Speech Network Regional Differences in Bulbar Amyotrophic Lateral Sclerosis: Neuroimaging and Neuropathology Investigations

by

Sanjana Shellikeri

A thesis submitted in conformity with the requirements for the degree of Doctorate of Philosophy
Rehabilitation Sciences Institute (Speech-Language Pathology)
University of Toronto

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Abstract

Bulbar ALS has devastating consequences on survival and quality of life, and may be linked to increased burden of extramotor deficits. This dissertation is comprised of three studies addressing the goal of better understanding the neural anatomical underpinnings of bulbar Amyotrophic Lateral Sclerosis (ALS) with a particular focus on the cortical speech network (SpN). The work has significant impact on ALS subtyping which is crucial for understanding disease pathogenesis, and clinical implications for diagnosis, prognosis, and recruitment into clinical trials. The first study characterized structural abnormalities in the SpN regions with relation to bulbar motor dysfunction using T1 and DTI neuroimaging in 16 patients with bulbar ALS. The results revealed left-lateralized differences in extramotor SpN regions with thinning in left inferior frontal gyrus (IFG) and diffusivity abnormalities underlying left primary auditory cortex (PAC) and posterior superior temporal gyrus (pSTG). Greater bulbar motor dysfunction was associated with greater structural abnormalities in selected SpN regions, while limb and disease severity were not. The second study systematically reviewed and compared published neuropathology data between bulbar-onset ALS (bALS) and spinal-onset ALS (sALS) in order to distinguish bulbar from spinal ALS. Neuropathology in IFG and pSTG were variable in bALS cases, however consistently spared in sALS. A subset of bALS cases also showed widespread
tauopathy. Study three compared the anatomic distribution and types of neuropathology between 3 groups, namely: 3 bALS cases, 3 sALS with antemortem bulbar dysfunction (sALSwB), and 3 sALS without antemortem bulbar dysfunction (sALSnoB). SpN regions were most severely and extensively affected in the bALS cases, followed by sALSwB cases. Neuropathology in SpN regions was absent in sALSnoB cases. Two of the three bALS cases presented with atypical proteinopathy. Findings from the studies suggested that cortical SpN may be exclusively affected in bulbar ALS. The extent and severity of damage in SpN regions may be related to the severity of bulbar motor disease. Further, bALS may be associated with unique morphology and co-existing proteinopathy.
Acknowledgments

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<td>AD</td>
<td>Axial Diffusivity</td>
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<tr>
<td>ALS</td>
<td>Amyotrophic Lateral Sclerosis</td>
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<tr>
<td>ALSFRS-R</td>
<td>ALS Functional Rating Scale – Revised</td>
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<tr>
<td>bALS</td>
<td>Bulbar-onset ALS</td>
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<tr>
<td>CNVmo</td>
<td>Trigeminal Motor Cranial Nucleus</td>
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<tr>
<td>CNVII</td>
<td>Facial Cranial Nucleus</td>
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<tr>
<td>CNXII</td>
<td>Hypoglossal Cranial Nucleus</td>
</tr>
<tr>
<td>DDK</td>
<td>Dysdiadochokinetic</td>
</tr>
<tr>
<td>DIVA</td>
<td>Directions and Velocities of Articulators - neurocomputational model of speech production</td>
</tr>
<tr>
<td>DN</td>
<td>Dystrophic Neurites</td>
</tr>
<tr>
<td>DTI</td>
<td>Diffusion Tensor Imaging</td>
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<tr>
<td>EMG</td>
<td>Electromyography</td>
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<tr>
<td>FA</td>
<td>Fractional Anisotropy</td>
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<tr>
<td>FTD</td>
<td>Frontotemporal Dementia</td>
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<tr>
<td>FTLD</td>
<td>Frontotemporal Lobar Degeneration</td>
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<tr>
<td>GM</td>
<td>Grey Matter</td>
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<tr>
<td>HE/LFB</td>
<td>Luxol fast blue-hematoxylin-eosin stain</td>
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<tr>
<td>IFG</td>
<td>Inferior Frontal Gyrus</td>
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<td>LMN</td>
<td>Lower Motor Neuron</td>
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<td>Abbreviation</td>
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<tr>
<td>MD</td>
<td>Mean Diffusivity</td>
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<tr>
<td>MoCA</td>
<td>Montreal Cognitive Assessment</td>
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<td>NCI</td>
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<td>PART</td>
<td>Primary Age Related Tauopathy</td>
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<td>Primary Motor Cortex</td>
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<td>PSC</td>
<td>Primary Somatosensory Cortex</td>
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<td>pSTG</td>
<td>Posterior Superior Temporal Gyrus</td>
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<td>RD</td>
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<td>sALS</td>
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<td>sALSwB</td>
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<tr>
<td>SpN</td>
<td>Speech Network</td>
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<tr>
<td>TDP-43</td>
<td>Phosphorylated 43-kDa TAR DNA-binding Protein</td>
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<tr>
<td>TT</td>
<td>Transverse Temporal</td>
</tr>
<tr>
<td>UMN</td>
<td>Upper Motor Neuron</td>
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<tr>
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Chapter 1
Introduction

Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig’s disease, was first described by Jean Martin Charcot in 1869 as a motor neuron disease characterized by progressive degeneration of upper and lower motor neurons in the brain and spinal cord (Charcot & Joffroy, 1869). In the last decade, ALS has been established as a multi-system disorder that affects both motor and extramotor systems (Strong et al., 2017). The hallmark of ALS is the degeneration of motor neurons, which results in a progressive paralysis of muscles affecting limb, bulbar, and respiratory functions. The paralytic nature of the disease leads to eventual death, commonly due to respiratory failure (Corcia et al., 2008; Jokelainen, 1976).

The incidence rate of ALS in Canada is estimated to be 2/100,000 people per year (Hudson, Davenport & Hader, 1986). Approximately 3000 Canadians over 18 years of age currently live with this disease (Hudson, Davenport & Hader, 1986). ALS can affect males and females equally (McCombe & Henderson, 2010) and can strike at all ages but is most commonly diagnosed in middle and late adulthood with a mean age of onset of 65 years (Chiò et al., 2009). In approximately 90% of ALS patients, there is no known family history (sporadic ALS), and the cause of disease is mostly unknown. The remaining 10% of patients can be identified as relatives of other ALS cases (familial ALS). At least 15 genes have been identified that are implicated in two-thirds of familial ALS cases, including SOD1, C9ORF72, TARDBP, FUS, and IBQLN2 among others (Chen et al., 2013; Turner et al., 2017). Some of these genes are noted for familial ALS have also been linked to approximately 14% of sporadic ALS cases (Turner et al., 2017).

The disease is typically fast progressing with a median survival of 3-5 years from disease onset (Millul et al., 2005). Currently, ALS has no effective cure and few pharmaceutical therapies exist to slow the rate of disease progression (Distad et al., 2008; Naganska & Matyja, 2011). Two drugs have been government approved in ALS, Riluzole and Edaravone, both only minimally affecting survival (Rothstein, 2017; Miller et al., 2003). The absence of effective therapeutics 150 years after the first descriptions of the disease necessitates further research into mechanisms of disease.
1.1 Motor Manifestations of ALS

The motor manifestation of ALS is characterized by the progressive loss of upper motor neurons (UMN) originating in the primary motor cortex (PMC) and lower motor neurons (LMN) within the brainstem and the anterior horn of the spinal cord (Brooks et al., 2000). The UMN degeneration in ALS leads to increased muscle stiffness (spasticity) and hyperreflexia. LMN degeneration leads to muscle weakness and atrophy, often with prominent muscle twitching (fasciculations) and reduced or absent deep tendon reflexes, and eventual paralysis (van Es et al., 2017). The degree of LMN/UMN involvement leading to a mixed presentation of flaccid and spastic muscles varies between individuals (Chio et al., 2011).

There is vast heterogeneity in the clinical presentations of ALS due to varying sites of onset and degree of UMN/LMN involvement (Ravits & La Spada, 2009). ALS is often classified by body region of onset, relative mix of UMN and LMN involvement, and rate of progression. Symptoms begin in the spinal muscles (arms, legs, or trunk) in 70% of patients, referred to as spinal-onset ALS, and are characterized by foot drop, imbalance/impaired mobility, reduced grip strength, and/or impaired fine motor control. For the remaining cases (~20-30%), symptoms begin in the bulbar muscles of the face, mouth, tongue, pharynx and/or larynx, affecting speech and swallowing functions and referred to as bulbar-onset ALS (Chio et al., 2011). Patients can also be categorized as progressive muscular atrophy (an asymmetrical LMN phenotype), primary lateral sclerosis (an often symmetrical UMN phenotype), or ALS showing both UMN and LMN degeneration. Other phenotypes are also recognized, such as the flail arm syndrome which is a symmetrical, predominantly proximal LMN weakness affecting the upper limbs, and flail leg syndrome, which is a symmetrical, predominantly distal LMN weakness affecting the lower limbs.

A clinical diagnosis of definite ALS requires evidence of UMN and LMN degeneration in three separate anatomic regions, by clinical, and/or electrophysiological examination (Brooks et al., 2000). Loss of LMN can be assessed clinically using electromyography (EMG) (Mamede de Carvalho et al., 2005). UMN signs are more difficult to detect clinically, as they can be masked by LMN signs (Mamede de Carvalho, 2012). Transcranial magnetic stimulation, where available, may help in the identification of patients with subclinical UMN dysfunction (Attarian, Vedel & Pouget, 2008; Claus, Kerling, & Henschel, 1995). Neuroimaging (MRI) of the brain is typically used to exclude syndromes that mimic ALS, but have recently been used to identify
early UMN involvement in ALS (Agosta, Spinelli & Filippi, 2018; Mitsumoto et al., 2007; Kalra & Arnold, 2003).

