bertuzzo, D., Galmozzi, F., . . . Valle d'Aosta Register for Amyotrophic Lateral, S. (2014). Amyotrophic lateral sclerosis outcome measures and the role of albumin and creatinine: a population-based study. *JAMA Neurol, 71*(9), 1134-1142. doi:10.1001/jamaneurol.2014.1129

Chio, A., Calvo, A., Moglia, C., Mazzini, L., Mora, G., & group, P. s. (2011). Phenotypic heterogeneity of amyotrophic lateral sclerosis: a population based study. *J Neurol Neurosurg Psychiatry, 82*(7), 740-746. doi:10.1136/jnnp.2010.235952

Chio, A., Logroscino, G., Hardiman, O., Swingler, R., Mitchell, D., Beghi, E., . . . Eurals, C. (2009). Prognostic factors in ALS: A critical review. *Amyotroph Lateral Scler, 10*(5-6), 310-323. doi:10.3109/17482960802566824

Chio, A., Logroscino, G., Traynor, B. J., Collins, J., Simeone, J. C., Goldstein, L. A., & White, L. A. (2013). Global epidemiology of amyotrophic lateral sclerosis: a systematic review of the published literature. *Neuroepidemiology, 41*(2), 118-130. doi:10.1159/000351153

Christidi, F., Karavasilis, E., Riederer, F., Zalonis, I., Ferentinos, P., Velonakis, G., . . . Evdokimidis, I. (2018). Gray matter and white matter changes in non-demented amyotrophic lateral sclerosis patients with or without cognitive impairment: A combined voxel-based morphometry and tract-based spatial statistics whole-brain analysis. *Brain Imaging Behav, 12*(2), 547-563. doi:10.1007/s11682-017-9722-y

Christidi, F., Karavasilis, E., Velonakis, G., Rentzos, M., Zambelis, T., Zouvelou, V., . . . Karandreas, N. (2018). Motor and extra-motor gray matter integrity may underlie neurophysiologic parameters of motor function in amyotrophic lateral sclerosis: a combined voxel-based morphometry and transcranial stimulation study. *Brain Imaging Behav*. doi:10.1007/s11682-018-9841-0

Christidi, F., Zalonis, I., Kyriazi, S., Rentzos, M., Karavasilis, E., Wilde, E. A., & Evdokimidis, I. (2014). Uncinate fasciculus microstructure and verbal episodic memory in amyotrophic lateral sclerosis: a diffusion tensor imaging and neuropsychological study. *Brain Imaging Behav, 8*(4), 497-505. doi:10.1007/s11682-013-9271-y

Christidi, F., Zalonis, I., Smyrnis, N., & Evdokimidis, I. (2012). Selective attention and the three-process memory model for the interpretation of verbal free recall in amyotrophic lateral sclerosis. *J Int Neuropsychol Soc, 18*(5), 809-818. doi:10.1017/S1355617712000562

Chung, M. J., & Suh, Y. L. (2002). Ultrastructural changes of mitochondria in the skeletal muscle of patients with amyotrophic lateral sclerosis. *Ultrastruct Pathol, 26*(1), 3-7. doi:10.1080/01913120252934260

Cleary, S., Misiaszek, J. E., Kalra, S., Wheeler, S., & Johnston, W. (2013). The effects of lung volume recruitment on coughing and pulmonary function in patients with ALS. *Amyotroph Lateral Scler Frontotemporal Degener, 14*(2), 111-115. doi:10.3109/17482968.2012.720262

Comalli, P. E., Jr., Wapner, S., & Werner, H. (1962). Interference effects of Stroop color-word test in childhood, adulthood, and aging. *J Genet Psychol, 100*, 47-53.

Consonni, M., Iannaccone, S., Cerami, C., Frasson, P., Lacerenza, M., Lunetta, C., . . . Cappa, S. F. (2013). The cognitive and behavioural profile of amyotrophic lateral sclerosis: Application of the consensus criteria. *Behavioural Neurology, 27*(2), 143-153.

Consonni, M., Rossi, S., Cerami, C., Marcone, A., Iannaccone, S., Francesco Cappa, S., & Perani, D. (2017). Executive dysfunction affects word list recall performance: Evidence from amyotrophic lateral sclerosis and other neurodegenerative diseases. *J Neuropsychol, 11*(1), 74-90.

Costa, J., Swash, M., & de Carvalho, M. (2012). Awaji criteria for the diagnosis of amyotrophic lateral sclerosis:a systematic review. *Arch Neurol, 69*(11), 1410-1416. doi:10.1001/archneurol.2012.254

Crespi, C., Cerami, C., Dodich, A., Canessa, N., Arpone, M., Iannaccone, S., . . . Cappa, S. F. (2014). Microstructural white matter correlates of emotion recognition impairment in Amyotrophic Lateral Sclerosis. *Cortex, 53*, 1-8. doi:10.1016/j.cortex.2014.01.002

Crespi, C., Cerami, C., Dodich, A., Canessa, N., Iannaccone, S., Corbo, M., . . . Cappa, S. F. (2016). Microstructural Correlates of Emotional Attribution Impairment in Non-Demented Patients with Amyotrophic Lateral Sclerosis. *PLoS One, 11*(8), e0161034. doi:10.1371/journal.pone.0161034

Crockford, C., Newton, J., Lonergan, K., Madden, C., Mays, I., O'Sullivan, M., . . . Abrahams, S. (2018). Measuring reliable change in cognition using the Edinburgh Cognitive and Behavioural ALS Screen (ECAS). *Amyotroph Lateral Scler Frontotemporal Degener, 19*(1-2), 65-73. doi:10.1080/21678421.2017.1407794

Crockford, C. J., Kleynhans, M., Wilton, E., Radakovic, R., Newton, J., Niven, E. H., . . . Abrahams, S. (2018). ECAS A-B-C: alternate forms of the Edinburgh Cognitive and Behavioural ALS Screen. *Amyotroph Lateral Scler Frontotemporal Degener, 19*(1-2), 57-64. doi:10.1080/21678421.2017.1407793

Cui, F., Zhu, W., Zhou, Z., Ren, Y., Li, Y., Li, M., . . . Huang, X. (2015). Frequency and risk factor analysis of cognitive and anxiety-depressive disorders in patients with

amyotrophic lateral sclerosis/motor neuron disease. *Neuropsychiatr Dis Treat, 11*, 2847-2854. doi:10.2147/NDT.S90520

Daianu, M., Mezher, A., Mendez, M. F., Jahanshad, N., Jimenez, E. E., & Thompson, P. M. (2016). Disrupted rich club network in behavioral variant frontotemporal dementia and early-onset Alzheimer's disease. *Hum Brain Mapp, 37*(3), 868-883. doi:10.1002/hbm.23069

Dajani, D. R., & Uddin, L. Q. (2015). Demystifying cognitive flexibility: Implications for clinical and developmental neuroscience. *Trends Neurosci, 38*(9), 571-578. doi:10.1016/j.tins.2015.07.003

Dalakas, M. C., Hatazawa, J., Brooks, R. A., & Di Chiro, G. (1987). Lowered cerebral glucose utilization in amyotrophic lateral sclerosis. *Ann Neurol, 22*(5), 580-586. doi:10.1002/ana.410220504

Davidson, Y., Kelley, T., Mackenzie, I. R., Pickering-Brown, S., Du Plessis, D., Neary, D., . . . Mann, D. M. (2007). Ubiquitinated pathological lesions in frontotemporal lobar degeneration contain the TAR DNA-binding protein, TDP-43. *Acta Neuropathol, 113*(5), 521-533. doi:10.1007/s00401-006-0189-y

de Carvalho, M., Dengler, R., Eisen, A., England, J. D., Kaji, R., Kimura, J., . . . Swash, M. (2008). Electrodiagnostic criteria for diagnosis of ALS. *Clin Neurophysiol, 119*(3), 497-503. doi:10.1016/j.clinph.2007.09.143

Deiber, M. P., Passingham, R. E., Colebatch, J. G., Friston, K. J., Nixon, P. D., & Frackowiak, R. S. (1991). Cortical areas and the selection of movement: a study with positron emission tomography. *Exp Brain Res, 84*(2), 393-402.

del Aguila, M. A., Longstreth, W. T., Jr., McGuire, V., Koepsell, T. D., & van Belle, G. (2003). Prognosis in amyotrophic lateral sclerosis: a population-based study. *Neurology, 60*(5), 813-819.

Dengler, R. (2012). Electromyography and muscle ultrasound in ALS diagnosis, complementary or competitive? *Clin Neurophysiol, 123*(8), 1485-1486. doi:10.1016/j.clinph.2011.12.007

Desai, J., & Swash, M. (1999). Extrapyramidal involvement in amyotrophic lateral sclerosis: backward falls and retropulsion. *J Neurol Neurosurg Psychiatry, 67*(2), 214-216.

Desport, J. C., Preux, P. M., Truong, T. C., Vallat, J. M., Sautereau, D., & Couratier, P. (1999). Nutritional status is a prognostic factor for survival in ALS patients. *Neurology, 53*(5), 1059-1063.

Dimond, D., Ishaque, A., Chenji, S., Mah, D., Chen, Z., Seres, P., . . . Kalra, S. (2017). White matter structural network abnormalities underlie executive dysfunction in amyotrophic lateral sclerosis. *Hum Brain Mapp, 38*(3), 1249-1268. doi:10.1002/hbm.23452

Dombroski, B. A., Galasko, D. R., Mata, I. F., Zabetian, C. P., Craig, U. K., Garruto, R. M., . . . Schellenberg, G. D. (2013). C9orf72 hexanucleotide repeat expansion and

Guam amyotrophic lateral sclerosis-Parkinsonism-dementia complex. *JAMA Neurol, 70*(6), 742-745. doi:10.1001/jamaneurol.2013.1817

Donaghy, C., Pinnock, R., Abrahams, S., Cardwell, C., Hardiman, O., Patterson, V., . . . Gibson, J. M. (2010). Slow saccades in bulbar-onset motor neurone disease. *J Neurol, 257*(7), 1134-1140. doi:10.1007/s00415-010-5478-7

Donaghy, C., Thurtell, M. J., Pioro, E. P., Gibson, J. M., & Leigh, R. J. (2011). Eye movements in amyotrophic lateral sclerosis and its mimics: a review with illustrative cases. *J Neurol Neurosurg Psychiatry, 82*(1), 110-116. doi:10.1136/jnnp.2010.212407

Douaud, G., Filippini, N., Knight, S., Talbot, K., & Turner, M. R. (2011). Integration of structural and functional magnetic resonance imaging in amyotrophic lateral sclerosis. *Brain, 134*(Pt 12), 3470-3479.

Droppelmann, C. A., Keller, B. A., Campos-Melo, D., Volkening, K., & Strong, M. J. (2013). Rho guanine nucleotide exchange factor is an NFL mRNA destabilizing factor that forms cytoplasmic inclusions in amyotrophic lateral sclerosis. *Neurobiol Aging, 34*(1), 248-262. doi:10.1016/j.neurobiolaging.2012.06.021

Dubois, B., Slachevsky, A., Litvan, I., & Pillon, B. (2000). The FAB: a Frontal Assessment Battery at bedside. *Neurology, 55*(11), 1621-1626.

Eisen, A., Schulzer, M., MacNeil, M., Pant, B., & Mak, E. (1993). Duration of amyotrophic lateral sclerosis is age dependent. *Muscle Nerve, 16*(1), 27-32. doi:10.1002/mus.880160107

Ekman, P., & Friesen, W. (1976). *Pictures of facial affect*: Consulting Psychologists Press.

Elamin, M., Bede, P., Byrne, S., Jordan, N., Gallagher, L., Wynne, B., . . . Hardiman, O. (2013). Cognitive changes predict functional decline in ALS: A population-based longitudinal study. *Neurology, 80*(17), 1590-1597.

Elamin, M., Phukan, J., Bede, P., Jordan, N., Byrne, S., Pender, N., & Hardiman, O. (2011). Executive dysfunction is a negative prognostic indicator in patients with ALS without dementia. *Neurology, 76*(14), 1263-1269.

Elamin, M., Pinto-Grau, M., Burke, T., Bede, P., Rooney, J., O'Sullivan, M., . . . Hardiman, O. (2017). Identifying behavioural changes in ALS: Validation of the Beaumont Behavioural Inventory (BBI). *Amyotroph Lateral Scler Frontotemporal Degener, 18*(1-2), 68-73. doi:10.1080/21678421.2016.1248976

Ellison, D., Love, S., Chimelli, L. M. C., Harding, B., Lowe, J. S., Vinters, H. V., . . . Yong, W. H. (2012). *Neuropathology E-Book: A Reference Text of CNS Pathology* (Third ed.). Italy: Elsevier.

Evans, J., Olm, C., McCluskey, L., Elman, L., Boller, A., Moran, E., . . . Grossman, M. (2015). Impaired cognitive flexibility in amyotrophic lateral sclerosis. *Cogn Behav Neurol, 28*(1), 17-26. doi:10.1097/WNN.0000000000000049

Fatima, M., Tan, R., Halliday, G. M., & Kril, J. J. (2015). Spread of pathology in amyotrophic lateral sclerosis: assessment of phosphorylated TDP-43 along axonal pathways. *Acta Neuropathol Commun, 3*, 47. doi:10.1186/s40478-015-0226-y

Feldman, M. J., & Drasgow, J. (1951). A visual-verbal test for schizophrenia. *Psychiatric Quarterly Supplement*.

Felice, K. J. (1997). A longitudinal study comparing thenar motor unit number estimates to other quantitative tests in patients with amyotrophic lateral sclerosis. *Muscle Nerve, 20*(2), 179-185.

Fellows, L. K. (2006). Deciding how to decide: ventromedial frontal lobe damage affects information acquisition in multi-attribute decision making. *Brain, 129*(Pt 4), 944-952. doi:10.1093/brain/awl017

Ferraiuolo, L., Kirby, J., Grierson, A. J., Sendtner, M., & Shaw, P. J. (2011). Molecular pathways of motor neuron injury in amyotrophic lateral sclerosis. *Nat Rev Neurol, 7*(11), 616-630. doi:10.1038/nrneurol.2011.152

Fischl, B., & Dale, A. M. (2000). Measuring the thickness of the human cerebral cortex from magnetic resonance images. *Proc Natl Acad Sci U S A, 97*(20), 11050-11055. doi:10.1073/pnas.200033797

Fisk, J. E., & Warr, P. (1996). Age and working memory: the role of perceptual speed, the central executive, and the phonological loop. *Psychol Aging, 11*(2), 316-323.

Ford, K. A., Goltz, H. C., Brown, M. R., & Everling, S. (2005). Neural processes associated with antisaccade task performance investigated with event-related FMRI. *J Neurophysiol, 94*(1), 429-440. doi:10.1152/jn.00471.2004

Forsberg, K., Andersen, P. M., Marklund, S. L., & Brannstrom, T. (2011). Glial nuclear aggregates of superoxide dismutase-1 are regularly present in patients with amyotrophic lateral sclerosis. *Acta Neuropathol, 121*(5), 623-634. doi:10.1007/s00401-011-0805-3

Friedman, N. P., & Miyake, A. (2017). Unity and diversity of executive functions: Individual differences as a window on cognitive structure. *Cortex, 86*, 186-204. doi:10.1016/j.cortex.2016.04.023

Frith, C. D., & Frith, U. (2012). Mechanisms of social cognition. *Annu Rev Psychol, 63*, 287-313. doi:10.1146/annurev-psych-120710-100449

Gallo, V., Bueno-De-Mesquita, H. B., Vermeulen, R., Andersen, P. M., Kyrozis, A., Linseisen, J., . . . Riboli, E. (2009). Smoking and risk for amyotrophic lateral sclerosis: analysis of the EPIC cohort. *Ann Neurol, 65*(4), 378-385. doi:10.1002/ana.21653

Garruto, R. M., & Yanagihara, R. (2009). Contributions of isolated Pacific populations to understanding neurodegenerative diseases. *Folia Neuropathol, 47*(2), 149-170.

Gauthier, C. J., & Fan, A. P. (2018). BOLD signal physiology: Models and applications. *Neuroimage*. doi:10.1016/j.neuroimage.2018.03.018

Geevasinga, N., Loy, C. T., Menon, P., de Carvalho, M., Swash, M., Schrooten, M., . . . Vucic, S. (2016). Awaji criteria improves the diagnostic sensitivity in amyotrophic lateral sclerosis: A systematic review using individual patient data. *Clin Neurophysiol, 127*(7), 2684-2691. doi:10.1016/j.clinph.2016.04.005

Geser, F., Lee, V. M., & Trojanowski, J. Q. (2010). Amyotrophic lateral sclerosis and frontotemporal lobar degeneration: a spectrum of TDP-43 proteinopathies. *Neuropathology, 30*(2), 103-112. doi:10.1111/j.1440-1789.2009.01091.x

Geser, F., Prvulovic, D., O'Dwyer, L., Hardiman, O., Bede, P., Bokde, A. L., . . . Hampel, H. (2011). On the development of markers for pathological TDP-43 in amyotrophic lateral sclerosis with and without dementia. *Prog Neurobiol, 95*(4), 649-662. doi:10.1016/j.pneurobio.2011.08.011

Gibbons, Z. C., Richardson, A., Neary, D., & Snowden, J. S. (2008). Behaviour in amyotrophic lateral sclerosis. *Amyotroph Lateral Scler, 9*(2), 67-74. doi:10.1080/17482960701642437

Gibbons, Z. C., Snowden, J. S., Thompson, J. C., Happe, F., Richardson, A., & Neary, D. (2007). Inferring thought and action in motor neurone disease. *Neuropsychologia, 45*(6), 1196-1207. doi:10.1016/j.neuropsychologia.2006.10.008

Girardi, A., Macpherson, S. E., & Abrahams, S. (2011). Deficits in emotional and social cognition in amyotrophic lateral sclerosis. *Neuropsychology, 25*(1), 53-65. doi:10.1037/a0020357

Goldstein, L. H., & Abrahams, S. (2013). Changes in cognition and behaviour in amyotrophic lateral sclerosis: nature of impairment and implications for assessment. *Lancet Neurol, 12*(4), 368-380. doi:10.1016/S1474-4422(13)70026-7

Goodin, D. S., Rowley, H. A., & Olney, R. K. (1988). Magnetic resonance imaging in amyotrophic lateral sclerosis. *Ann Neurol, 23*(4), 418-420. doi:10.1002/ana.410230424

Gordon, P. H. (2011). Amyotrophic lateral sclerosis: pathophysiology, diagnosis and management. *CNS Drugs, 25*(1), 1-15. doi:10.2165/11586000-000000000-00000

Gorno-Tempini, M. L., Hillis, A. E., Weintraub, S., Kertesz, A., Mendez, M., Cappa, S. F., . . . Grossman, M. (2011). Classification of primary progressive aphasia and its variants. *Neurology, 76*(11), 1006-1014. doi:10.1212/WNL.0b013e31821103e6

Govaarts, R., Beeldman, E., Kampelmacher, M. J., van Tol, M. J., van den Berg, L. H., van der Kooi, A. J., . . . Raaphorst, J. (2016). The frontotemporal syndrome of ALS is associated with poor survival. *J Neurol, 263*(12), 2476-2483. doi:10.1007/s00415-016-8290-1

Grace, J., & Malloy, P. (2000). *Frontal systems behavior scale: professional manual*: Psychological Assessment Resources, Incorporated.

Gregory, C., Lough, S., Stone, V., Erzinclioglu, S., Martin, L., Baron-Cohen, S., & Hodges, J. R. (2002). Theory of mind in patients with frontal variant frontotemporal dementia and Alzheimer's disease: theoretical and practical implications. *Brain, 125*(Pt 4), 752-764.

Grober, E., & Sliwinski, M. (1991). Development and validation of a model for estimating premorbid verbal intelligence in the elderly. *J Clin Exp Neuropsychol, 13*(6), 933-949. doi:10.1080/01688639108405109

Grossman, A. B., Woolley-Levine, S., Bradley, W. G., & Miller, R. G. (2007). Detecting neurobehavioral changes in amyotrophic lateral sclerosis. *Amyotrophic Lateral Sclerosise, 8*(1), 56-61.

Grossman, M., Elman, L., McCluskey, L., McMillan, C. T., Boller, A., Powers, J., . . . Trojanowski, J. Q. (2014). Phosphorylated tau as a candidate biomarker for amyotrophic lateral sclerosis. *JAMA Neurol, 71*(4), 442-448. doi:10.1001/jamaneurol.2013.6064

Hammer, R. P., Jr., Tomiyasu, U., & Scheibel, A. B. (1979). Degeneration of the human Betz cell due to amyotrophic lateral sclerosis. *Exp Neurol, 63*(2), 336-346.

Hanagasi, H. A., Gurvit, I. H., Ermutlu, N., Kaptanoglu, G., Karamursel, S., Idrisoglu, H. A., . . . Demiralp, T. (2002). Cognitive impairment in amyotrophic lateral sclerosis: Evidence from neuropsychological investigation and event-related potentials. *Cognitive Brain Research, 14*(2), 234-244.

Happe, F. G. (1994). An advanced test of theory of mind: understanding of story characters' thoughts and feelings by able autistic, mentally handicapped, and normal children and adults. *J Autism Dev Disord, 24*(2), 129-154.

Hardiman, O., & van den Berg, L. H. (2017). Edaravone: a new treatment for ALS on the horizon? *Lancet Neurol, 16*(7), 490-491. doi:10.1016/S1474-4422(17)30163-1

Hardiman, O., van den Berg, L. H., & Kiernan, M. C. (2011). Clinical diagnosis and management of amyotrophic lateral sclerosis. *Nat Rev Neurol, 7*(11), 639-649. doi:10.1038/nrneurol.2011.153

Hartikainen, P., Helkala, E. L., Soininen, H., & Riekkinen, P., Sr. (1993). Cognitive and memory deficits in untreated Parkinson's disease and amyotrophic lateral sclerosis patients: a comparative study. *J Neural Transm Park Dis Dement Sect, 6*(2), 127-137.

Hasegawa, M., Arai, T., Nonaka, T., Kametani, F., Yoshida, M., Hashizume, Y., . . . Akiyama, H. (2008). Phosphorylated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Ann Neurol, 64*(1), 60-70. doi:10.1002/ana.21425

Hatazawa, J., Brooks, R. A., Dalakas, M. C., Mansi, L., & Di Chiro, G. (1988). Cortical motor-sensory hypometabolism in amyotrophic lateral sclerosis: a PET study. *J Comput Assist Tomogr, 12*(4), 630-636.

Haverkamp, L. J., Appel, V., & Appel, S. H. (1995). Natural history of amyotrophic lateral sclerosis in a database population. Validation of a scoring system and a model for survival prediction. *Brain, 118 (Pt 3)*, 707-719.

Heeger, D. J., & Ress, D. (2002). What does fMRI tell us about neuronal activity? *Nat Rev Neurosci, 3*(2), 142-151. doi:10.1038/nrn730

Henry, J. D., von Hippel, W., Molenberghs, P., Lee, T., & Sachdev, P. S. (2016). Clinical assessment of social cognitive function in neurological disorders. *Nat Rev Neurol, 12*(1), 28-39. doi:10.1038/nrneurol.2015.229

Hensley, K., Mhatre, M., Mou, S., Pye, Q. N., Stewart, C., West, M., & Williamson, K. S. (2006). On the relation of oxidative stress to neuroinflammation: lessons learned

from the G93A-SOD1 mouse model of amyotrophic lateral sclerosis. *Antioxid Redox Signal, 8*(11-12), 2075-2087. doi:10.1089/ars.2006.8.2075

Henstridge, C. M., Sideris, D. I., Carroll, E., Rotariu, S., Salomonsson, S., Tzioras, M., . . . Spires-Jones, T. L. (2018). Synapse loss in the prefrontal cortex is associated with cognitive decline in amyotrophic lateral sclerosis. *Acta Neuropathol, 135*(2), 213-226. doi:10.1007/s00401-017-1797-4

Higashi, S., Iseki, E., Yamamoto, R., Minegishi, M., Hino, H., Fujisawa, K., . . . Arai, H. (2007). Appearance pattern of TDP-43 in Japanese frontotemporal lobar degeneration with ubiquitin-positive inclusions. *Neurosci Lett, 419*(3), 213-218. doi:10.1016/j.neulet.2007.04.051

Horner, R. D., Kamins, K. G., Feussner, J. R., Grambow, S. C., Hoff-Lindquist, J., Harati, Y., . . . Kasarskis, E. J. (2003). Occurrence of amyotrophic lateral sclerosis among Gulf War veterans. *Neurology, 61*(6), 742-749.