### 1.2 Extramotor Manifestations of ALS

While the core feature of ALS is a relentless loss of motor function, behavioural and cognitive-linguistic changes in ALS are now recognized as a frequent feature of the disease (Goldstein & Abrahams, 2013). Up to 65% of ALS patients exhibit extramotor impairments consistent with frontotemporal dysfunction (Goldstein & Abrahams, 2013; Ringholz et al., 2005). Of these, 6–15% of individuals fulfill the diagnostic criteria for overt Frontotemporal Dementia (FTD), typically a behavioural variant (Montuschi et al., 2015; Trojsi et al., 2017; Raaphorst et al., 2010).

Based on the common co-occurrence of extramotor features in ALS and FTD, an ALS-FTD disease continuum has been described in recent years and consensus criteria have been proposed to categorize the various forms of cognitive and behavioural impairments associated with ALS (Swinnen & Robberecht, 2014; Strong, 2009; Strong et al., 2017). The extramotor presentation in ALS is heterogeneous with vast individual differences in the pattern and severity of impairments (Phukan, Pender & Hardiman, 2007; Taylor et al., 2013). Behavioural impairments are reported as mental rigidity, disinhibition, impulsivity, loss of insight, lack of foresight and planning, distractibility or apathy, and/or altered emotional expressiveness (Raaphorst et al., 2010).

Cognitive-linguistic deficits are described as changes in the domains of executive function, primarily verbal fluency, language, memory, and social cognition (Montuschi et al., 2015; Phukan, Pender & Hardiman, 2007; Goldstein & Abrahams, 2013; Girardi, Macpherson, & Abrahams, 2011; Beeldman et al., 2018). Executive dysfunction is the most commonly investigated cognitive domain in this population, with reports of executive dysfunction in up to one-third of non-demented ALS patients (Ringholz et al., 2005; Strong et al., 2009; Phukan et al., 2012). Recently, large population-based studies have found that language deficits are one of the most frequently occurring extramotor impairments in non-demented ALS cases, with a possibly higher prevalence rate than executive dysfunction (Abrahams 2013; Taylor et al., 2013; Pinto-Grau, Hardiman, & Pender, 2018). Most commonly, impairments in syntactic processing and comprehension have been detected in up to 72% of patients with ALS (Yoshizawa et al.,
2014a; Kamminga et al., 2016; Phukan et al., 2012). Other language deficits in this population include impairments in verb naming and action verb processing (Bak et al., 2001; Grossman, 2008; York et al., 2014; Papeo et al., 2015; Cobble, 1998), and errors in spelling (Ichikawa et al., 2008; Ferguson & Boller, 1977). These deficits have also been observed in ALS cases without detectable executive dysfunction (Consonni et al., 2013; Taylor et al., 2013; Ash et al., 2015), suggesting that language dysfunction may be an independent and prominent extramotor feature of ALS.

Ascertaining the development and type of extramotor changes in ALS is of direct importance as its presence has vast prognostic and implications for clinical care (Lomen-Hoerth et al., 2003; Elamin et al., 2011; Elamin et al., 2013), affects the design and outcome of pharmacological clinical trials (Gladman, Cudkowicz & Zinman, 2012; Leigh et al., 2004), and can contribute to our understanding of the underlying neural mechanisms and pathogenesis of the disease process. Currently, the identification of extramotor involvement is based on a brief screening assessment for global cognitive status (i.e., the Montreal Cognitive Assessment (MoCA, (Nasreddine et al., 2005)). Scores below a predetermined cut-off score indicate the need for further neuropsychological testing. Neuropsychological testing is limited in its sensitivity however, especially in a population with severe motor disability such as ALS (Gillingham et al., 2017). Recently, the use of in vivo structural MRI has become increasingly more popular in the assessment of extramotor pathology in the brain (Chang et al., 2005a; Bede et al., 2013; Turner, Agosta & Govind, 2012). Neuroimaging studies are reviewed below in Section 1.4 of this chapter.

1.3 Bulbar ALS

Bulbar ALS is a subtype of ALS that affects speech and swallowing functions. Although only 30% of patients present with bulbar symptoms at onset, more than 80% of spinal-onset ALS patients develop bulbar impairments with disease progression (Hillel & Miller, 1989; Ruoppolo et al., 2013). The pathophysiology of bulbar ALS is characterized by degenerative changes in the bulbar region of the primary motor cortex (bulbar PMC) and/or descending corticobulbar tracts, and cranial nerve motor nuclei (V, VII, IX, X, XII) in the pons and medulla oblongata. Clinically, the UMN changes to the bulbar PMC and corticobulbar tracts presents as pseudobulbar palsy, resulting in spasticity of the bulbar muscles, emotional lability, and a brisk
jaw jerk. Degeneration of the brainstem LMN results in a bulbar palsy with flaccid paresis, muscular atrophy, and tongue fasciculations (Kühnlein et al., 2008).

Dysarthria and dysphagia are the most common bulbar symptoms in ALS. Studies have reported dysarthria in 93% and dysphagia in 86% of patients with bulbar ALS (Carpenter 3rd, McDonald & Howard Jr., 1978; Chen & Garrett, 2005b). ALS patients usually present with a mixed dysarthria of spastic-flaccid type which is characterized by slow speech, imprecise consonants, marked hypernasality, and a strained/strangled voice (Kent et al., 2000). Additionally, decreased respiratory function can lead to a weak (soft, low volume) voice and abnormal vowel production can result in monopitch, short phrases, distorted vowels, monoloudness, and ‘breathy’ voice quality (Kent et al., 2000; Strong et al., 1996a). Specific clinical phenotypes (e.g., Primary Lateral Sclerosis or Kennedy’s disease) can present with pure spastic or flaccid dysarthria. Dysphagia in ALS is characterized by oropharyngeal weakness contributing to compromised airway safety and bolus inefficiency (Tabor et al., 2016).

Bulbar ALS is arguably one of the most devastating subtypes of the disease as the onset of bulbar signs and symptoms is associated with a faster rate of functional decline resulting in a shorter survival time (Haverkamp, Appel & Appel, 1995; Norris et al., 1993). The mechanisms underlying this worse prognosis in bulbar subtype is not clear, but can be partially explained by the associations between greater bulbar symptoms and an increased risk of aspiration pneumonia (Hardiman, 2011), which is one of the main causes of death in this population. Further, patients with ALS report detrimental effects of bulbar dysfunction on their overall quality of life (Bach, 2006; Hillemacher et al., 2004; Hecht et al., 2002; Mitsumoto & Del Bene, 2000). Dysarthria has been consistently associated with low mood, withdrawal from activities and social isolation (Tomik & Guiloff, 2010; Watts & Vanryckeghem, 2001), while dysphagia often leads to weight loss, complications of choking and/or malnutrition (Muscaritoli et al., 2012). Yet, bulbar ALS remains the least studied and understood subtype of ALS in terms of underlying neural correlates and objective markers for identification and tracking of bulbar disease.

Recent guidelines on the best practices for the evaluation of dysarthria, a motor speech disorder, in ALS consist of a the cranial nerve examination, sustained phonation, a diadochokinetic rate task, a spontaneous speech sample, and the reading of a short, standardized passage (Yunusova et al., 2019; Pattee et al., 2019). A careful neurological examination of cranial nerves is performed which focuses on providing clinical impressions of strength, range, and speed of
movements as well as symmetry of oral musculature (e.g., jaw, lips, tongue, soft palate) (Makkonen et al., 2016). Sustained phonation, oral reading, and conversational speech are used to evaluate voice quality (e.g., breathiness, harshness, etc), duration of phonation and speaking phrases, and speech loudness. Diadochokinetic (DDK) rate in syllable repetition tasks is used to evaluate articulatory rate and rhythm. Further, needle EMG of the genioglossus muscle can provide physiological evidence supporting bulbar LMN involvement (Finsterer & Mamoli, 1997; Lambert & Mulder, 1957).

Speech studies in ALS have demonstrated that objective time-based speech measures of bulbar dysfunction are more sensitive to to changes in disease severity and are better at detecting early changes and monitoring longitudinal changes than subjective assessments of dysarthria (Shellikeri et al., 2016; Rong et al., 2015b; Yunusova et al., 2019). These measures include articulatory rates, speech phrase and pause durations, and percent pause time (see Green et al., 2004; Yunusova et al., 2016). Furthermore, these measures appear to respond to pharmaceutical interventions such as dextromethorphan/quinidine (Nuedexta) therapy (Smith et al., 2017).

Recent work has shown that DDK rate showed changes earlier in the disease course than speaking rate (Rong et al., 2015b), which is the longstanding clinical gold-standard of measuring speech deterioration in ALS (Ball et al., 2002). DDK rate showed excellent sensitivity to early, even presymptomatic, changes of bulbar disease (Allison et al., 2017; Rong et al., 2019). Further, DDK rate changed in a linear manner with disease progression (Rong et al., 2015a) and was able to distinguish between patients with slow versus fast progressing disease (Rong, Yunusova & Green, 2015). Overall, DDK rate performance emerged as one of the most useful indices of bulbar motor dysfunction.