Hosler, B. A., Siddique, T., Sapp, P. C., Sailor, W., Huang, M. C., Hossain, A., . . . Brown, R. H., Jr. (2000). Linkage of familial amyotrophic lateral sclerosis with frontotemporal dementia to chromosome 9q21-q22. *JAMA, 284*(13), 1664-1669.

Hu, W. T., Shelnutt, M., Wilson, A., Yarab, N., Kelly, C., Grossman, M., . . . Glass, J. (2013). Behavior matters-Cognitive predictors of survival in amyotrophic lateral sclerosis. *PLoS One, 8*(2), Art e57584-57588.

Hudson, A. J., Davenport, A., & Hader, W. J. (1986). The incidence of amyotrophic lateral sclerosis in southwestern Ontario, Canada. *Neurology, 36*(11), 1524-1528.

Ikeda, K., Akiyama, H., Arai, T., Ueno, H., Tsuchiya, K., & Kosaka, K. (2002). Morphometrical reappraisal of motor neuron system of Pick's disease and amyotrophic lateral sclerosis with dementia. *Acta Neuropathol, 104*(1), 21-28. doi:10.1007/s00401-001-0513-5

Ilzecka, J., Stelmasiak, Z., & Dobosz, B. (2001). [Matrix metalloproteinase-9 (MMP-9) activity in cerebrospinal fluid of amyotrophic lateral sclerosis patients]. *Neurol Neurochir Pol, 35*(6), 1035-1043.

Irwin, D. J., McMillan, C. T., Brettschneider, J., Libon, D. J., Powers, J., Rascovsky, K., . . . Grossman, M. (2013). Cognitive decline and reduced survival in C9orf72 expansion frontotemporal degeneration and amyotrophic lateral sclerosis. *Journal of Neurology & Psychiatry, 84*(2), 163-169.

Jackson, M., Lennox, G., & Lowe, J. (1996). Motor neurone disease-inclusion dementia. *Neurodegeneration, 5*(4), 339-350.

Jelsone-Swain, L., Persad, C., Burkard, D., & Welsh, R. C. (2015). Action processing and mirror neuron function in patients with amyotrophic lateral sclerosis: an fMRI study. *PLoS One, 10*(4), e0119862. doi:10.1371/journal.pone.0119862

Jelsone-Swain, L., Persad, C., Votruba, K. L., Weisenbach, S. L., Johnson, T., Gruis, K. L., & Welsh, R. C. (2012). The relationship between depressive symptoms, disease state, and cognition in amyotrophic lateral sclerosis. *Frontiers in Psychology, 3 Dec*, Art 542-510.

Jokic, N., Gonzalez de Aguilar, J. L., Pradat, P. F., Dupuis, L., Echaniz-Laguna, A., Muller, A., . . . Meininger, V. (2005). Nogo expression in muscle correlates with amyotrophic lateral sclerosis severity. *Ann Neurol, 57*(4), 553-556. doi:10.1002/ana.20420

Kabashi, E., Valdmanis, P. N., Dion, P., Spiegelman, D., McConkey, B. J., Vande Velde, C., . . . Rouleau, G. A. (2008). TARDBP mutations in individuals with sporadic and familial amyotrophic lateral sclerosis. *Nat Genet, 40*(5), 572-574. doi:10.1038/ng.132

Kalra, S., & Arnold, D. (2003). Neuroimaging in amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Other Motor Neuron Disord, 4*(4), 243-248. doi:10.1080/14660820310011269

Kalra, S., Cashman, N. R., Genge, A., & Arnold, D. L. (1998). Recovery of N-acetylaspartate in corticomotor neurons of patients with ALS after riluzole therapy. *Neuroreport, 9*(8), 1757-1761.

Kalra, S., Hanstock, C. C., Martin, W. R., Allen, P. S., & Johnston, W. S. (2006). Detection of cerebral degeneration in amyotrophic lateral sclerosis using high-field magnetic resonance spectroscopy. *Arch Neurol, 63*(8), 1144-1148. doi:10.1001/archneur.63.8.1144

Kamel, F., Umbach, D. M., Bedlack, R. S., Richards, M., Watson, M., Alavanja, M. C., . . . Sandler, D. P. (2012). Pesticide exposure and amyotrophic lateral sclerosis. *Neurotoxicology, 33*(3), 457-462. doi:10.1016/j.neuro.2012.04.001

Kasarskis, E. J., Scarlata, D., Hill, R., Fuller, C., Stambler, N., & Cedarbaum, J. M. (1999). A retrospective study of percutaneous endoscopic gastrostomy in ALS patients during the BDNF and CNTF trials. *J Neurol Sci, 169*(1-2), 118-125.

Kasper, E., Schuster, C., Machts, J., Kaufmann, J., Bittner, D., Vielhaber, S., . . . Prudlo, J. (2014). Microstructural white matter changes underlying cognitive and behavioural impairment in ALS--an in vivo study using DTI. *PLoS One, 9*(12), e114543. doi:10.1371/journal.pone.0114543

Kassubek, J., Muller, H. P., Del Tredici, K., Lule, D., Gorges, M., Braak, H., & Ludolph, A. C. (2018). Imaging the pathoanatomy of amyotrophic lateral sclerosis in vivo: targeting a propagation-based biological marker. *J Neurol Neurosurg Psychiatry, 89*(4), 374-381. doi:10.1136/jnnp-2017-316365

Kaufmann, P., Pullman, S. L., Shungu, D. C., Chan, S., Hays, A. P., Del Bene, M. L., . . . Mitsumoto, H. (2004). Objective tests for upper motor neuron involvement in amyotrophic lateral sclerosis (ALS). *Neurology, 62*(10), 1753-1757.

Kawashima, T., Kikuchi, H., Takita, M., Doh-ura, K., Ogomori, K., Oda, M., & Iwaki, T. (1998). Skein-like inclusions in the neostriatum from a case of amyotrophic lateral sclerosis with dementia. *Acta Neuropathol, 96*(5), 541-545.

Keller, J., Bohm, S., Aho-Ozhan, H. E. A., Loose, M., Gorges, M., Kassubek, J., . . . Lule, D. (2017). Functional reorganization during cognitive function tasks in patients with amyotrophic lateral sclerosis. *Brain Imaging Behav*. doi:10.1007/s11682-017-9738-3

Kew, J. J., Goldstein, L. H., Leigh, P. N., Abrahams, S., Cosgrave, N., Passingham, R. E., . . . Brooks, D. J. (1993). The relationship between abnormalities of cognitive function and cerebral activation in amyotrophic lateral sclerosis. A neuropsychological and positron emission tomography study. *Brain, 116 (Pt 6)*, 1399-1423.

Kew, J. J., Leigh, P. N., Playford, E. D., Passingham, R. E., Goldstein, L. H., Frackowiak, R. S., & Brooks, D. J. (1993). Cortical function in amyotrophic lateral sclerosis. A positron emission tomography study. *Brain, 116 (Pt 3)*, 655-680.

Kiernan, M. C., Vucic, S., Cheah, B. C., Turner, M. R., Eisen, A., Hardiman, O., . . . Zoing, M. C. (2011). Amyotrophic lateral sclerosis. *Lancet, 377*(9769), 942-955. doi:10.1016/S0140-6736(10)61156-7

Kihira, T., Yoshida, S., Kondo, T., Iwai, K., Wada, S., Morinaga, S., . . . Kuzuhara, S. (2012). An increase in ALS incidence on the Kii Peninsula, 1960-2009: a possible link to change in drinking water source. *Amyotroph Lateral Scler, 13*(4), 347-350. doi:10.3109/17482968.2012.674140

Kilani, M., Micallef, J., Soubrouillard, C., Rey-Lardiller, D., Demattei, C., Dib, M., . . . Blin, O. (2004). A longitudinal study of the evolution of cognitive function and affective state in patients with amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Other Motor Neuron Disord, 5*(1), 46-54. doi:10.1080/14660820310017560

Kimura, F., Fujimura, C., Ishida, S., Nakajima, H., Furutama, D., Uehara, H., . . . Hanafusa, T. (2006). Progression rate of ALSFRS-R at time of diagnosis predicts

survival time in ALS. *Neurology, 66*(2), 265-267. doi:10.1212/01.wnl.0000194316.91908.8a

Kraat, A. W. (1990). Augmentative and alternative communication: Does it have a future in aphasia rehabilitation? *Aphasiology, 4*(4), 321-338.

Kurland, L. T., & Mulder, D. W. (1954). Epidemiologic investigations of amyotrophic lateral sclerosis. I. Preliminary report on geographic distribution, with special reference to the Mariana Islands, including clinical and pathologic observations. *Neurology, 4*(5), 355-378.

Kurland, L. T., & Mulder, D. W. (1955). Epidemiologic investigations of amyotrophic lateral sclerosis. 2. Familial aggregations indicative of dominant inheritance. I. *Neurology, 5*(3), 182-196.

Kuther, G., Rodiek, S. O., & Struppler, A. (1987). CT-scanning of skeletal muscles in amyotrophic lateral sclerosis. *Adv Exp Med Biol, 209*, 143-148.

Lang, P. J., Bradley, M. M., & Cuthbert, B. N. (2008). *International affective picture system (IAPS): Affective ratings of pictures and instruction manual.* Gainesville, FL: University of Florida.

Lasiene, J., & Yamanaka, K. (2011). Glial cells in amyotrophic lateral sclerosis. *Neurol Res Int, 2011*, 718987. doi:10.1155/2011/718987

Lee, A., Eurich, D., Mah, D., Hanstock, C., & Kalra, S. (2016). *Advanced MRI in a Multicentre Study: Assessing the Reliability of Candidate Biomarkers in ALS*. ALS Canada Forum Toronto.

Leemans, A., Jeurissen, B., Sijbers, J., & Jones, D. K. (2009). *ExploreDTI: a graphical toolbox for processing, analyzing, and visualizing diffusion MR data.* Paper presented at the 17th Annual Meeting of Intl Soc Mag Reson Med, Hawaii, USA.

Leigh, P. N., Anderton, B. H., Dodson, A., Gallo, J. M., Swash, M., & Power, D. M. (1988). Ubiquitin deposits in anterior horn cells in motor neurone disease. *Neurosci Lett, 93*(2-3), 197-203.

Lepow, L., Van Sweringen, J., Strutt, A. M., Jawaid, A., MacAdam, C., Harati, Y., . . . York, M. K. (2010). Frontal and temporal lobe involvement on verbal fluency measures in amyotrophic lateral sclerosis. *J Clin Exp Neuropsychol, 32*(9), 913-922. doi:10.1080/13803391003596439

Leveille, A., Kiernan, J., Goodwin, J. A., & Antel, J. (1982). Eye movements in amyotrophic lateral sclerosis. *Arch Neurol, 39*(11), 684-686.

Lezak, M. D., Howieson, D. B., Loring, D. W., & Fischer, J. S. (2004). *Neuropsychological assessment*: Oxford University Press, USA.

Lillo, P., Mioshi, E., Burrell, J. R., Kiernan, M. C., Hodges, J. R., & Hornberger, M. (2012). Grey and white matter changes across the amyotrophic lateral sclerosis-frontotemporal dementia continuum. *PLoS One, 7*(8), e43993. doi:10.1371/journal.pone.0043993

Lillo, P., Mioshi, E., & Hodges, J. R. (2012). Caregiver burden in amyotrophic lateral sclerosis is more dependent on patients' behavioral changes than physical disability: a comparative study. *BMC Neurol, 12*, 156. doi:10.1186/1471-2377-12-156

Lillo, P., Mioshi, E., Zoing, M. C., Kiernan, M. C., & Hodges, J. R. (2011). How common are behavioural changes in amyotrophic lateral sclerosis? *Amyotroph Lateral Scler, 12*(1), 45-51. doi:10.3109/17482968.2010.520718

Lillo, P., Savage, S., Mioshi, E., Kiernan, M. C., & Hodges, J. R. (2012). Amyotrophic lateral sclerosis and frontotemporal dementia: A behavioural and cognitive continuum. *Amyotroph Lateral Scler, 13*(1), 102-109. doi:10.3109/17482968.2011.639376

Lin, C. L., Bristol, L. A., Jin, L., Dykes-Hoberg, M., Crawford, T., Clawson, L., & Rothstein, J. D. (1998). Aberrant RNA processing in a neurodegenerative disease: the cause for absent EAAT2, a glutamate transporter, in amyotrophic lateral sclerosis. *Neuron, 20*(3), 589-602.

Liu, R., Althaus, J. S., Ellerbrock, B. R., Becker, D. A., & Gurney, M. E. (1998). Enhanced oxygen radical production in a transgenic mouse model of familial amyotrophic lateral sclerosis. *Ann Neurol, 44*(5), 763-770. doi:10.1002/ana.410440510

Logroscino, G., Traynor, B. J., Hardiman, O., Chio, A., Couratier, P., Mitchell, J. D., . . . Eurals. (2008). Descriptive epidemiology of amyotrophic lateral sclerosis: new evidence and unsolved issues. *J Neurol Neurosurg Psychiatry, 79*(1), 6-11. doi:10.1136/jnnp.2006.104828

Lomen-Hoerth, C., Murphy, J., Langmore, S., Kramer, J. H., Olney, R. K., & Miller, B. (2003). Are amyotrophic lateral sclerosis patients cognitively normal? *Neurology, 60*(7), 1094-1097.

Loose, M., Burkhardt, C., Aho-Ozhan, H., Keller, J., Abdulla, S., Bohm, S., . . . Lule, D. (2016). Age and education-matched cut-off scores for the revised German/Swiss-German version of ECAS. *Amyotroph Lateral Scler Frontotemporal Degener*, 1-3. doi:10.3109/21678421.2016.1162814

Lowe, J., Lennox, G., Jefferson, D., Morrell, K., McQuire, D., Gray, T., . . . Mayer, R. J. (1988). A filamentous inclusion body within anterior horn neurones in motor neurone disease defined by immunocytochemical localisation of ubiquitin. *Neurosci Lett, 94*(1-2), 203-210.

Ludolph, A. C., Langen, K. J., Regard, M., Herzog, H., Kemper, B., Kuwert, T., . . . Feinendegen, L. (1992). Frontal lobe function in amyotrophic lateral sclerosis: a neuropsychologic and positron emission tomography study. *Acta Neurol Scand, 85*(2), 81-89.

Lulé, D., Bohm, S., Muller, H. P., Aho-Ozhan, H., Keller, J., Gorges, M., . . . Ludolph, A. C. (2018). Cognitive phenotypes of sequential staging in amyotrophic lateral sclerosis. *Cortex, 101*, 163-171. doi:10.1016/j.cortex.2018.01.004

Lulé, D., Burkhardt, C., Abdulla, S., Bohm, S., Kollewe, K., Uttner, I., . . . Ludolph, A. C. (2015). The Edinburgh Cognitive and Behavioural Amyotrophic Lateral Sclerosis Screen: a cross-sectional comparison of established screening tools in a German-

Swiss population. *Amyotroph Lateral Scler Frontotemporal Degener, 16*(1-2), 16-23. doi:10.3109/21678421.2014.959451

Lulé, D., Diekmann, V., Anders, S., Kassubek, J., Kubler, A., Ludolph, A. C., & Birbaumer, N. (2007). Brain responses to emotional stimuli in patients with amyotrophic lateral sclerosis (ALS). *J Neurol, 254*(4), 519-527. doi:10.1007/s00415-006-0409-3

Lulé, D., Kurt, A., Jurgens, R., Kassubek, J., Diekmann, V., Kraft, E., . . . Anders, S. (2005). Emotional responding in amyotrophic lateral sclerosis. *J Neurol, 252*(12), 1517-1524. doi:10.1007/s00415-005-0907-8

Machts, J., Bittner, V., Kasper, E., Schuster, C., Prudlo, J., Abdulla, S., . . . Bittner, D. M. (2014). Memory deficits in amyotrophic lateral sclerosis are not exclusively caused by executive dysfunction: a comparative neuropsychological study of amnestic mild cognitive impairment. *BMC Neuroscience, 15*, 83.

Mackenzie, I. R., Ansorge, O., Strong, M., Bilbao, J., Zinman, L., Ang, L. C., . . . Neumann, M. (2011). Pathological heterogeneity in amyotrophic lateral sclerosis with FUS mutations: two distinct patterns correlating with disease severity and mutation. *Acta Neuropathol, 122*(1), 87-98. doi:10.1007/s00401-011-0838-7

Mackenzie, I. R., Rademakers, R., & Neumann, M. (2010). TDP-43 and FUS in amyotrophic lateral sclerosis and frontotemporal dementia. *Lancet Neurol, 9*(10), 995-1007. doi:10.1016/S1474-4422(10)70195-2

Maher, P., & Davis, J. B. (1996). The role of monoamine metabolism in oxidative glutamate toxicity. *J Neurosci, 16*(20), 6394-6401.

Mahurin, R. K., Velligan, D. I., & Miller, A. L. (1998). Executive-frontal lobe cognitive dysfunction in schizophrenia: a symptom subtype analysis. *Psychiatry Res, 79*(2), 139-149.

Maldjian, J. A., Laurienti, P. J., Kraft, R. A., & Burdette, J. H. (2003). An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. *Neuroimage, 19*(3), 1233-1239.

Mandrioli, J., Faglioni, P., Nichelli, P., & Sola, P. (2006). Amyotrophic lateral sclerosis: prognostic indicators of survival. *Amyotroph Lateral Scler, 7*(4), 211-220. doi:10.1080/17482960600947648

Marin, B., Boumediene, F., Logroscino, G., Couratier, P., Babron, M. C., Leutenegger, A. L., . . . Beghi, E. (2017). Variation in worldwide incidence of amyotrophic lateral sclerosis: a meta-analysis. *Int J Epidemiol, 46*(1), 57-74. doi:10.1093/ije/dyw061

Marinkovic, P., Reuter, M. S., Brill, M. S., Godinho, L., Kerschensteiner, M., & Misgeld, T. (2012). Axonal transport deficits and degeneration can evolve independently in mouse models of amyotrophic lateral sclerosis. *Proc Natl Acad Sci U S A, 109*(11), 4296-4301. doi:10.1073/pnas.1200658109

Mars, R. B., Neubert, F. X., Noonan, M. P., Sallet, J., Toni, I., & Rushworth, M. F. (2012). On the relationship between the "default mode network" and the "social brain". *Front Hum Neurosci, 6*, 189. doi:10.3389/fnhum.2012.00189

Martin, I., & McDonald, S. (2003). Weak coherence, no theory of mind, or executive dysfunction? Solving the puzzle of pragmatic language disorders. *Brain Lang, 85*(3), 451-466.

Massman, P. J., Sims, J., Cooke, N., Haverkamp, L. J., Appel, V., & Appel, S. H. (1996). Prevalence and correlates of neuropsychological deficits in amyotrophic lateral sclerosis. *J Neurol Neurosurg Psychiatry, 61*(5), 450-455.

Mathuranath, P. S., Nestor, P. J., Berrios, G. E., Rakowicz, W., & Hodges, J. R. (2000). A brief cognitive test battery to differentiate Alzheimer's disease and frontotemporal dementia. *Neurology, 55*(11), 1613-1620.

McColgan, P., Seunarine, K. K., Razi, A., Cole, J. H., Gregory, S., Durr, A., . . . Track, H. D. I. (2015). Selective vulnerability of Rich Club brain regions is an organizational principle of structural connectivity loss in Huntington's disease. *Brain, 138*(Pt 11), 3327-3344. doi:10.1093/brain/awv259

McDonald, S. (1999). Exploring the process of inference generation in sarcasm: a review of normal and clinical studies. *Brain Lang, 68*(3), 486-506. doi:10.1006/brln.1999.2124

McDonald, S., Bornhofen, C., Shum, D., Long, E., Saunders, C., & Neulinger, K. (2006). Reliability and validity of The Awareness of Social Inference Test (TASIT): a clinical test of social perception. *Disabil Rehabil, 28*(24), 1529-1542. doi:10.1080/09638280600646185

McGeer, P. L., & McGeer, E. G. (2002). Inflammatory processes in amyotrophic lateral sclerosis. *Muscle Nerve, 26*(4), 459-470. doi:10.1002/mus.10191

Meier, S. L., Charleston, A. J., & Tippett, L. J. (2010). Cognitive and behavioural deficits associated with the orbitomedial prefrontal cortex in amyotrophic lateral sclerosis. *Brain: A Journal of Neurology, 133*(11), 3444-3457.

Menke, R. A., Korner, S., Filippini, N., Douaud, G., Knight, S., Talbot, K., & Turner, M. R. (2014). Widespread grey matter pathology dominates the longitudinal cerebral MRI and clinical landscape of amyotrophic lateral sclerosis. *Brain, 137*(Pt 9), 2546-2555.

Menke, R. A., Proudfoot, M., Wuu, J., Andersen, P. M., Talbot, K., Benatar, M., & Turner, M. R. (2016). Increased functional connectivity common to symptomatic amyotrophic lateral sclerosis and those at genetic risk. *J Neurol Neurosurg Psychiatry, 87*(6), 580-588. doi:10.1136/jnnp-2015-311945

Mezzapesa, D. M., D'Errico, E., Tortelli, R., Distaso, E., Cortese, R., Tursi, M., . . . Simone, I. L. (2013). Cortical thinning and clinical heterogeneity in amyotrophic lateral sclerosis. *PLoS One, 8*(11), e80748. doi:10.1371/journal.pone.0080748

Millecamps, S., Boillee, S., Le Ber, I., Seilhean, D., Teyssou, E., Giraudeau, M., . . . Salachas, F. (2012). Phenotype difference between ALS patients with expanded repeats in C9ORF72 and patients with mutations in other ALS-related genes. *J Med Genet, 49*(4), 258-263. doi:10.1136/jmedgenet-2011-100699

Miller, R. G., Jackson, C. E., Kasarskis, E. J., England, J. D., Forshew, D., Johnston, W., . . . Quality Standards Subcommittee of the American Academy of, N. (2009). Practice parameter update: the care of the patient with amyotrophic lateral sclerosis: multidisciplinary care, symptom management, and cognitive/behavioral impairment (an evidence-based review): report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology, 73*(15), 1227-1233. doi:10.1212/WNL.0b013e3181bc01a4

Miller, R. G., Mitchell, J. D., & Moore, D. H. (2012). Riluzole for amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND). *Cochrane Database Syst Rev*(3), CD001447. doi:10.1002/14651858.CD001447.pub3

Mioshi, E., Caga, J., Lillo, P., Hsieh, S., Ramsey, E., Devenney, E., . . . Kiernan, M. C. (2014). Neuropsychiatric changes precede classic motor symptoms in ALS and do not affect survival. *Neurology, 82*(2), 149-155. doi:10.1212/WNL.0000000000000023

Mioshi, E., Dawson, K., Mitchell, J., Arnold, R., & Hodges, J. R. (2006). The Addenbrooke's Cognitive Examination Revised (ACE-R): a brief cognitive test battery for dementia screening. *Int J Geriatr Psychiatry, 21*(11), 1078-1085. doi:10.1002/gps.1610

Mioshi, E., Hsieh, S., Caga, J., Ramsey, E., Chen, K., Lillo, P., . . . Kiernan, M. C. (2014). A novel tool to detect behavioural symptoms in ALS. *Amyotroph Lateral Scler Frontotemporal Degener, 15*(3-4), 298-304. doi:10.3109/21678421.2014.896927

Mioshi, E., Lillo, P., Yew, B., Hsieh, S., Savage, S., Hodges, J. R., . . . Hornberger, M. (2013). Cortical atrophy in ALS is critically associated with neuropsychiatric and cognitive changes. *Neurology, 80*(12), 1117-1123.