1.3.1 Bulbar ALS and Extramotor Impairments

Beyond the devastating motor consequences associated with bulbar ALS, neuropsychological investigations have indicated a link between bulbar ALS and a development of greater extramotor impairments. An association with bulbar-onset ALS has been demonstrated. Specifically, more severe impairments of working memory (Strong et al., 1999; Screiber et al., 2005), attention/inhibition (Ogawa et al., 2009; Schreiber et al., 2005) and verbal fluency (Lomen-Hoerth et al., 2003; Schreiber et al., 2005; Ringholz et al., 2005) were found in bulbar-onset patients compared to spinal-onset patients. Additionally, greater language deficits which include object naming (Portet et al., 2001), spelling (Ichikawa et al., 2008), sentence
comprehension (Yoshizawa et al., 2014), and grammar (syntactic errors) (Kamminga et al., 2016) have been also reported. Furthermore, one study found a higher prevalence of frontotemporal dysfunction early in the disease course of ALS in patients with bulbar-onset ALS compared to those with spinal-onset disease (Schreiber et al., 2005). However, the association between bulbar-onset and greater extramotor impairments has been questioned by other studies which failed to find differences between disease onset subtypes (Frank et al., 1997; Massman et al., 1996a; Ringholz et al., 2005; Rippon et al., 2006; Zalonis et al., 2012) or showed that an absence of bulbar signs was not a definite predictor of intact cognition (Strong et al., 1999).

In contrast to differences between onset subtypes, findings from other studies have suggested that extramotor impairments may be related to the presence of bulbar motor dysfunction regardless of an individual’s site of symptom onset. Sterling and colleagues (2010) found significantly reduced cognitive performance on a number of neuropsychological tests (e.g., Rey Copy Test assessing memory, attention, and working memory among other domains; Stroop Test assessing selective attention and cognitive flexibility) in dysarthric individuals irrespective of the onset type as compared to ALS patients without dysarthria. This relationship was maintained after controlling for motor impairment, indicating that the cognitive dysfunction was not an artifact of their motor dysfunction. Massman et al. (1996) observed that cognitive impairment was more prevalent in ALS patients with dysarthria (48.5%) than in those without it (27.4%). The association with dysarthria has also been debated, however. A number of studies failed to find an association between the presence or degree of bulbar motor disease and cognitive status (Gordon et al., 2010; Abrahams et al., 1996; Ringholz et al., 2005; Rippon et al., 2006; Robinson et al., 2006).

Lastly, some studies reported greater cognitive-linguistic deficits in patients with pseudobulbar palsy (i.e., corticobulbar neuronal damage) compared to patients with classic ALS, which suggests that extramotor cortical pathology may be especially related to UMN involvement in bulbar regions (Gallassi et al., 1989; David & Gillham, 1986; Abrahams, Goldstein, Pickering, et al., 1997; Abrahams et al., 2005).

Overall, the relationship between bulbar ALS and extramotor changes remains unclear and is complicated by the challenges of assessing cognitive function in a population with severe motor speech impairments (Gillingham et al., 2016; Strong et al., 1996b; Beeldman et al., 2016). Dysarthria can confound results of standardized neuropsychological tests, which require verbal
responses and are typically scored from timed tasks (see Abrahams & Bak, 2013). Further, neuropsychological testing may be inherently limited in its sensitivity to subtle subclinical impairments. Assessing the link neuroanatomically through direct investigations of the brain may help elucidate the nature of this relationship.

1.4 Structural Neuroimaging in ALS

Although neuroimaging currently plays only a supportive role in the diagnosis of ALS, it provides an ideal tool to explore objective measures of subclinical cortical involvement in a non-invasive way. It aims to detect extramotor pathology in the cortex, leading to emerging methods that are able to identify unique patterns of brain changes that are characteristic of ALS (Review: (Agosta, Spinelli & Filippi, 2018). One of the methods for assessing in vivo changes in the brain is through structural MRI of the cortex. Voxel-based and surface-based morphometry are widely-used methods that use high resolution 3D T1-weighted MR images to identify focal grey matter (GM) alterations. White matter (WM) integrity is most commonly evaluated by diffusion tensor imaging (DTI). Upper motor degeneration in ALS is characterized by cortical thinning and GM volume reduction in the PMC, and underlying WM abnormalities of the corticospinal and corticobulbar tracts (Shen et al., 2016; Foerster, Welsh, and Feldman 2013; Zhang et al., 2018).

Beyond the consensus on motor cortex involvement, many studies also detect multifocal frontotemporal and parietal GM and WM changes, and WM changes in the corpus callosum (Bede, Bokde, Byrne, et al., 2013; Bede, Bokde, Elamin, et al., 2013; Thivard et al., 2007; Abe et al., 2004; Agosta et al., 2007; Ciccarelli et al., 2009; Filippini et al., 2010; Sage et al., 2009; Sato et al., 2010). GM abnormalities have also been identified in subcortical structures such as the hippocampus (Bede, Bokde, Byrne, et al., 2013; Bede et al., 2013; Westeneng et al., 2016), amygdala (Christidi et al., 2018), thalamus (Bede et al., 2013; Thivard et al., 2007; Bede, Bokde, Byrne, et al., 2013; Chang et al., 2005b) as well as WM changes underlying these regions (Keil et al., 2012; Sage et al., 2009; Schuster et al., 2013; Sach et al., 2004; Sarica et al., 2014). Reports of changes in occipital (Thivard et al., 2007; Agosta et al., 2012; Mezzapesa et al., 2013; Mezzapesa et al., 2007) and cerebellar (Thivard et al., 2007; Christidi et al., 2018; Müller et al., 2016) regions are emerging. Further, the observed changes in extramotor cortical regions have been linked to structure-specific cognitive and behavioural impairments (Murphy, Henry &
Lomen-Hoerth, 2007; Menke et al., 2014). Both GM and WM extramotor changes have also been identified in patients without overt FTD (Christidi et al., 2018).

Recent connectivity-based analyses have provided evidence of cortical network degeneration in ALS as opposed to selective, focal GM and WM pathology (Verstraete et al., 2011; Douaud et al., 2011). An impaired “prefrontal-motor-subcortical” network with reduced structural connectivity to bilateral primary motor regions, bilateral supplementary motor regions, regions of the basal ganglia, the left hippocampus, right posterior cingulate, and right precuneus was observed, with changes predominantly localized around the motor cortex (Verstraete et al., 2011; Buchanan et al., 2015). Longitudinal investigations demonstrated a similar subnetwork of affected structural connections with a central onset site in the PMC and sequential progression to adjacent and non-adjacent connected regions in the frontal, temporal, and parietal lobes (Verstraete et al., 2014). However, despite the vast heterogeneity in ALS presentations, network-based characterization of ALS subtypes has not yet been investigated. With the ultimate goal in ALS research being to design therapy that effectively stops disease progression, understanding disease spread within the brain between subtypes is crucial to this quest. Further, characterizing disease patterns of ALS subtypes may aid in improved understanding of disease pathogenesis, potentially explain the wide variability in motor and extramotor features between patients, and provide important diagnostic and prognostic information (Ravits & La Spada, 2009).

1.5 Neuropathology in ALS

A neuropathological diagnosis of ALS is confirmed through post-mortem examination of the cortex, brainstem, and spinal cord (El Escorial, 2000). At macroscopic examination, the evidence of atrophy of the precentral gyrus and spinal cord is expected (Ellison, 2012). Microscopy typically reveals pathology consistent with neurodegenerative changes, characterized by neuronal loss and concomitant gliosis, as well as the presence of intraneuronal cytoplasmic misfolded protein inclusions seen in 80% of cases (Arai et al., 2006). Histological changes are seen in the PMC, anterior horn cells of the spinal cord, and the brainstem motor nuclei (Esiri, Squier, & Perl, 2006). Small eosinophilic inclusions are observed in the surviving LMN, known as Bunina Bodies (Okamoto, 1993).
Twenty five to 50% of ALS cases also show co-existing Frontotemporal Lobar Degeneration (FTLD), the neuropathological correlate of FTD, characterized by degenerative changes in frontal and temporal lobes (Seelaar et al., 2011). FTLD in ALS is characterized by similar degenerative changes in extramotor frontal and temporal lobes (Mackenzie & Feldman, 2003; Okamoto et al., 1991). Cortical superficial spongiosis affecting the first and second cortical layers is a common pathological marker in FTD (Wilson et al., 2001) but is also observed in these ALS-FTD cases (Yoshida, 2004).

The phosphorylated 43-kDa TAR DNA-binding protein (TDP-43) was identified as the major component in ubiquitin-positive inclusions found in the surviving neurons and glia in sporadic ALS (Neumann et al., 2006). TDP-43 aggregates are also commonly seen in FTLD associated with FTD phenotypes (Neumann et al., 2006; Arai et al., 2006), further supporting an ALS-FTD disease continuum. The severity and distribution of TDP-43 in the brain has been shown to be well-correlated with antemortem cognitive profiles, giving insight into the phenotypic presentations of the disease and representing a clinicopathologic spectrum that ranges from pure motor neuron disease to FTD (Cykowski et al., 2017; Strong & Yang, 2011). Recently, a large-N autopsy study suggested a sequential regional staging pattern of TDP-43 proteins within the cortex (Brettschneider et al., 2013). TDP-43 pathology appears to disseminate from a focal site of onset within the PMC and spreads rostrally and caudally towards the spinal cord and brainstem, and then to frontal, parietal, and temporal lobes at later stages, mirroring the structural MRI findings of an expanding network within the cortex.

1.6 Current Understanding of Bulbar ALS Neuroanatomy

Our current understanding of the neural correlates underlying bulbar ALS is severely limited and mainly derived from neuroimaging investigations. Most existing structural MRI studies in bulbar ALS are focused mainly on the motor system and have reported bilateral GM volume reduction and cortical thinning of the bulbar PMC (Bede et al., 2013; Schuster et al., 2013; Schuster, Kasper, Machts, et al., 2014), and reduced fractional anisotropy of CBT (Aoki et al., 2005; Iwata, Aoki & Masutani, 2005) and corticospinal tracts (Agosta et al., 2009; Judith & Julio, 2014; Ellis et al., 1999; Prell et al., 2013) in patients with bulbar-onset ALS compared to spinal-onset ALS. These anatomical changes were moderately associated with the severity of bulbar motor symptoms (Menke et al., 2014; Roccatagliata et al., 2009; Schuster et al., 2013). Few studies have linked bulbar ALS to cortical extramotor neuroanatomy (beyond the PMC).
reporting thinning of the left inferior cingulate, left inferior frontal gyrus, right insula, and right anterior and middle temporal gyri in relation to increased severity of bulbar symptoms (Chen, Liu & Ma, 2018; Schuster, Kasper, Machts, et al., 2014; Verstraete et al., 2012). The characterization of bulbar dysfunction in these neuroimaging investigations was restricted to the bulbar subscore on the ALS Functional Rating Scale-Revised (ALSFRS-R) (Cedarbaum et al., 1999), which may not be sensitive to early and subtle changes in bulbar function (Allison et al., 2017).