Mitrushina, M., Boone, K. B., Razani, J., & D'Elia, L. F. (2005). *Handbook of normative data for neuropsychological assessment* (2nd ed.). New York, NY, US: Oxford University Press.

Mitsumoto, H., Factor-Litvak, P., Andrews, H., Goetz, R. R., Andrews, L., Rabkin, J. G., . . . Group, A. C. S. (2014). ALS Multicenter Cohort Study of Oxidative Stress (ALS COSMOS): study methodology, recruitment, and baseline demographic and disease characteristics. *Amyotroph Lateral Scler Frontotemporal Degener, 15*(3-4), 192-203. doi:10.3109/21678421.2013.864312

Mitsumoto, H., Ulug, A. M., Pullman, S. L., Gooch, C. L., Chan, S., Tang, M. X., . . . Shungu, D. C. (2007). Quantitative objective markers for upper and lower motor neuron dysfunction in ALS. *Neurology, 68*(17), 1402-1410. doi:10.1212/01.wnl.0000260065.57832.87

Miyake, A., Friedman, N. P., Emerson, M. J., Witzki, A. H., Howerter, A., & Wager, T. D. (2000). The unity and diversity of executive functions and their contributions to complex "Frontal Lobe" tasks: a latent variable analysis. *Cogn Psychol, 41*(1), 49-100. doi:10.1006/cogp.1999.0734

Mohammadi, B., Kollewe, K., Samii, A., Krampfl, K., Dengler, R., & Munte, T. F. (2009). Changes of resting state brain networks in amyotrophic lateral sclerosis. *Exp Neurol, 217*(1), 147-153.

Morris, R. G., Downes, J. J., Sahakian, B. J., Evenden, J. L., Heald, A., & Robbins, T. W. (1988). Planning and spatial working memory in Parkinson's disease. *J Neurol Neurosurg Psychiatry, 51*(6), 757-766.

Müller, H. P., Agosta, F., Riva, N., Spinelli, E. G., Comi, G., Ludolph, A. C., . . . Kassubek, J. (2018a). Fast progressive lower motor neuron disease is an ALS variant: A two-centre tract of interest-based MRI data analysis. *Neuroimage Clin, 17*, 145-152. doi:10.1016/j.nicl.2017.10.008

Müller, H. P., Gorges, M., Kassubek, R., Dorst, J., Ludolph, A. C., & Kassubek, J. (2018b). Identical patterns of cortico-efferent tract involvement in primary lateral sclerosis and amyotrophic lateral sclerosis: A tract of interest-based MRI study. *NeuroImage: Clinical, 18*, 762-769.

Müller, H. P., Turner, M. R., Grosskreutz, J., Abrahams, S., Bede, P., Govind, V., . . . Neuroimaging Society in, A. L. S. D. T. I. S. G. (2016). A large-scale multicentre cerebral diffusion tensor imaging study in amyotrophic lateral sclerosis. *J Neurol Neurosurg Psychiatry, 87*(6), 570-579. doi:10.1136/jnnp-2015-311952

Murdock Jr, B. B. (1964). Proactive inhibition in short-term memory. *J Exp Psychol, 68*(2), 184.

Nasreddine, Z. S., Phillips, N. A., Bedirian, V., Charbonneau, S., Whitehead, V., Collin, I., . . . Chertkow, H. (2005). The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. *J Am Geriatr Soc, 53*(4), 695-699. doi:10.1111/j.1532-5415.2005.53221.x

Neary, D., Snowden, J. S., Gustafson, L., Passant, U., Stuss, D., Black, S., . . . Benson, D. F. (1998). Frontotemporal lobar degeneration: a consensus on clinical diagnostic criteria. *Neurology, 51*(6), 1546-1554.

Neumann, M., Sampathu, D. M., Kwong, L. K., Truax, A. C., Micsenyi, M. C., Chou, T. T., . . . Lee, V. M. (2006). Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science, 314*(5796), 130-133. doi:10.1126/science.1134108

Neuwirth, C., Barkhaus, P. E., Burkhardt, C., Castro, J., Czell, D., de Carvalho, M., . . . Weber, M. (2015). Tracking motor neuron loss in a set of six muscles in amyotrophic lateral sclerosis using the Motor Unit Number Index (MUNIX): a 15-month longitudinal multicentre trial. *J Neurol Neurosurg Psychiatry, 86*(11), 1172-1179. doi:10.1136/jnnp-2015-310509

Nihei, K., McKee, A. C., & Kowall, N. W. (1993). Patterns of neuronal degeneration in the motor cortex of amyotrophic lateral sclerosis patients. *Acta Neuropathol, 86*(1), 55-64.

Nijboer, F., Sellers, E. W., Mellinger, J., Jordan, M. A., Matuz, T., Furdea, A., . . . Kubler, A. (2008). A P300-based brain-computer interface for people with amyotrophic

lateral sclerosis. *Clin Neurophysiol, 119*(8), 1909-1916. doi:10.1016/j.clinph.2008.03.034

Niven, E., Newton, J., Foley, J., Colville, S., Swingler, R., Chandran, S., . . . Abrahams, S. (2015). Validation of the Edinburgh Cognitive and Behavioural Amyotrophic Lateral Sclerosis Screen (ECAS): A cognitive tool for motor disorders. *Amyotroph Lateral Scler Frontotemporal Degener, 16*(3-4), 172-179. doi:10.3109/21678421.2015.1030430

Norman, D. A., & Shallice, T. (1986). Attention to action *Consciousness and self-regulation* (pp. 1-18): Springer.

O'Dowd, S., Curtin, D., Waite, A. J., Roberts, K., Pender, N., Reid, V., . . . Lynch, T. (2012). C9ORF72 expansion in amyotrophic lateral sclerosis/frontotemporal dementia also causes parkinsonism. *Mov Disord, 27*(8), 1072-1074. doi:10.1002/mds.25022

O'Rourke, J. G., Bogdanik, L., Yanez, A., Lall, D., Wolf, A. J., Muhammad, A. K., . . . Baloh, R. H. (2016). C9orf72 is required for proper macrophage and microglial function in mice. *Science, 351*(6279), 1324-1329. doi:10.1126/science.aaf1064

Ohki, M., Kanayama, R., Nakamura, T., Okuyama, T., Kimura, Y., & Koike, Y. (1994). Ocular abnormalities in amyotrophic lateral sclerosis. *Acta Otolaryngol Suppl, 511*, 138-142.

Okamoto, K., Hirai, S., Amari, M., Iizuka, T., Watanabe, M., Murakami, N., & Takatama, M. (1993). Oculomotor nuclear pathology in amyotrophic lateral sclerosis. *Acta Neuropathol, 85*(5), 458-462.

Okamoto, K., Hirai, S., Amari, M., Watanabe, M., & Sakurai, A. (1993). Bunina bodies in amyotrophic lateral sclerosis immunostained with rabbit anti-cystatin C serum. *Neurosci Lett, 162*(1-2), 125-128.

Okamoto, K., Hirai, S., Yamazaki, T., Sun, X. Y., & Nakazato, Y. (1991). New ubiquitin-positive intraneuronal inclusions in the extra-motor cortices in patients with amyotrophic lateral sclerosis. *Neurosci Lett, 129*(2), 233-236.

Okamoto, K., Mizuno, Y., & Fujita, Y. (2008). Bunina bodies in amyotrophic lateral sclerosis. *Neuropathology, 28*(2), 109-115. doi:10.1111/j.1440-1789.2007.00873.x

Owen, A. M. (2005). *Cognitive planning in humans: New insights from the Tower of London (TOL) task*. New York, NY: Psychology Press.

Pasinetti, G. M., Ungar, L. H., Lange, D. J., Yemul, S., Deng, H., Yuan, X., . . . Ho, L. (2006). Identification of potential CSF biomarkers in ALS. *Neurology, 66*(8), 1218-1222. doi:10.1212/01.wnl.0000203129.82104.07

Patel, A. N., & Sampson, J. B. (2015). Cognitive Profile of C9orf72 in Frontotemporal Dementia and Amyotrophic Lateral Sclerosis. *Curr Neurol Neurosci Rep, 15*(9), 59. doi:10.1007/s11910-015-0582-9

Patin, F., Corcia, P., Madji Hounoum, B., Veyrat-Durebex, C., Respaud, E., Piver, E., . . . Blasco, H. (2015). Biological follow-up in amyotrophic lateral sclerosis: decrease in creatinine levels and increase in ferritin levels predict poor prognosis. *Eur J Neurol, 22*(10), 1385-1390. doi:10.1111/ene.12754

Pfister, T., Sekhon, R., White, M., Scott, P., Munro, S., Johnston, M., . . . Korngut, L. (2013). Familial amyotrophic lateral sclerosis in Alberta, Canada. *Amyotroph Lateral Scler Frontotemporal Degener, 14*(4), 273-277. doi:10.3109/21678421.2012.754044

Phukan, J., Elamin, M., Bede, P., Jordan, N., Gallagher, L., Byrne, S., . . . Hardiman, O. (2012). The syndrome of cognitive impairment in amyotrophic lateral sclerosis: A population-based study. *Journal of Neurology & Psychiatry, 83*(1), 102-108.

Piao, Y. S., Wakabayashi, K., Kakita, A., Yamada, M., Hayashi, S., Morita, T., . . . Takahashi, H. (2003). Neuropathology with clinical correlations of sporadic amyotrophic lateral sclerosis: 102 autopsy cases examined between 1962 and 2000. *Brain Pathol, 13*(1), 10-22.

Pinto-Grau, M., Burke, T., Lonergan, K., McHugh, C., Mays, I., Madden, C., . . . Pender, N. (2017). Screening for cognitive dysfunction in ALS: validation of the Edinburgh Cognitive and Behavioural ALS Screen (ECAS) using age and education adjusted normative data. *Amyotroph Lateral Scler Frontotemporal Degener, 18*(1-2), 99-106. doi:10.1080/21678421.2016.1249887

Pioro, E. P., Antel, J. P., Cashman, N. R., & Arnold, D. L. (1994). Detection of cortical neuron loss in motor neuron disease by proton magnetic resonance spectroscopic imaging in vivo. *Neurology, 44*(10), 1933-1938.

Plato, C. C., Garruto, R. M., Galasko, D., Craig, U. K., Plato, M., Gamst, A., . . . Wiederholt, W. (2003). Amyotrophic lateral sclerosis and parkinsonism-dementia complex of Guam: changing incidence rates during the past 60 years. *Am J Epidemiol, 157*(2), 149-157.

Poletti, B., Solca, F., Carelli, L., Madotto, F., Lafronza, A., Faini, A., . . . Silani, V. (2016). The validation of the Italian Edinburgh Cognitive and Behavioural ALS Screen (ECAS). *Amyotroph Lateral Scler Frontotemporal Degener, 17*(7-8), 489-498. doi:10.1080/21678421.2016.1183679

Poletti, M., Enrici, I., & Adenzato, M. (2012). Cognitive and affective Theory of Mind in neurodegenerative diseases: neuropsychological, neuroanatomical and neurochemical levels. *Neurosci Biobehav Rev, 36*(9), 2147-2164. doi:10.1016/j.neubiorev.2012.07.004

Poloni, M., Capitani, E., Mazzini, L., & Ceroni, M. (1986). Neuropsychological measures in amyotrophic lateral sclerosis and their relationship with CT scan-assessed cerebral atrophy. *Acta Neurol Scand, 74*(4), 257-260.

Raaphorst, J., Beeldman, E., Schmand, B., Berkhout, J., Linssen, W. H., van den Berg, L. H., . . . de Haan, R. J. (2012). The ALS-FTD-Q: a new screening tool for behavioral

disturbances in ALS. *Neurology, 79*(13), 1377-1383. doi:10.1212/WNL.0b013e31826c1aa1

Raaphorst, J., de Visser, M., Linssen, W. H., de Haan, R. J., & Schmand, B. (2010a). The cognitive profile of amyotrophic lateral sclerosis: A meta-analysis. *Amyotrophic Lateral Sclerosis, 11*(1-2), 27-37.

Raaphorst, J., de Visser, M., Linssen, W. H., de Haan, R. J., & Schmand, B. (2010b). The cognitive profile of amyotrophic lateral sclerosis: A meta-analysis. *Amyotroph Lateral Scler, 11*(1-2), 27-37. doi:10.3109/17482960802645008

Rabinovici, G. D., & Miller, B. L. (2010). Frontotemporal lobar degeneration: epidemiology, pathophysiology, diagnosis and management. *CNS Drugs, 24*(5), 375-398. doi:10.2165/11533100-000000000-00000

Radakovic, R., Stephenson, L., Colville, S., Swingler, R., Chandran, S., & Abrahams, S. (2016). Multidimensional apathy in ALS: validation of the Dimensional Apathy Scale. *J Neurol Neurosurg Psychiatry, 87*(6), 663-669. doi:10.1136/jnnp-2015-310772

Radakovic, R., Stephenson, L., Newton, J., Crockford, C., Swingler, R., Chandran, S., & Abrahams, S. (2017). Multidimensional apathy and executive dysfunction in amyotrophic lateral sclerosis. *Cortex, 94*, 142-151. doi:10.1016/j.cortex.2017.06.023

Radunovic, A., Mitsumoto, H., & Leigh, P. N. (2007). Clinical care of patients with amyotrophic lateral sclerosis. *Lancet Neurol, 6*(10), 913-925. doi:10.1016/S1474-4422(07)70244-2

Rafalowska, J., & Dziewulska, D. (1996). White matter injury in amyotrophic lateral sclerosis (ALS). *Folia Neuropathol, 34*(2), 87-91.

Rascovsky, K., Hodges, J. R., Knopman, D., Mendez, M. F., Kramer, J. H., Neuhaus, J., . . . Miller, B. L. (2011). Sensitivity of revised diagnostic criteria for the behavioural variant of frontotemporal dementia. *Brain, 134*(Pt 9), 2456-2477. doi:10.1093/brain/awr179

Rechtman, L., Jordan, H., Wagner, L., Horton, D. K., & Kaye, W. (2015). Racial and ethnic differences among amyotrophic lateral sclerosis cases in the United States. *Amyotroph Lateral Scler Frontotemporal Degener, 16*(1-2), 65-71. doi:10.3109/21678421.2014.971813

Riccio, C. A., Reynolds, C. R., Lowe, P., & Moore, J. J. (2002). The continuous performance test: a window on the neural substrates for attention? *Arch Clin Neuropsychol, 17*(3), 235-272.

Roberts-South, A., Findlater, K., Strong, M. J., & Orange, J. B. (2012). Longitudinal changes in discourse production in amyotrophic lateral sclerosis. *Semin Speech Lang, 33*(1), 79-94. doi:10.1055/s-0031-1301165

Robinson, K. M., Lacey, S. C., Grugan, P., Glosser, G., Grossman, M., & McCluskey, L. F. (2006). Cognitive functioning in sporadic amyotrophic lateral sclerosis: a six

month longitudinal study. *J Neurol Neurosurg Psychiatry, 77*(5), 668-670. doi:10.1136/jnnp.2005.073403

Roccatagliata, L., Bonzano, L., Mancardi, G., Canepa, C., & Caponnetto, C. (2009). Detection of motor cortex thinning and corticospinal tract involvement by quantitative MRI in amyotrophic lateral sclerosis. *Amyotroph Lateral Scler, 10*(1), 47-52. doi:10.1080/17482960802267530

Roche, J. C., Rojas-Garcia, R., Scott, K. M., Scotton, W., Ellis, C. E., Burman, R., . . . Al-Chalabi, A. (2012). A proposed staging system for amyotrophic lateral sclerosis. *Brain, 135*(Pt 3), 847-852. doi:10.1093/brain/awr351

Rosen, D. R., Siddique, T., Patterson, D., Figlewicz, D. A., Sapp, P., Hentati, A., . . . et al. (1993). Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature, 362*(6415), 59-62. doi:10.1038/362059a0

Rothstein, J. D. (2017). Edaravone: A new drug approved for ALS. *Cell, 171*(4), 725. doi:10.1016/j.cell.2017.10.011

Rottig, D., Leplow, B., Eger, K., Ludolph, A. C., Graf, M., & Zierz, S. (2006). Only subtle cognitive deficits in non-bulbar amyotrophic lateral sclerosis patients. *J Neurol, 253*(3), 333-339.

Rutkove, S. (2009). Electrical impedance myography as a biomarker for ALS. *Lancet Neurol, 8*(3), 226; author reply 227. doi:10.1016/S1474-4422(09)70030-4

Saberi, S., Stauffer, J. E., Schulte, D. J., & Ravits, J. (2015). Neuropathology of Amyotrophic Lateral Sclerosis and Its Variants. *Neurol Clin, 33*(4), 855-876. doi:10.1016/j.ncl.2015.07.012

Santangelo, G., Raimo, S., Siciliano, M., D'Iorio, A., Piscopo, F., Cuoco, S., . . . Trojano, L. (2017). Assessment of apathy independent of physical disability: validation of the Dimensional Apathy Scale in Italian healthy sample. *Neurol Sci, 38*(2), 303-309. doi:10.1007/s10072-016-2766-8

Sarro, L., Agosta, F., Canu, E., Riva, N., Prelle, A., Copetti, M., . . . Filippi, M. (2011). Cognitive functions and white matter tract damage in amyotrophic lateral sclerosis: a diffusion tensor tractography study. *Ajnr: American Journal of Neuroradiology, 32*(10), 1866-1872.

Sasaki, S., & Maruyama, S. (1993). Ultrastructural study of Bunina bodies in the anterior horn neurons of patients with amyotrophic lateral sclerosis. *Neurosci Lett, 154*(1-2), 117-120.

Savage, S. A., Lillo, P., Kumfor, F., Kiernan, M. C., Piguet, O., & Hodges, J. R. (2014). Emotion processing deficits distinguish pure amyotrophic lateral sclerosis from frontotemporal dementia. *Amyotroph Lateral Scler Frontotemporal Degener, 15*(1-2), 39-46. doi:10.3109/21678421.2013.809763

Saxon, J. A., Harris, J. M., Thompson, J. C., Jones, M., Richardson, A. M. T., Langheinrich, T., . . . Snowden, J. S. (2017). Semantic dementia, progressive non-

fluent aphasia and their association with amyotrophic lateral sclerosis. *J Neurol Neurosurg Psychiatry, 88*(8), 711-712. doi:10.1136/jnnp-2016-314912

Scarmeas, N., Shih, T., Stern, Y., Ottman, R., & Rowland, L. P. (2002). Premorbid weight, body mass, and varsity athletics in ALS. *Neurology, 59*(5), 773-775.

Schaffer, S. G., Wisniewski, A., Dahdah, M., & Froming, K. B. (2009). The comprehensive affect testing system-abbreviated: effects of age on performance. *Arch Clin Neuropsychol, 24*(1), 89-104. doi:10.1093/arclin/acp012

Schmidt, R., de Reus, M. A., Scholtens, L. H., van den Berg, L. H., & van den Heuvel, M. P. (2016). Simulating disease propagation across white matter connectome reveals anatomical substrate for neuropathology staging in amyotrophic lateral sclerosis. *Neuroimage, 124*(Pt A), 762-769. doi:10.1016/j.neuroimage.2015.04.005

Schmolck, H., Mosnik, D., & Schulz, P. (2007). Rating the approachability of faces in ALS. *Neurology, 69*(24), 2232-2235. doi:10.1212/01.wnl.0000296001.16603.b3

Schreiber, H., Gaigalat, T., Wiedemuth-Catrinescu, U., Graf, M., Uttner, I., Muche, R., & Ludolph, A. C. (2005). Cognitive function in bulbar- and spinal-onset amyotrophic lateral sclerosis. A longitudinal study in 52 patients. *J Neurol, 252*(7), 772-781.

Schuster, C., Kasper, E., Dyrba, M., Machts, J., Bittner, D., Kaufmann, J., . . . Prudlo, J. (2014). Cortical thinning and its relation to cognition in amyotrophic lateral sclerosis. *Neurobiol Aging, 35*(1), 240-246. doi:10.1016/j.neurobiolaging.2013.07.020

Schuster, C., Kasper, E., Machts, J., Bittner, D., Kaufmann, J., Benecke, R., . . . Prudlo, J. (2013). Focal thinning of the motor cortex mirrors clinical features of amyotrophic lateral sclerosis and their phenotypes: a neuroimaging study. *J Neurol, 260*(11), 2856-2864. doi:10.1007/s00415-013-7083-z

Senda, J., Ito, M., Watanabe, H., Atsuta, N., Kawai, Y., Katsuno, M., . . . Sobue, G. (2009). Correlation between pyramidal tract degeneration and widespread white matter involvement in amyotrophic lateral sclerosis: a study with tractography and diffusion-tensor imaging. *Amyotroph Lateral Scler, 10*(5-6), 288-294. doi:10.3109/17482960802651717

Sgobio, C., Trabalza, A., Spalloni, A., Zona, C., Carunchio, I., Longone, P., & Ammassari-Teule, M. (2008). Abnormal medial prefrontal cortex connectivity and defective fear extinction in the presymptomatic G93A SOD1 mouse model of ALS. *Genes, Brain, & Behavior, 7*(4), 427-434.

Shallice, T. (1982). Specific impairments of planning. *Philos Trans R Soc Lond B Biol Sci, 298*(1089), 199-209.

Shallice, T., & Burgess, P. (1996). The domain of supervisory processes and temporal organization of behaviour. *Philos Trans R Soc Lond B Biol Sci, 351*(1346), 1405-1411; discussion 1411-1402. doi:10.1098/rstb.1996.0124

Shaunak, S., Orrell, R. W., O'Sullivan, E., Hawken, M. B., Lane, R. J., Henderson, L., & Kennard, C. (1995). Oculomotor function in amyotrophic lateral sclerosis: evidence for frontal impairment. *Ann Neurol, 38*(1), 38-44. doi:10.1002/ana.410380109

Shen, D., Cui, L., Fang, J., Cui, B., Li, D., & Tai, H. (2016). Voxel-Wise Meta-Analysis of Gray Matter Changes in Amyotrophic Lateral Sclerosis. *Front Aging Neurosci, 8*, 64. doi:10.3389/fnagi.2016.00064

Shoesmith, C. L., Findlater, K., Rowe, A., & Strong, M. J. (2007). Prognosis of amyotrophic lateral sclerosis with respiratory onset. *J Neurol Neurosurg Psychiatry, 78*(6), 629-631. doi:10.1136/jnnp.2006.103564

Si, Y., Cui, X., Kim, S., Wians, R., Sorge, R., Oh, S. J., . . . King, P. H. (2014). Smads as muscle biomarkers in amyotrophic lateral sclerosis. *Ann Clin Transl Neurol, 1*(10), 778-787. doi:10.1002/acn3.117

Siciliano, M., Trojano, L., Trojsi, F., Greco, R., Santoro, M., Basile, G., . . . Santangelo, G. (2017). Edinburgh Cognitive and Behavioural ALS Screen (ECAS)-Italian version: regression based norms and equivalent scores. *Neurol Sci, 38*(6), 1059-1068. doi:10.1007/s10072-017-2919-4

Snowden, J. S., Gibbons, Z. C., Blackshaw, A., Doubleday, E., Thompson, J., Craufurd, D., . . . Neary, D. (2003). Social cognition in frontotemporal dementia and Huntington's disease. *Neuropsychologia, 41*(6), 688-701.

Soares, J. M., Marques, P., Alves, V., & Sousa, N. (2013). A hitchhiker's guide to diffusion tensor imaging. *Front Neurosci, 7*, 31. doi:10.3389/fnins.2013.00031

Song, S. K., Sun, S. W., Ju, W. K., Lin, S. J., Cross, A. H., & Neufeld, A. H. (2003). Diffusion tensor imaging detects and differentiates axon and myelin degeneration in mouse optic nerve after retinal ischemia. *Neuroimage, 20*(3), 1714-1722.

Sorenson, E. J., Stalker, A. P., Kurland, L. T., & Windebank, A. J. (2002). Amyotrophic lateral sclerosis in Olmsted County, Minnesota, 1925 to 1998. *Neurology, 59*(2), 280-282.