Another method of establishing neuroanatomical correlates is through post-mortem examination of the distribution of pathology within the brain. An existing large sample autopsy study found that bulbar-onset ALS was statistically associated with greater temporal lesions, temporal inclusions and neostriatal inclusions (Piao et al., 2003). Dystrophic neurites were found to be associated with dementia and bulbar-onset subtype. The existing investigation however was restricted to the temporal lobe and PMC within the cortex. Overall, there is an imminent need to further characterize the neuroanatomy of bulbar ALS by examining hypothesis-specific regions in the brain that may be vulnerable in bulbar disease.

1.7 Neuroanatomy of Speech Production

The act of speaking is commonly defined as the act of expressing concepts, feelings, and/or perceptions through the articulation of words. Speech and language, as used in the scientific literature, are not synonymous: Speech production concerns the process of taking a linguistic message and articulating it via the vocal tract, whereas language encompasses the linguistic meaning – sound contrast (phonology), word content (semantics), form (grammar), and use (pragmatics) of the utterance (Bloom & Lahey, 1978). The specific processes underlying speech motor control consist of motor planning, programming, initiation and neuromuscular execution of speech output (Duffy, 2013).

The neuroanatomy of speech and language has been a topic of intense investigation for over 130 years. The “classic model”, often referred to as the “Broca-Wernicke-Geschwind model” originated in the late 19th century and was based on observations of brain lesions and their associated behavioural consequences. It was composed of an anterior inferior frontal area, referred to as “Broca’s area”, a posterior temporal area, referred to as “Wernicke’s area”, and a single white-matter pathway connecting the two called the arcuate fasciculus (Fedorenko,
Duncan & Kanwisher, 2012; Lichtheim, 2009; Broca, 1861; Wernicke & Eggert, 1874). Since then, advances in brain imaging and brain stimulation techniques have contributed tremendously to uncovering the sophisticated neural system that underlies the production and processing of speech and language (Burton & Small, 2006; Hickok & Poeppel, 2000; Scott & Johnsrude, 2003; Sörös et al., 2006; Lotze et al., 2000; Geranmayeh et al., 2014).

Among speech production models, the Directions Into Velocities of Articulators (DIVA) computational model provides the most detailed and thoroughly tested account of the neural processes underlying speech motor control (Guenther, Ghosh & Tourville, 2006a; Guenther 1994; Golfinopoulos, Tourville & Guenther, 2010; Bohland, Bullock & Guenther, 2010). Speech production begins in the model with the activation of a “speech sound map cell” in left ventral premotor and adjacent inferior frontal gyrus (IFG). Information is then projected in a feedforward loop to the “articulator velocity map cells” in the ventral primary motor cortex (bulbar PMC). In addition to its projections to the feedforward control loop, the “speech sound map” also projects to auditory and somatosensory “target and error maps”, currently hypothesized to lie in the posterior superior temporal gyrus (pSTG) and the ventral supramarginal gyrus. The sensory “error maps” receive inputs from sensory “state maps” in auditory and somatosensory cortex. The auditory “state map” is hypothesized to lie along Heschl’s gyrus, a region associated with the primary auditory cortex (PAC) in the transverse temporal gyrus, and the somatosensory “state map” is distributed along the ventral postcentral gyrus (bulbar PSC). The forward control also involves subcortical structures, like the basal ganglia and cerebellum. Although parts of the model remain speculative, it accounts for a wide range of kinematic, acoustic, and neuroimaging data collected during speech production (Guenther, Ghosh & Tourville, 2006a). Together, the existing framework provides a set of connected motor and extramotor regions that are involved in speech production and processing, hereby referred to as the speech network (SpN).

Due to the high occurrence of speech production deficits in ALS as a result of bulbar dysfunction (Carpenter 3rd, McDonald & Howard Jr., 1978; Chen & Garrett, 2005a) and the frequently reported involvement of cerebral networks in ALS (Trojsi et al., 2018b), an impairment of SpN in bulbar ALS is highly likely. Further, preferential involvement of SpN in bulbar ALS may explain the reported association between bulbar symptoms and cognitive-linguistic deficits that are functionally mapped to selected SpN regions (e.g., IFG and pSTG).
Establishing the neural underpinnings of bulbar ALS is essential for the development of objective markers of bulbar disease identification and tracking. The work has significant implications for understanding disease pathogenesis, particularly for its most debilitating subtype – bulbar ALS, which has significant clinical and research implications.

1.8 Dissertation Research

The overall goal of this work was to further our understanding of bulbar ALS and its neuroanatomical underpinnings. Specifically, changes in cortical SpN regions were examined in bulbar ALS through in vivo structural neuroimaging and post-mortem neuropathology methods.

1.8.1 Dissertation Overview: Specific Studies and Hypotheses

In the first study (Chapter 2 of the dissertation), we conducted a region-of-interest structural MRI study examining GM and WM changes in cortical regions part of the SpN. We compared volumetric, surface-based and DTI metrics of the SpN regions in 16 patients with varying degrees of bulbar motor disease and 19 healthy controls. Associations between structural neuroanatomy and clinical measures of bulbar motor, limb motor, and disease severity were examined. We hypothesized that SpN regions would show GM and WM abnormalities in ALS when compared to controls and structural integrity of SpN regions would be associated with bulbar motor dysfunction, but not with limb or disease severity.

In the second study (Chapter 3), we conducted a systematic review of existing literature on neuropathology of the brain and brainstem in ALS and compared the anatomical distribution of pathology and histological features in published data between bulbar-onset ALS and spinal-onset ALS cases. We hypothesized that bulbar-onset cases would differ from spinal-onset cases with greater changes in regions part of the SpN; histological features between the two subtypes would be similar, however.

The third study (Chapter 4) examined post-mortem neuropathological changes of regions part of the SpN in cases with bulbar-onset ALS (bALS), spinal-onset ALS with antemortem bulbar dysfunction (sALSwB), spinal-onset ALS without antemortem bulbar dysfunction (sALSnoB), and controls. We hypothesized that SpN regions would show greater degenerative changes in
cases with antemortem bulbar dysfunction (bALS and sALSwB) than cases with preserved bulbar function (sALSnoB).

In Chapter 5, the overall work is summarized in the context of the existing knowledge on bulbar ALS and pathogenesis of disease, and the outlook for future research is discussed.

1.8.2 Submitted and Accepted Publications of the Dissertation

The first study (Neuroimaging, Chapter 2 of the dissertation) has been published by Taylor & Francis in Amyotrophic Lateral Sclerosis and Frontotemporal Dementia. Permission to reprint has been granted. The full citation is as follows:


The second study (Systematic Review, Chapter 3 of the dissertation) has been published by Elsevier in Neuroscience and Biobehavioral Reviews. Permission to reprint has been granted. The full citation is as follows:


The third study (Neuropathology, Chapter 4 of the dissertation) has been submitted to Journal of Neuropathology and Experimental Neurology by Oxford Academic. Permission to reprint has not been requested. It will be requested, should the study be accepted for publication before dissertation submission deadline.
Chapter 2
Speech Network Regional Involvement in Bulbar ALS: A Multimodal Structural MRI Study

This is an Accepted Manuscript of an article published by Taylor & Francis in Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration on April 26th, 2019, available online: http://www.tandfonline.com/10.1080/21678421.2019.1612920.

2.1 Abstract

OBJECTIVE: To examine grey (GM) and white matter (WM) structural changes in regions of the speech network (SpN) in ALS patients with varying degree of bulbar disease. METHODS: T1 and DTI images were obtained for 19 ALS participants and 13 neurologically-intact controls. Surface-based, volumetric, and DTI metrics were obtained for 6 regions-of-interest (ROIs) including the primary motor cortex (PMC), pars triangularis (parsT), pars opercularis (ParsO), posterior superior temporal gyrus (pSTG), and transverse temporal (TT). Disease-effects and brain-behavioural correlates between neuroanatomy and clinical measures of bulbar, limb, and overall disability were examined using linear models. RESULTS: Structural changes were observed in the right oral and limb PMC and left ParsT, TT, and pSTG in ALS. Bulbar motor dysfunction was associated with WM abnormalities in the right oral PMC and left pSTG, and GM changes in bilateral TT. In contrast, symptom progression rate predicted GM and WM changes in bilateral pars opercularis (part of Broca’s area). Grip strength and disease duration models were non-significant. CONCLUSIONS: The findings suggested that regions of the left-dominant SpN may be implicated in ALS and degeneration of these areas are related to bulbar disease severity. Involvement of regions that overlap across multiple connectomes such as Broca’s area, however, may be dependent on the rate of disease progression. The work contributes to our understanding of the bulbar ALS subtype, which is crucial for predicting disease progression, delivering targeted clinical care, and appropriate recruitment into clinical trials.

2.2 Introduction

ALS is a multisystem neurodegenerative disease with motor and extramotor involvement (Strong et al., 2009; Strong et al., 2017). In addition to upper and lower motor neuron deterioration affecting spinal and bulbar musculature, nearly 50% of all patients present with
cognitive impairments encompassing domains of executive function (Flaherty-Craig et al., 2006; Kew et al., 1993; Strong et al., 1999), language (Abrahams, Leigh & Goldstein, 2005; Abrahams et al., 2000; Ichikawa et al., 2012; Yoshizawa et al., 2014b), memory (Gordon et al., 2010; Phukan, Pender & Hardiman, 2007) and behaviour (Abrahams et al., 2014; Elamin et al., 2017). Among these, language deficits—characterized by impairments in syntactic processing and comprehension, naming, and spelling (see review: Pinto-Grau, Hardiman & Pender, 2018)—may be the most prominent feature of non-demented ALS presentation (Abrahams 2013; Leslie et al., 2015; Phukan et al., 2012; Taylor et al., 2013; Tsermentseli et al., 2016). A number of neuropsychological investigations have proposed that bulbar motor signs and symptoms (i.e., dysarthria) may be associated with an increased burden of extramotor deficits (Massman et al., 1996b; Neary, Snowden & Mann, 2000; Ogawa, Tanaka & Hirata, 2009; Sterling et al., 2010), suggesting a link between motor and extramotor deterioration, tied by the notion of neural networks (Bak & Chandran, 2012a; Buchanan et al., 2014; Verstraete et al., 2014). The studies to date however are limited, and there is a need for direct assessment of neuroanatomy in patients with bulbar ALS to explore the potential structural underpinnings of the disease process.

Existing MRI studies investigating neuroanatomical changes in bulbar ALS have focused mainly on the motor system and have reported bilateral grey matter (GM) volume reduction and cortical thinning of the ventral precentral gyri (i.e., oral portion of the primary motor cortex (oral PMC)) (Bede, Bokde, Elamin, et al., 2013; Schuster et al., 2013; Schuster, Kasper, Dyrba et al., 2014) and reduced fractional anisotropy (FA) of corticobulbar (Iwata et al., 2005; Aoki et al., 2005) and corticospinal white matter (WM) tracts (Agosta et al., 2009; Cardenas-Blanco et al., 2014; Ellis et al., 1999; Prell et al., 2013) in patients with bulbar-onset as compared to patients with spinal-onset ALS. These anatomical changes were moderately associated with the severity of bulbar motor symptoms (Menke et al., 2014; Luca Roccatagliata et al., 2009; Schuster et al., 2013). Few studies have linked bulbar ALS to extramotor neuroanatomy reporting thinning of the left inferior cingulate, left inferior frontal (IFG), right insula, and right anterior and middle temporal gyri in relation to increased severity of bulbar symptoms (Chen, Liu & Ma, 2018; Schuster, Kasper, Dyrba et al., 2014; Verstraete et al., 2012). However, investigations were performed using “whole-brain” analyses, which did not allow examination of hypothesis-specific regions-of-interest (ROIs). Further, the characterization of bulbar dysfunction in these studies was restricted to the bulbar subscore on the ALS – Functional Rating Scale – Revised
ALSFRS-R), which may not be sensitive to early and subtle changes in bulbar function (Allison et al., 2017).

A rapidly growing body of neuroimaging literature on speech function in neurologically intact individuals and lesion-based examinations have identified key motor and extramotor cortical regions responsible for the production and processing of meaningless syllables, sentences, and complex utterances of real-life speech, together forming a densely-connected specialized network, hereafter referred to as the speech network (SpN) (Bohland & Guenther, 2006b; Dronkers et al., 2004; Guenther, Ghosh & Tourville, 2006b; Bohland & Guenther, 2006a; Fuertinger, Horwitz & Simonyan, 2015). The SpN includes the oral PMC, as well as frontal, temporal, and parietal extramotor regions including, bilaterally, the ventral post-central gyrus (i.e., “oral” somatosensory cortex), supplementary motor and premotor areas in the prefrontal cortex, primary auditory cortex (i.e., Heschl’s gyrus of the transverse temporal cortex), posterior superior temporal gyrus (pSTG), parietal-temporal junction (i.e., area SPT (Hickok, Okada & Serences, 2009)), angular cortex (i.e., Geschwind’s area), cingulate cortex, and IFG (both, pars triangularis and pars opercularis) (Bohland & Guenther, 2006b; Hickok, 2001). On account of the high occurrence of bulbar dysfunction affecting speech production in ALS and the reported link between bulbar ALS and cognitive-linguistic impairments functionally mapped to selected SpN areas, patients with bulbar disease may present with increased SpN changes.

The main objective of this work was to examine structural changes in motor and extramotor regions of the SpN in relation to bulbar ALS. Specific objectives of this study were to: (1) examine GM and WM changes in SpN regions in patients with bulbar motor disease; and (2) evaluate associations between MRI metrics of SpN regions and clinical measures of bulbar motor, spinal motor, and overall disease severity. We hypothesized that both motor and extramotor SpN regions would be implicated in patients with bulbar ALS, and the severity of degenerative changes will correlate with bulbar motor disease but not with spinal or overall disease severity.

2.3 Materials and Methods

2.3.1 Participants

A total of 16 patients with ALS (Brooks et al., 2000) and 19 sex-, age- and education-matched neurologically-intact controls (HC) participated in this study. All participants were native
English speakers and right-hand dominant. Patients were excluded if they presented with additional neurological diagnoses, showed a forced vital capacity below 80%, or tested positive for any known genetic mutations (i.e., C9ORF72). Further, only those with normal hearing and without evidence of overt dementia, as determined by a minimum score of 26 on the Montreal Cognitive Assessment (Nasreddine et al., 2005) were included. An extended neuropsychological assessment was performed with patients and controls (see Gillingham et al., 2017 for details), but the presence of cognitive deficits, detected with a novel computerised battery, was not a variable of interest for this study.

2.3.2 Procedures

2.3.2.1 Clinical Protocol

A neurological assessment of upper motor neuron (UMN) integrity was conducted by an experienced neurologist (Author LZ). The upper motor neuron (UMN) score (/10) was calculated based on the assessment of increased tone (0=normal, 1=increased), exaggerated reflexes (0=absent, 1=reduced; 2 = normal, 3= brisk, 4= very brisk), pseudobulbar affect (0=normal, 1=present), and spastic dysarthria (0= normal, 4=severe). None of the patients presented with apraxia of speech, as determined by an experienced speech-language pathologist (Author YY).

The effect of overall motor impairment on daily functions was assessed using the ALSFRS-R (Cedarbaum et al., 1999). Disease progression rate was calculated as (48 – ALSFRS-R total score/ disease duration).

A dysdiadochokinetic (DDK) rate test was conducted to assess bulbar dysfunction. This measure was chosen to index bulbar dysfunction over the more typically used ALSFRS-R bulbar subscore as recent studies have demonstrated its excellent sensitivity to early detection of bulbar disease (Allison et al., 2017) and linear decline over time (Rong et al., 2015a). The participants were asked to repeat syllable /ta/ as clear and as fast as possible on one breath; the number of syllables per second was calculated (SPS).

Left and right hand grip strength was quantified measuring the amount of static force with a hand held dynamometer. Strength was normalized to body weight (kg/BMI) to remove sex and age effects (Beck et al., 1999; Sevene et al., 2017). The highest force across three trials was used for analysis and represented the primary measure of limb dysfunction (Beck et al., 1999).
2.3.2.2 MRI Protocol

All imaging and clinical data were collected either on the same day or, if not possible, within not more than 2 weeks of each other. 3D T1-weighted, PD-T2 and diffusion tensor imaging (DTI) data were acquired on a 3T Philips Achieva Medical Scanner (Philips Medical Systems, Best, Netherlands). For T1-weighted images, 186 axial slices were obtained using the following parameters: 25 ms TR, 1.99 ms TE, 30° flip angle, field-of-view (FOV) 240x240x130 mm, isometric voxel size of 1x1x1 mm, no inter-slice gap. Interleaved dual-echo spin-echo PD and T2 images were acquired axially with the following parameters: 54 slices, 2500 ms TR, 11 and 102 ms TE, FOV 224x224x120 mm, in plane resolution of 1x1x1.5 mm, no gap. Diffusion weighted spin echo, echo planar images (EPI) were acquired with a standard head coil for signal reception. Eighty-six axial slices were obtained using the following parameters: 35812 ms TR, 70 ms TE, 90° flip angle, FOV 224x224x120 mm, in plane resolution of 1.4x1.4x1.4 mm, no gap. Diffusion weighting was performed along 36 optimized non-collinear directions. A single b value of 1000 s/mm² was applied. A reference image with no diffusion weighting was also obtained (b0 image).

2.3.2.3 MRI Data Processing

Brain extraction was accomplished using a previously-described procedure that used PD-T2 images to modify the initial skull-stripping to effectively minimize potential segmentation errors due to focal atrophy (Kovacevic et al., 2002; Ramirez et al., 2011). MRI-derived tissue segmentation for GM, WM, cerebrospinal fluid (CSF), ventricular CSF, and WM hyperintensities of presumed vascular origin (WMH) were obtained using a comprehensive, previously published, and rigorously validated image processing pipeline called SABRE (Kovacevic et al., 2002; Ramirez et al., 2011; Ramirez, Scott & Black 2013). The DTI data were corrected for motion and eddy-current distortions using the nonlinear registration tool provided in the FMRIB software library (www.fmrib.ox.ac.uk/fsl).

Using FreeSurfer’s automated cortical parcellation, each hemisphere was segmented into 34 anatomical regions (Desikan et al., 2006) (V.5.0.0, http://surfer.nmr.mgh.harvard.edu/). ROIs corresponding to the bulbar and limb areas of the PMC and the posterior superior temporal gyrus (pSTG) (i.e., Wernicke area) were determined using a custom semi-automatic partitioning method developed for this study (see Appendix A: Neuroimaging Supplementary Material). The bulbar and limb PMC ROIs were identified with excellent inter-rater reliability and clinically
validated with moderate to strong associations with clinical measures of motor dysfunction (Appendix A: Neuroimaging Supplementary Material).