Sreedharan, J., Blair, I. P., Tripathi, V. B., Hu, X., Vance, C., Rogelj, B., . . . Shaw, C. E. (2008). TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. *Science, 319*(5870), 1668-1672. doi:10.1126/science.1154584

Staios, M., Fisher, F., Lindell, A. K., Ong, B., Howe, J., & Reardon, K. (2013). Exploring sarcasm detection in amyotrophic lateral sclerosis using ecologically valid measures. *Front Hum Neurosci, 7*, 178. doi:10.3389/fnhum.2013.00178

Stefanelli, M., Lim, C., Sloka, J., Whelan, G., Murphy, D., & Goodridge, A. e. a. (2005). *Iron horse disease: are we overrun? The incidence of ALS in Newfoundland and Labrador, Canada*. Paper presented at the World Congr Neurol, Sydney.

Stone, V. E., Baron-Cohen, S., & Knight, R. T. (1998). Frontal lobe contributions to theory of mind. *J Cogn Neurosci, 10*(5), 640-656.

Strimbu, K., & Tavel, J. A. (2010). What are biomarkers? *Curr Opin HIV AIDS, 5*(6), 463-466. doi:10.1097/COH.0b013e32833ed177

Strong, M. J. (2010). The evidence for altered RNA metabolism in amyotrophic lateral sclerosis (ALS). *J Neurol Sci, 288*(1-2), 1-12. doi:10.1016/j.jns.2009.09.029

Strong, M. J., Abrahams, S., Goldstein, L. H., Woolley, S., McLaughlin, P., Snowden, J., . . . Turner, M. R. (2017). Amyotrophic lateral sclerosis - frontotemporal spectrum

disorder (ALS-FTSD): Revised diagnostic criteria. *Amyotroph Lateral Scler Frontotemporal Degener, 18*(3-4), 153-174. doi:10.1080/21678421.2016.1267768

Strong, M. J., Grace, G. M., Freedman, M., Lomen-Hoerth, C., Woolley, S., Goldstein, L. H., . . . Figlewicz, D. (2009). Consensus criteria for the diagnosis of frontotemporal cognitive and behavioural syndromes in amyotrophic lateral sclerosis. *Amyotroph Lateral Scler, 10*(3), 131-146.

Strong, M. J., Grace, G. M., Orange, J. B., & Leeper, H. A. (1996). Cognition, language, and speech in amyotrophic lateral sclerosis: a review. *J Clin Exp Neuropsychol, 18*(2), 291-303. doi:10.1080/01688639608408283

Stukovnik, V., Zidar, J., Podnar, S., & Repovs, G. (2010). Amyotrophic lateral sclerosis patients show executive impairments on standard neuropsychological measures and an ecologically valid motor-free test of executive functions. *Journal of Clinical Neuropsychology, 32*(10), 1095-1109.

Sundar, P. D., Yu, C. E., Sieh, W., Steinbart, E., Garruto, R. M., Oyanagi, K., . . . Schellenberg, G. D. (2007). Two sites in the MAPT region confer genetic risk for Guam ALS/PDC and dementia. *Hum Mol Genet, 16*(3), 295-306. doi:10.1093/hmg/ddl463

Sutedja, N. A., Veldink, J. H., Fischer, K., Kromhout, H., Heederik, D., Huisman, M. H., . . . van den Berg, L. H. (2009). Exposure to chemicals and metals and risk of amyotrophic lateral sclerosis: a systematic review. *Amyotroph Lateral Scler, 10*(5-6), 302-309. doi:10.3109/17482960802455416

Svenson, L. W., Cwik, V. A., & Martin, W. R. (1999). The prevalence of motor neurone disease in the Province of Alberta. *Can J Neurol Sci, 26*(2), 119-122.

Swinnen, B., & Robberecht, W. (2014). The phenotypic variability of amyotrophic lateral sclerosis. *Nat Rev Neurol, 10*(11), 661-670. doi:10.1038/nrneurol.2014.184

Talbot, P. R., Goulding, P. J., Lloyd, J. J., Snowden, J. S., Neary, D., & Testa, H. J. (1995). Inter-relation between "classic" motor neuron disease and frontotemporal dementia: neuropsychological and single photon emission computed tomography study. *J Neurol Neurosurg Psychiatry, 58*(5), 541-547.

Tarasiuk, J., Kulakowska, A., Drozdowski, W., Kornhuber, J., & Lewczuk, P. (2012). CSF markers in amyotrophic lateral sclerosis. *J Neural Transm (Vienna), 119*(7), 747-757. doi:10.1007/s00702-012-0806-y

Taylor, J. P. (2017). A PR plug for the nuclear pore in amyotrophic lateral sclerosis. *Proc Natl Acad Sci U S A, 114*(7), 1445-1447. doi:10.1073/pnas.1621085114

Taylor, L. J., Brown, R. G., Tsermentseli, S., AlChalabi, A., Shaw, C. E., Ellis, C. M., . . . Goldstein, L. H. (2013). Is language impairment more common than executive dysfunction in amyotrophic lateral sclerosis? *Journal of Neurology & Psychiatry, 84*(5), 494-498.

Tedeschi, G., Trojsi, F., Tessitore, A., Corbo, D., Sagnelli, A., Paccone, A., . . . Esposito, F. (2012). Interaction between aging and neurodegeneration in amyotrophic lateral sclerosis. *Neurobiol Aging, 33*(5), 886-898.

Thakore, N. J., & Pioro, E. P. (2017). Laughter, crying and sadness in ALS. *J Neurol Neurosurg Psychiatry, 88*(10), 825-831. doi:10.1136/jnnp-2017-315622

Thorpe, J. R., Tang, H., Atherton, J., & Cairns, N. J. (2008). Fine structural analysis of the neuronal inclusions of frontotemporal lobar degeneration with TDP-43 proteinopathy. *J Neural Transm (Vienna), 115*(12), 1661-1671. doi:10.1007/s00702-008-0137-1

Tomonaga, M., Saito, M., Yoshimura, M., Shimada, H., & Tohgi, H. (1978). Ultrastructure of the Bunina bodies in anterior horn cells of amyotrophic lateral sclerosis. *Acta Neuropathol, 42*(2), 81-86.

Toronov, V., Walker, S., Gupta, R., Choi, J. H., Gratton, E., Hueber, D., & Webb, A. (2003). The roles of changes in deoxyhemoglobin concentration and regional cerebral blood volume in the fMRI BOLD signal. *Neuroimage, 19*(4), 1521-1531.

Torralva, T., Roca, M., Gleichgerrcht, E., Bekinschtein, T., & Manes, F. (2009). A neuropsychological battery to detect specific executive and social cognitive impairments in early frontotemporal dementia. *Brain, 132*(Pt 5), 1299-1309. doi:10.1093/brain/awp041

Tortelli, R., Ruggieri, M., Cortese, R., D'Errico, E., Capozzo, R., Leo, A., . . . Simone, I. L. (2012). Elevated cerebrospinal fluid neurofilament light levels in patients with amyotrophic lateral sclerosis: a possible marker of disease severity and progression. *Eur J Neurol, 19*(12), 1561-1567. doi:10.1111/j.1468-1331.2012.03777.x

Trojsi, F., Di Nardo, F., Santangelo, G., Siciliano, M., Femiano, C., Passaniti, C., . . . Tedeschi, G. (2017). Resting state fMRI correlates of Theory of Mind impairment in amyotrophic lateral sclerosis. *Cortex, 97*, 1-16. doi:10.1016/j.cortex.2017.09.016

Trojsi, F., Esposito, F., de Stefano, M., Buonanno, D., Conforti, F. L., Corbo, D., . . . Tedeschi, G. (2015). Functional overlap and divergence between ALS and bvFTD. *Neurobiol Aging, 36*(1), 413-423. doi:10.1016/j.neurobiolaging.2014.06.025

Trojsi, F., Santangelo, G., Caiazzo, G., Siciliano, M., Ferrantino, T., Piccirillo, G., . . . Tedeschi, G. (2016). Neuropsychological assessment in different King's clinical stages of amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Frontotemporal Degener, 17*(3-4), 228-235. doi:10.3109/21678421.2016.1143513

Trojsi, F., Siciliano, M., Russo, A., Passaniti, C., Femiano, C., Ferrantino, T., . . . Santangelo, G. (2016). Theory of Mind and Its Neuropsychological and Quality of Life Correlates in the Early Stages of Amyotrophic Lateral Sclerosis. *Front Psychol, 7*, 1934. doi:10.3389/fpsyg.2016.01934

Trotti, D., Rolfs, A., Danbolt, N. C., Brown, R. H., Jr., & Hediger, M. A. (1999). SOD1 mutants linked to amyotrophic lateral sclerosis selectively inactivate a glial glutamate transporter. *Nat Neurosci, 2*(9), 848. doi:10.1038/12227

Troyer, A. K., Moscovitch, M., & Winocur, G. (1997). Clustering and switching as two components of verbal fluency: evidence from younger and older healthy adults. *Neuropsychology, 11*(1), 138-146.

Troyer, A. K., Moscovitch, M., Winocur, G., Alexander, M. P., & Stuss, D. (1998). Clustering and switching on verbal fluency: the effects of focal frontal- and temporal-lobe lesions. *Neuropsychologia, 36*(6), 499-504.

Tsermentseli, S., Leigh, P. N., Taylor, L. J., Radunovic, A., Catani, M., & Goldstein, L. H. (2016). Syntactic processing as a marker for cognitive impairment in amyotrophic lateral sclerosis. *Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration, 17*(1-2), 69-76.

Turner, M. R. (2013). Increased premorbid physical activity and amyotrophic lateral sclerosis: born to run rather than run to death, or a seductive myth? *J Neurol Neurosurg Psychiatry, 84*(9), 947. doi:10.1136/jnnp-2013-304935

Turner, M. R., Hardiman, O., Benatar, M., Brooks, B. R., Chio, A., de Carvalho, M., . . . Kiernan, M. C. (2013). Controversies and priorities in amyotrophic lateral sclerosis. *Lancet Neurol, 12*(3), 310-322. doi:10.1016/S1474-4422(13)70036-X

Turner, M. R., & Swash, M. (2015). The expanding syndrome of amyotrophic lateral sclerosis: a clinical and molecular odyssey. *J Neurol Neurosurg Psychiatry, 86*(6), 667-673. doi:10.1136/jnnp-2014-308946

Usman, U., Choi, C., Camicioli, R., Seres, P., Lynch, M., Sekhon, R., . . . Kalra, S. (2011). Mesial prefrontal cortex degeneration in amyotrophic lateral sclerosis: a high-field proton MR spectroscopy study. *AJNR Am J Neuroradiol, 32*(9), 1677-1680. doi:10.3174/ajnr.A2590

Utevsky, A. V., Smith, D. V., & Huettel, S. A. (2014). Precuneus is a functional core of the default-mode network. *J Neurosci, 34*(3), 932-940. doi:10.1523/JNEUROSCI.4227-13.2014

van Bogaert, L. (1925). Mental disturbances in amyotrophic lateral sclerosis. *Encephale, 20*(27).