Using Freesurfer’s automated surface-to-volume WM parcellation, WM volumes underlying each cortical ROI were automatically labeled according to the surface label of the nearest cortical voxel (Salat et al., 2009). The volumes did not include WM from the centrum semiovale and periventricular regions (Salat et al., 2009). The WM labels were then used as masks to extract intensity information from the DTI maps for each WM ROI.

2.3.2.4 MRI Measures

Surface-based and volumetric measures were obtained using Freesurfer for each GM ROI, as they previously showed high sensitivity to disease-related GM changes in ALS (Turner et al., 2012; Grosskreutz et al., 2006; Lule et al., 2005; Turner et al., 2007; Verstraete et al., 2010, 2012). Specifically, GM changes were assessed using measures of cortical thickness (mm) (Fischl 2012), surface area (mm²), and GM volume (mm³) (Dale, Fischl & Sereno, 1999). To assess WM integrity, a diffusion tensor model was fitted at each voxel of the subcortical WM volumes and eigenvalue (λ1, λ2, λ3) maps were generated to obtain measures of anisotropy and diffusivity. DTI metrics included average values of fractional anisotropy (FA), axial diffusivity (AD), radial diffusivity (RD), and mean diffusivity (MD) per WM ROI.

2.3.2.5 Regions-of-Interest (ROIs)

Select bilateral motor and extramotor regions part of the SpN were chosen a priori. Regions with strong structural connectivity to the oral PMC were chosen for analysis (Vassal et al., 2016). In addition, the medial PMC was also included to examine limb-related motor changes. Figure 2-1 shows the six cortical ROIs (and 6 corresponding WM ROIs) examined in this study.

The WM ROIs subcortical to the bulbar and limb PMC included portions of the corona radiata, and corticobulbar and corticospinal tracts, respectively. WM subcortical to parsO, ParsT, TT, and pSTG may include terminal fibers of the arcuate/ superior longitudinal, uncinate, inferior fronto-occipital, and middle longitudinal fasciculi (see Friederici 2009).
Figure 2-1. A 3D volume surface-rendered left hemisphere cerebral cortex showing the six cortical regions-of-interest (ROIs) chosen for analysis *a priori*. Six additional WM ROIs subcortical to each cortical ROI were also included. PMC = Primary Motor Cortex. Identical ROIs were analyzed for the opposite hemisphere.
2.3.3 Statistical Analyses

Analyses were conducted using SPSS Statistics v. 23 (for Windows, Version 19.0). Estimated total intracranial volume (eTIV) served as a fixed covariate for all volumetric and surface area models. All DTI indices are reported as raw values multiplied by 1000. All models were tested against an α level of .05.

GM and WM changes in ROIs were examined using univariate linear regression models. Group differences between ALS and HC were analyzed for all GM and WM ROIs per hemisphere. Age and sex were not included as covariates for any GM models as the two groups were age- and sex- matched for all T1-derived metrics in this dataset. DTI images were not usable for 6 HC; for these WM models, both age and sex were included as covariates to control for variance introduced by differences in demographics between the two groups.

Associations were examined between MRI metrics for each ROI and clinical measures of bulbar, limb, and overall motor disability using univariate linear regression models. A main predictor of DDK rate (SPS) to represent bulbar motor dysfunction, composite scores of left and right grip strength (kg/BMI) to represent limb motor dysfunction, and ALSFRS-R scores to represent overall disease severity were used. Associations with disease progression rates were also included, as previous studies have reported a link between faster rates and increased burden of extramotor impairments (Elamin et al., 2013; Gordon et al., 2010). Sex and age were both included as fixed covariates.

2.4 Results

Participant demographics and disease-related clinical information are presented in Table 2-1.
Table 2-1. Demographic information and disease-related clinical information for ALS and neurologically-intact healthy control (HC) participants. * = significant group differences between ALS and HC as determined by Student’s T-test at alpha = .05; Data is reported as medians (IQR). † = Disease duration was defined as the time period in months between patient-reported symptom onset and date of testing; M = males; F = females; Bi = bilateral; R = Right; L = Left; NR = Not Reported; NA = Not Applicable. ALSFRS-R = Revised Amyotrophic Lateral Sclerosis Functional Rating Scale; NA = Not Applicable; DDK = Diadochokinetic; SPS = Syllables per second.

<table>
<thead>
<tr>
<th>Measure</th>
<th>ALS (N = 16)</th>
<th>HC (N=19)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n, Sex, M/F</td>
<td>7/9</td>
<td>9/10</td>
</tr>
<tr>
<td>Age (years)</td>
<td>61 (57.75)</td>
<td>65 (60)</td>
</tr>
<tr>
<td>Years of Education</td>
<td>14 (13)</td>
<td>14 (13.75)</td>
</tr>
<tr>
<td><strong>Clinical Information</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n, Site (Side) of Onset</td>
<td>7 Arm (1Bi, 1R, 1L, 4NR)</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>7 Leg (4R, 2L, 1NR)</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>2 Bulbar</td>
<td></td>
</tr>
<tr>
<td>Disease duration (months)†</td>
<td>37.5 (20)</td>
<td>NA</td>
</tr>
<tr>
<td>ALSFRS-R, total (/48)</td>
<td>40 (35.75)</td>
<td>NA</td>
</tr>
<tr>
<td>Progression rate (unit/month)</td>
<td>.29 (.16)</td>
<td>NA</td>
</tr>
<tr>
<td>Total UMN score (/10)</td>
<td>3.5 (3)</td>
<td>NA</td>
</tr>
<tr>
<td>DDK Rate (SPS)</td>
<td>5.21 (3.61)</td>
<td>5.27 (3.95)</td>
</tr>
<tr>
<td>*Grip Strength Index (kg/BMI)</td>
<td>*L = .48 (.20)</td>
<td>L = .97 (.69)</td>
</tr>
<tr>
<td></td>
<td>*R = .70 (.23)</td>
<td>R = 1.16 (.68)</td>
</tr>
</tbody>
</table>
2.4.1 Neuroanatomical Regional Differences in ALS

Compared to HC, the ALS group presented with reduced GM volume of the right limb PMC, and cortical thinning in the right oral PMC and the left parsT. Left hemispheric white matter changes were observed for the fibers underlying the TT and pSTG (see Table 2-2).
Table 2-2. Results of the univariate linear regressions showing group differences in regional GM and WM between ALS (served as reference group) and neurologically intact healthy control controls (HC). †= adjusted for eTIV; ^= adjusted for age and sex. Only statistically significant results are shown. GM = Grey matter; PMC = Primary Motor Cortex; ParsT = Pars Triangularis; TT = Tranverse Temporal gyrus; pSTG = Posterior Superior Temporal Gyrus; R = Right; L = Left.

<table>
<thead>
<tr>
<th>Region, Hemisphere</th>
<th>ALS (Mean, SD)</th>
<th>Controls (Mean, SD)</th>
<th>β</th>
<th>t (df; regression, residual)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MRI measures ~ Group</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>GM Volume (mm³)†</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limb PMC, R</td>
<td>4043.87, 872.02</td>
<td>4713.12, 614.96</td>
<td>486.22</td>
<td>2.17 (2, 29)</td>
<td>.04</td>
</tr>
<tr>
<td><strong>Cortical Thickness (mm)†</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral PMC, R</td>
<td>2.33, .14</td>
<td>2.40, .15</td>
<td>.13</td>
<td>2.39 (1, 25)</td>
<td>.03</td>
</tr>
<tr>
<td>ParsT, L</td>
<td>2.30, .15</td>
<td>2.41, .09</td>
<td>.12</td>
<td>2.70 (1, 32)</td>
<td>.01</td>
</tr>
<tr>
<td><strong>AD^</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT, L</td>
<td>.61, .07</td>
<td>.63, .05</td>
<td>-.05</td>
<td>-2.23 (3, 23)</td>
<td>.04</td>
</tr>
<tr>
<td><strong>MD^</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT, L</td>
<td>.40, .03</td>
<td>.38, .02</td>
<td>-.02</td>
<td>-2.40 (3, 23)</td>
<td>.03</td>
</tr>
<tr>
<td><strong>RD^</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pSTG, L</td>
<td>.24, .03</td>
<td>.22, .03</td>
<td>-.02</td>
<td>-2.17 (3, 23)</td>
<td>.04</td>
</tr>
</tbody>
</table>
2.4.2 Associations between Neuroanatomy and Motor Dysfunction

2.4.2.1 Associations with DDK Rate

DDK rate was associated with WM changes underlying the right oral PMC and the left pSTG. Slower DDK rate was also associated with reduced surface area and GM volume of bilateral TT (See Table 2-3). Figure 2-2 shows the predicted values for surface area and GM volume of bilateral TT based on DDK rate.

Table 2-3. Results of the univariate linear regressions predicting GM and WM changes based on DDK rate (SPS). †= adjusted for age, sex, and eTIV; ^= adjusted for age and sex. Only statistically significant results are shown.