Van Deerlin, V. M., Leverenz, J. B., Bekris, L. M., Bird, T. D., Yuan, W., Elman, L. B., . . . Yu, C. E. (2008). TARDBP mutations in amyotrophic lateral sclerosis with TDP-43 neuropathology: a genetic and histopathological analysis. *Lancet Neurol, 7*(5), 409-416. doi:10.1016/S1474-4422(08)70071-1

van den Berg, J. P., Kalmijn, S., Lindeman, E., Veldink, J. H., de Visser, M., Van der Graaff, M. M., . . . Van den Berg, L. H. (2005). Multidisciplinary ALS care improves quality of life in patients with ALS. *Neurology, 65*(8), 1264-1267. doi:10.1212/01.wnl.0000180717.29273.12

van der Hulst, E. J., Bak, T. H., & Abrahams, S. (2014). Impaired affective and cognitive theory of mind and behavioural change in amyotrophic lateral sclerosis. *J Neurol Neurosurg Psychiatry*. doi:10.1136/jnnp-2014-309290

Vance, C., Rogelj, B., Hortobagyi, T., De Vos, K. J., Nishimura, A. L., Sreedharan, J., . . . Shaw, C. E. (2009). Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6. *Science, 323*(5918), 1208-1211. doi:10.1126/science.1165942

Varghese, A. M., Sharma, A., Mishra, P., Vijayalakshmi, K., Harsha, H. C., Sathyaprabha, T. N., . . . Raju, T. R. (2013). Chitotriosidase - a putative biomarker for sporadic amyotrophic lateral sclerosis. *Clin Proteomics, 10*(1), 19. doi:10.1186/1559-0275-10-19

Verde, F., Del Tredici, K., Braak, H., & Ludolph, A. (2017). The multisystem degeneration amyotrophic lateral sclerosis - neuropathological staging and clinical translation. *Arch Ital Biol, 155*(4), 118-130. doi:10.12871/00039829201746

Verma, A., & Tandan, R. (2013). RNA quality control and protein aggregates in amyotrophic lateral sclerosis: a review. *Muscle Nerve, 47*(3), 330-338. doi:10.1002/mus.23673

Verstraete, E., Veldink, J. H., Hendrikse, J., Schelhaas, H. J., van den Heuvel, M. P., & van den Berg, L. H. (2012). Structural MRI reveals cortical thinning in amyotrophic lateral sclerosis. *J Neurol Neurosurg Psychiatry, 83*(4), 383-388. doi:10.1136/jnnp-2011-300909

Visser, J., van den Berg-Vos, R. M., Franssen, H., van den Berg, L. H., Wokke, J. H., de Jong, J. M., . . . de Visser, M. (2007). Disease course and prognostic factors of progressive muscular atrophy. *Arch Neurol, 64*(4), 522-528. doi:10.1001/archneur.64.4.522

Vucic, S., Cheah, B. C., Yiannikas, C., & Kiernan, M. C. (2011). Cortical excitability distinguishes ALS from mimic disorders. *Clin Neurophysiol, 122*(9), 1860-1866. doi:10.1016/j.clinph.2010.12.062

Vucic, S., Lin, C. S., Cheah, B. C., Murray, J., Menon, P., Krishnan, A. V., & Kiernan, M. C. (2013). Riluzole exerts central and peripheral modulating effects in amyotrophic lateral sclerosis. *Brain, 136*(Pt 5), 1361-1370. doi:10.1093/brain/awt085

Wagner, L., Rechtman, L., Jordan, H., Ritsick, M., Sanchez, M., Sorenson, E., & Kaye, W. (2015). State and metropolitan area-based amyotrophic lateral sclerosis (ALS) surveillance. *Amyotroph Lateral Scler Frontotemporal Degener, 17*(1-2), 128-134. doi:10.3109/21678421.2015.1074699

Walhout, R., Westeneng, H. J., Verstraete, E., Hendrikse, J., Veldink, J. H., van den Heuvel, M. P., & van den Berg, L. H. (2015). Cortical thickness in ALS: towards a marker for upper motor neuron involvement. *J Neurol Neurosurg Psychiatry, 86*(3), 288-294. doi:10.1136/jnnp-2013-306839

Warrington, E. K. (1984). *Recognition memory test*. Windsor: NFER-Nelson.

Watermeyer, T. J., Brown, R. G., Sidle, K. C., Oliver, D. J., Allen, C., Karlsson, J., . . . Goldstein, L. H. (2015a). Impact of disease, cognitive and behavioural factors on caregiver outcome in amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Frontotemporal Degener, 16*(5-6), 316-323. doi:10.3109/21678421.2015.1051990

Watermeyer, T. J., Brown, R. G., Sidle, K. C., Oliver, D. J., Allen, C., Karlsson, J., . . . Goldstein, L. H. (2015b). Executive dysfunction predicts social cognition impairment in amyotrophic lateral sclerosis. *J Neurol, 262*(7), 1681-1690. doi:10.1007/s00415-015-7761-0

Wei, Q., Chen, X., Zheng, Z., Huang, R., Guo, X., Cao, B., . . . Shang, H. (2015). Screening for cognitive impairment in a Chinese ALS population. *Amyotroph Lateral Scler Frontotemporal Degener, 16*(1-2), 40-45. doi:10.3109/21678421.2014.966311

Weisskopf, M. G., McCullough, M. L., Morozova, N., Calle, E. E., Thun, M. J., & Ascherio, A. (2005). Prospective study of occupation and amyotrophic lateral sclerosis mortality. *Am J Epidemiol, 162*(12), 1146-1152. doi:10.1093/aje/kwi343

Westeneng, H. J., Walhout, R., Straathof, M., Schmidt, R., Hendrikse, J., Veldink, J. H., . . . van den Berg, L. H. (2016). Widespread structural brain involvement in ALS is not limited to the C9orf72 repeat expansion. *J Neurol Neurosurg Psychiatry, 87*(12), 1354-1360. doi:10.1136/jnnp-2016-313959

Whiting, M. G. (1964). Food Practices in Als Foci in Japan, the Marianas, and New Guinea. *Fed Proc, 23*, 1343-1345.

Wicks, P., Abrahams, S., Papps, B., Al-Chalabi, A., Shaw, C. E., Leigh, P. N., & Goldstein, L. H. (2009). SOD1 and cognitive dysfunction in familial amyotrophic lateral sclerosis. *J Neurol, 256*(2), 234-241.

Wightman, G., Anderson, V. E., Martin, J., Swash, M., Anderton, B. H., Neary, D., . . . Leigh, P. N. (1992). Hippocampal and neocortical ubiquitin-immunoreactive inclusions in amyotrophic lateral sclerosis with dementia. *Neurosci Lett, 139*(2), 269-274.

Wijesekera, L. C., & Leigh, P. N. (2009). Amyotrophic lateral sclerosis. *Orphanet J Rare Dis, 4*, 3. doi:10.1186/1750-1172-4-3

Wilke, C., Deuschle, C., Rattay, T. W., Maetzler, W., & Synofzik, M. (2015). Total tau is increased, but phosphorylated tau not decreased, in cerebrospinal fluid in amyotrophic lateral sclerosis. *Neurobiol Aging, 36*(2), 1072-1074. doi:10.1016/j.neurobiolaging.2014.10.019

Wimmer, H., & Perner, J. (1983). Beliefs about beliefs: representation and constraining function of wrong beliefs in young children's understanding of deception. *Cognition, 13*(1), 103-128.

Witgert, M., Salamone, A. R., Strutt, A. M., Jawaid, A., Massman, P. J., Bradshaw, M., . . . Schulz, P. E. (2010). Frontal-lobe mediated behavioral dysfunction in amyotrophic lateral sclerosis. *European Journal of Neurology, 17*(1), 103-110.

Wolfson, C., Kilborn, S., Oskoui, M., & Genge, A. (2009). Incidence and prevalence of amyotrophic lateral sclerosis in Canada: a systematic review of the literature. *Neuroepidemiology, 33*(2), 79-88. doi:10.1159/000222089

Woo, J. H., Wang, S., Melhem, E. R., Gee, J. C., Cucchiara, A., McCluskey, L., & Elman, L. (2014). Linear associations between clinically assessed upper motor neuron disease and diffusion tensor imaging metrics in amyotrophic lateral sclerosis. *PLoS One, 9*(8), e105753. doi:10.1371/journal.pone.0105753

Woolley, S. C., Moore, D. H., & Katz, J. S. (2010). Insight in ALS: awareness of behavioral change in patients with and without FTD. *Amyotrophic Lateral Sclerosis, 11*(1-2), 52-56.

Woolley, S. C., York, M. K., Moore, D. H., Strutt, A. M., Murphy, J., Schulz, P. E., & Katz, J. S. (2010). Detecting frontotemporal dysfunction in ALS: utility of the ALS Cognitive Behavioral Screen (ALS-CBS). *Amyotroph Lateral Scler, 11*(3), 303-311. doi:10.3109/17482961003727954

Worms, P. M. (2001). The epidemiology of motor neuron diseases: a review of recent studies. *J Neurol Sci, 191*(1-2), 3-9.

Writing, G., & Edaravone, A. L. S. S. G. (2017). Safety and efficacy of edaravone in well defined patients with amyotrophic lateral sclerosis: a randomised, double-blind, placebo-controlled trial. *Lancet Neurol, 16*(7), 505-512. doi:10.1016/S1474-4422(17)30115-1

Xu, Z., Alruwaili, A. R. S., Henderson, R. D., & McCombe, P. A. (2017). Screening for cognitive and behavioural impairment in amyotrophic lateral sclerosis: Frequency of abnormality and effect on survival. *J Neurol Sci, 376*, 16-23. doi:10.1016/j.jns.2017.02.061

Yamanaka, K., Chun, S. J., Boillee, S., Fujimori-Tonou, N., Yamashita, H., Gutmann, D. H., . . . Cleveland, D. W. (2008). Astrocytes as determinants of disease progression in inherited amyotrophic lateral sclerosis. *Nat Neurosci, 11*(3), 251-253. doi:10.1038/nn2047

Ye, S., Ji, Y., Li, C., He, J., Liu, X., & Fan, D. (2016). The Edinburgh Cognitive and Behavioural ALS Screen in a Chinese Amyotrophic Lateral Sclerosis Population. *PLoS One, 11*(5), e0155496. doi:10.1371/journal.pone.0155496

York, C., Olm, C., Boller, A., McCluskey, L., Elman, L., Haley, J., . . . Grossman, M. (2014). Action verb comprehension in amyotrophic lateral sclerosis and Parkinson's disease. *J Neurol, 261*(6), 1073-1079. doi:10.1007/s00415-014-7314-y

Yoshino, H., & Kimura, A. (2006). Investigation of the therapeutic effects of edaravone, a free radical scavenger, on amyotrophic lateral sclerosis (Phase II study). *Amyotrophic Lateral Sclerosis, 7*(4), 247-251.

Yoshizawa, K., Yasuda, N., Fukuda, M., Yukimoto, Y., Ogino, M., Hata, W., . . . Higashikawa, M. (2014). Syntactic comprehension in patients with amyotrophic lateral sclerosis. *Behavioural Neurology, 2014*, 230578.

Zalonis, I., Christidi, F., Paraskevas, G., Zabelis, T., Evdokimidis, I., & Kararizou, E. (2012). Can executive cognitive measures differentiate between patients with spinal- and bulbar-onset amyotrophic lateral sclerosis? *Archives of Clinical Neuropsychology, 27*(3), 348-354.

Zarei, S., Carr, K., Reiley, L., Diaz, K., Guerra, O., Altamirano, P. F., . . . Chinea, A. (2015). A comprehensive review of amyotrophic lateral sclerosis. *Surg Neurol Int, 6*, 171. doi:10.4103/2152-7806.169561

Zetterstrom, P., Andersen, P. M., Brannstrom, T., & Marklund, S. L. (2011). Misfolded superoxide dismutase-1 in CSF from amyotrophic lateral sclerosis patients. *J Neurochem, 117*(1), 91-99. doi:10.1111/j.1471-4159.2011.07177.x

Zhang, H., Avants, B. B., Yushkevich, P. A., Woo, J. H., Wang, S., McCluskey, L. F., . . . Gee, J. C. (2007). High-dimensional spatial normalization of diffusion tensor images improves the detection of white matter differences: an example study using amyotrophic lateral sclerosis. *IEEE Trans Med Imaging, 26*(11), 1585-1597. doi:10.1109/TMI.2007.906784

Zhang, H., Yushkevich, P. A., Rueckert, D., & Gee, J. C. (2007). Unbiased white matter atlas construction using diffusion tensor images. *Med Image Comput Comput Assist Interv, 10*(Pt 2), 211-218.

Zhang, Y., Schuff, N., Woolley, S. C., Chiang, G. C., Boreta, L., Laxamana, J., . . . Weiner, M. W. (2011). Progression of white matter degeneration in amyotrophic lateral sclerosis: A diffusion tensor imaging study. *Amyotroph Lateral Scler, 12*(6), 421-429. doi:10.3109/17482968.2011.593036

Zhou, F., Xu, R., Dowd, E., Zang, Y., Gong, H., & Wang, Z. (2014). Alterations in regional functional coherence within the sensory-motor network in amyotrophic lateral sclerosis. *Neuroscience letters, 558*, 192-196.

Zimmerman, E. K., Eslinger, P. J., Simmons, Z., & Barrett, A. M. (2007). Emotional perception deficits in amyotrophic lateral sclerosis. *Cogn Behav Neurol, 20*(2), 79-82. doi:10.1097/WNN.0b013e31804c700b