<table>
<thead>
<tr>
<th>Region, Hemisphere</th>
<th>β</th>
<th>p-value</th>
<th>Adjusted R²</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Surface Area (mm²)†</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT, R</td>
<td>40.20</td>
<td>.02</td>
<td>.61</td>
</tr>
<tr>
<td>TT, L</td>
<td>38.004</td>
<td>.049</td>
<td>.37</td>
</tr>
<tr>
<td><strong>GM Volume (mm³)†</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT, R</td>
<td>64.40</td>
<td>.03</td>
<td>.78</td>
</tr>
<tr>
<td>TT, L</td>
<td>88.56</td>
<td>.02</td>
<td>.50</td>
</tr>
<tr>
<td><strong>FA ^=</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral PMC, R</td>
<td>.02</td>
<td>.007</td>
<td>.60</td>
</tr>
<tr>
<td><strong>AD ^=</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pSTG, L</td>
<td>.013</td>
<td>.03</td>
<td>.23</td>
</tr>
<tr>
<td><strong>RD ^=</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral PMC, R</td>
<td>-.012</td>
<td>.045</td>
<td>.37</td>
</tr>
</tbody>
</table>
Figure 2-2. Scatterplots showing predicted values (adjusted for age, sex, and eTIV) for surface area (mm$^2$; left panel) and GM volume (mm$^3$; right panel) of the TT bilaterally, based on DDK rate (SPS) for the ALS group.
2.4.2.2 Associations with Grip Strength and ALSFRS-R

MRI measures were not significantly associated with composite grip strength (left + right) or ALSFRS-R scores.

2.4.2.3 Associations with Disease Progression Rate

Faster progression rates were associated with greater cortical thinning of bilateral parsO and increased MD and RD of WM underlying the left ParsO (See Table 2-4).

Table 2-4. Results of the univariate linear regressions predicting GM and WM changes based on disease progression rate (units/month). †= adjusted for age, sex, and eTIV; ^ = adjusted for age and sex. Only statistically significant results are shown.

<table>
<thead>
<tr>
<th>Region, Hemisphere</th>
<th>β</th>
<th>p-value</th>
<th>Adjusted R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRI measures~ Disease progression rate (units/month)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortical Thickness (mm) †</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>parsO, L</td>
<td>-.30</td>
<td>.02</td>
<td>.26</td>
</tr>
<tr>
<td>parsO, R</td>
<td>-.34</td>
<td>.03</td>
<td>.40</td>
</tr>
<tr>
<td>MD^</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ParsO, L</td>
<td>.05</td>
<td>.02</td>
<td>.50</td>
</tr>
<tr>
<td>RD^</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ParsO, L</td>
<td>.06</td>
<td>.01</td>
<td>.62</td>
</tr>
</tbody>
</table>

2.5 Discussion

2.5.1 Summary of Findings

This study examined GM and WM changes in regions associated with speech network (SpN) in patients with ALS and varying degree of bulbar disease. Group differences were found in the right oral and limb PMC and left extramotor regions of ParsT, TT, and pSTG. Correlational analyses revealed that bulbar motor dysfunction was associated with WM abnormalities in the right oral PMC and left pSTG, and GM changes in bilateral TT. In contrast, symptom progression rate was associated with GM and WM changes in bilateral pars opercularis (part of
Broca’s area). These findings suggested that extramotor regions that are part of the SpN might be implicated in ALS and degeneration of these areas may relate specifically to bulbar disease severity and symptom progression rates, but not to overall disability or limb weakness.

2.5.2 Left-Dominant Speech network Vulnerability in ALS

Our findings support the existing “whole-brain” studies reporting frontal and temporal lobe changes in ALS including bilateral involvement of the motor cortex and extramotor regions (Turner, Agosta & Govind, 2012; Agosta et al., 2007; Bede, Bokde, Elamin, et al., 2013; Canu et al., 2011; Chang et al., 2005a; Chen, Liu & Ma, 2018; Ciccarelli, 2009; Kassubek et al., 2005; Keil et al., 2012; Mezzapesa et al., 2007). In our sample, only the right PMC – both oral and limb regions – showed group differences, lateralization which has been previously reported (Agosta et al., 2007; Chen et al., 2018; Kassubek et al., 2005).

In contrast, extramotor involvement of the SpN (i.e., regions beyond the PMC) was restricted to the left hemisphere. The observed left asymmetry of extramotor involvement was not explained by the initial side of onset as a comparable number of patients exhibited left, right, and bilateral onsets. Instead, left-lateralized speech and language processing networks encompassing these regions has been traditionally accepted in the cognitive neuroscience literature (Fuertinger, Horwitz & Simonyan, 2015; Lemaire et al., 2013; Zatorre et al., 1992) (although recently debated, see Ferstl et al., 2008). The observation supports the notion of increased vulnerability of neural networks in ALS, which has been suggested by recent MRI connectomic and neuropathology studies showing a sequential neuroanatomical spread of disease initiating in the PMC and propagating to connected regions in the cortex (Brettschneider et al., 2013; Rose et al., 2012; Tu et al., 2018; Verstraete et al., 2010, 2014). The full extent of SpN involvement needs to be further investigated however, including the somatosensory cortex, secondary motor areas in the prefrontal cortex, insula, anterior cingulate, and deep grey matter structures, as well as brainstem and cerebellum. Examining the SpN connection with the language network is also needed in future research.

For the first time, structural changes of the TT, also known as Heschl’s gyrus associated with the primary auditory cortex (PAC) of the SpN, was observed in this study (Braak, 1978; Penhune, Zatorre, MacDonald & Evans, 1996). The past fMRI and positron emission tomography studies indicated that the PAC was involved in speech perception (i.e., pitch discrimination, time
estimation of auditory stimuli, (Hyde, Peretz & Zatorre, 2008; Kanai, Lloyd, Bueti & Walsh, 2011) but was also activated during speaking presumably as part of the auditory feedback loop (Scott & Johnsrude, 2003; Tourville, Reilly & Guenther, 2008). Neurostructural changes in this area have not yet been reported in ALS, although functional connectivity alterations underlying the TT have been previously observed (Mohammadi, 2009). Further, auditory processing deficits, specifically delayed auditory latency and pitch discrimination errors, have been previously reported (Raggi et al., 2008). The involvement of the TT suggests that neurodegeneration in ALS goes beyond higher-order frontotemporal cognitive processing areas that are typically reported in ALS cases with overt cognitive deficits (i.e., ALS-FTD (Saxon et al., 2017)) and include lower-order sensory processing areas as well. These observations need to be validated with simultaneous clinical testing of speech, language, and multisensory processing.

2.5.3 Bulbar Dysfunction and Progression Rates are associated with Specific Extramotor Changes of the SpN

Areas associated with bulbar motor dysfunction included the oral PMC as well as extramotor bilateral TT (primary auditory cortex) and left pSTG (traditionally known as the Wernicke area). A double dissociative pattern was also observed in which extramotor degeneration was correlated with bulbar but not limb motor measures. This pattern suggests that the development of bulbar ALS may be distinct from spinal ALS with increased involvement of the SpN-specific regions. This finding should be interpreted with caution however. The true disentanglement of clinical variables (bulbar vs. overall motor dysfunction) is challenging due to the inherent multicollinearity between clinical measures. Further, our measure of limb dysfunction was restricted to the upper limbs while the limb PMC included leg representations. This work needs to be further validated in a longitudinal study with a large N and with a comprehensive workup regarding motor and extramotor disability; this work is currently in progress (Ishaque et al., 2018; Kalra et al., 2018).

Interestingly, disease progression rate emerged as another predictor of SpN regional involvement - a rapidly-progressing disease course was associated with increased GM and WM changes in bilateral ParsO, a portion of the Broca’s area that has been linked to semantic processing and action imitation, among other functions (Bookheimer, 2002; Molnar-Szakacs, Iacoboni, Koski & Mazziotta, 2004). Degeneration of this area has been observed in those
carrying the C9ORF72 hexanucleotide expansion (Bede, Bokde, et al., 2013; Westeneng et al., 2016), a genetic subtype associated with ALS and FTD, yet mutation carriers were excluded from this study. The finding in sporadic ALS cases is in line with previous reports of an association between disease progression rates and performance on a word generation task in this population (Gordon et al., 2010; Kwan et al., 2013), which has been functionally mapped to the IFG (Brannen et al., 2001). Healthy brain connectome studies indicate that the IFG is the most connected node to the PMC (Fuertinger et al., 2015) and is operational in many functional connectomes including the large-scale sensory and motor networks (De Luca et al., 2006; Flinker et al., 2015; Hampshire et al., 2010; Heim, Opitz & Friederici, 2003). These “heavy traffic” regions may undergo ‘nodal stress’ (Zhou et al., 2012) and may show a more involved disease course (Verstraete et al., 2010). Degeneration of high-traffic areas that may be preferentially vulnerable in ALS can be of potential prognostic importance.

2.5.4 Study Limitations

This study served as a pilot investigating the relationship between bulbar motor dysfunction and neuroanatomy of the SpN regions. We recognize the limitations posed by the small sample size and the cross-sectional design of the study. The study findings need to be confirmed using a larger cohort of ALS patients with longitudinal and simultaneous neuroimaging, motor speech, and detailed language testing. Furthermore, the DTI protocol employed in this study was limited in its number of directions and did not allow tractography analyses. Individual fiber tract tracing may be especially important in smaller, more variable WM pathways such as the corticobulbar tract.

2.5.5 Conclusions

Overall, the findings suggest a relationship between the severity of bulbar motor disease and degeneration in SpN regions. The study found that regions of the left-dominant SpN may be implicated in bulbar ALS, including higher-order regions such as portions of the IFG and pSTG (Broca and Wernicke areas), involved in speech but also language processing and production, as well as lower-order regions such as the PAC involved in auditory perception. Bulbar motor severity may be uniquely associated with neurodegeneration of SpN-specific regions. However, involvement of regions that overlap across multiple connectomes, such as Broca’s area (Flinker et al., 2015; Hampshire et al., 2010; Heim et al., 2003), may be dependent on the rate of disease progression. The work has significant clinical and research implications – understanding bulbar
subtype is crucial for predicting disease progression, delivering targeted clinical care, and improving patient stratification for recruitment into clinical trials.
Chapter 3  
The Neuropathological Signature of Bulbar-Onset ALS: A Systematic Review  

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3.1 Abstract 

ALS is a multisystem disorder affecting cognitive and motor functions. Bulbar-onset ALS (bALS) may be preferentially associated with language/cognitive impairments, compared with spinal-onset ALS (sALS), stemming from a potentially unique neuropathology. The objective of this systematic review was to compare neuropathology reported for bALS and sALS subtypes in studies of cadaveric brains. Using Cochrane guidelines, we reviewed articles in MEDLINE, Embase, and PsycINFO databases using standardized search terms for ALS and neuropathology, from inception until July 16th 2016. 17 studies were accepted. In summary, both subtypes presented with involvement in motor and frontotemporal cortices, deep cortical structures, and cerebellum, characterized by neuronal loss, spongiosis, myelin pallor, and ubiquitin+ and TDP43+ inclusion bodies. Changes in Broca and Wernicke areas, regions associated with speech and language processing, were noted exclusively in bALS. Further, some bALS cases presented with atypical pathology, neurofibrillary tangles and basophilic inclusions, which were not found in any sALS cases. Given the few studies, all with methodological biases, further work is required to better understand neuropathology of ALS subtypes. 

3.2 Introduction 

Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disease that affects upper and lower motor neurons in the brain, brainstem, and spinal cord, but has also been associated with extra-motor (i.e., cognitive and language) impairments, similar to those found in frontotemporal dementia (FTD) (Phukan et al., 2012; Schreiber et al., 2005). ALS has two typical presentations at disease onset – approximately 70% of patients present initially with the spinal form of the disease, characterized by muscle weakness and atrophy in the limbs and
trunk, when the remaining patients present with bulbar changes, affecting speech and swallowing musculature (Bonduelle, 1975). Nearly 85% of patients with spinal-onset ALS, however, exhibit bulbar changes with disease progression (Armon & Moses, 1998; Haverkamp, Appel & Appel, 1995). Approximately 50% of all patients diagnosed with ALS show cognitive and language impairments, while 10% of the patients present with clear signs of FTD (Massman et al., 1996; Ringholz et al., 2005). ALS is a complex disorder with considerable heterogeneity across affected individuals (Green et al., 2013; Robert et al., 1999). This heterogeneity is not well understood, however. Addressing the heterogeneity by developing accurate means of patient subtyping (Brooks et al., 1991) is essential for providing more targeted approaches to treatment development, recruitment into clinical trials, and disease management in a clinic (e.g., early identification of bulbar disease in order to plan supportive interventions and predict disease progression).

Bulbar ALS is arguably the most devastating variant of the disease as it is characterized by the fastest decline, the shortest survival (<2 years post diagnosis), and a significantly reduced quality of life (Goldstein, Atkins & Leigh, 2002; Mitsumoto & Del Bene, 2000). In addition to the rapid motor decline, some neuroimaging and behavioural studies have observed that bulbar ALS may present with an increased burden of cognitive/language impairments (Lomen-Hoerth et al., 2003; Massman et al., 1996; Ogawa et al., 2009; Ota, Tsuchiya, & Akiyama, 2005; Schreiber et al., 2005; Sterling et al., 2010; Strong et al., 1999). This latter finding remains disputed, however (Gordon et al., 2010; Taylor et al., 2013). Two hypotheses have been proposed regarding the association between motor and extramotor abnormalities in ALS, in relation to the disease subtype: 1) it has been suggested that the site of symptom onset may be related to the burden of extramotor impairments, with bulbar-onset ALS showing a unique neurodegenerative profile associated with specific and concomitant extramotor impairments (Ichikawa, Koyama, et al., 2008; Kato et al., 1994; Lomen-Hoerth et al., 2003; Massman et al., 1996; Ogawa et al., 2009; Portet, Cadilhac, Touchon, & Camu, 2001; Schreiber et al., 2005; Strong et al., 1999); and 2) the presence of bulbar motor dysfunction, regardless of site of onset, may be associated with extramotor impairments (Massman et al., 1996; Ota et al., 2005; Ringholz et al., 2005; Sterling et al., 2010). Neither of the two hypotheses has been investigated neuropathologically in cadaveric brain tissue.
Studies that examined the underlying neuropathology in cases with cognitive and language impairments showed that ALS cases typically present with frontotemporal lobar degeneration (FTLD) (Geser, Lee, & Trojanowski, 2010; Liscic et al., 2008). The pathology in the frontotemporal regions consisted of neuronal loss, marked gliosis, and intraneuronal inclusion bodies that were positive for ubiquitin and TAR DNA-binding protein 43 (TDP-43) (Arai et al., 2006; Liscic et al., 2008; Neumann et al., 2006). The severity and distribution of TDP-43 in the brain has been shown to be well-correlated with antemortem cognitive profiles, often giving insight into the phenotypic presentations of the disease and representing a clinicopathologic spectrum (Mackenzie, 2007; Mackenzie & Feldman, 2003; Prudlo et al., 2016; Yoshida, 2004) that ranges from pure motor neuron disease to frontotemporal dementia. The underlying neuropathology, however, has not been well-characterized in the context of bulbar- versus spinal-onset subtypes in the existing literature. An examination of the neuropathological findings from the subtype perspective might shed light into the underlying similarities and/or differences in clinical disease presentations.

This study aimed to contribute to our understanding of ALS subtypes through neuropathological examinations of cadaveric autopsy brains, and elucidate whether these subtypes are neuropathologically distinct or lie within a spectrum of the same disease. To do this, we conducted a systematic review investigating similarities and differences between neuropathological profiles of bulbar-onset ALS (bALS) and spinal-onset ALS (sALS) by regional distribution and types of pathology.

3.3 Methods

3.3.1 Operational Definitions

Our search was guided by the following operational definitions, determined a priori:

*Amyotrophic Lateral Sclerosis*, defined as a progressive neurological disease with upper and lower motor neuron involvement determined by clinical, electrophysiological or neuropathologic examination; and *cadaveric neuropathological examination*, defined as the post-mortem study of disease on the brain and brainstem by gross or microscopic examination.

3.3.2 Search Methodology

Studies were identified by searching the Medline (1946 to July 12th, 2016), Embase (1980 to July 12th, 2016), and PsycInfo (2002 to July 12th, 2016) databases. Main search terms included
Amyotrophic Lateral Sclerosis, ALS, Lou Gehrig’s disease, or motor neuron disease combined with neuropathology, histology, immunohistochemistry, or immunocytochemistry, limited to humans. The search strategy for Amyotrophic Lateral Sclerosis was adapted and modified from a previous Cochrane Review (Dal Bello-Haas, Florence & Krivickas, 2008). The search terms were adapted for each database to accommodate for differences in subject headings (see Appendix B for full search strategies). Citation lists of included articles were hand-searched for articles relevant to the systematic review.

3.3.3 Study Selection

Articles were excluded for this review if they: 1) had no abstract; 2) included no human participants (i.e. animal study); 3) were classified as a tutorial, educational report, or narrative review; 4) were a neuroimaging study (i.e., fMRI, DTI, PET, EEG, MEG, etc.); 5) were a genetic study (i.e., focusing on cases with SOD1/C9ORF72/FUS mutations only); 6) involved a population where >90% of subjects were not diagnosed with ALS (i.e., all forms of motor neuropathies, Alzheimer’s Disease, etc.); 7) involved a population where >90% of subjects were diagnosed with an atypical subtype of ALS (i.e., Parkinson-Dementia-ALS of the Guam complex, or juvenile ALS of the Madras subtype); or, 8) did not involve a pathological examination, either microscopic or at a gross macroscopic level, of cadaveric brains (i.e., blood serum analysis, DNA fragmentation, or skin microscopy). Two independent raters (authors SS and KV) reviewed all unique abstracts identified from the primary search. Discrepant ratings were resolved by consensus. All accepted abstracts were brought to full review.

During the full article review, articles were excluded for the same reasons as above and also if they: 1) did not investigate the brain or brainstem (i.e., investigated muscle or spinal cord only), 2) involved a population where the site of onset were not specified, 3) did not have any bALS cases (i.e., sALS or FTD-onset cases only), or, 4) did not allow for data extraction of bALS cases (i.e., only aggregate data across all subjects of varying onsets). A full review of each article was conducted by the same two independent raters (Authors SS and KV). Discrepant ratings were again resolved by consensus.

3.3.4 Quality Assessment

The methodological quality of each included full article was critically appraised using a combination of guidelines designed to improve the quality of reporting non-randomized studies:
The Effective Public Health Practice Project (EPHPP) Quality Assessment Tool for Quantitative studies (Thomas, Ciliska, Dobbins & Micucci, 2004), and the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) statement (Von Elm et al., 2007). Also, relevant quality assessment questions were adapted from the Cochrane Risk of Bias (Higgins et al., 2011) for domains pertaining to selective enrolment, validity of bALS and sALS group comparisons (i.e., a high risk of bias (ROB) rating for dissimilar sites of tissue sampling, unequal number of patients using artificial respiratory support (ARS) between the two groups, etc.), incomplete clinical information provided (i.e., a high ROB rating for missing data on the cognitive and/or bulbar status of the patients, inadequate genetic testing, etc.), and poor methodological descriptions thereby limiting reproducibility of the study. Risk of bias for each article was judged relative to the dates of gene discovery. For example, articles published after 2011 that did not screen for C9ORF72 mutations were given a high ROB rating, but articles predating 2011 were not (see Renton, Chio & Traynor, 2014) for a chronological timeline of each discovered gene in ALS).

3.3.5 Data Extraction

Only full articles that met the inclusion criteria outlined above underwent data extraction by a single rater (Author SS) and included: 1) study design; 2) diagnosis of the examined group and sample size; 3) demographic variables (i.e. mean age at onset/death, site of initial symptom onset, and sex; 4) disease characteristics (i.e. severity of disease, disease duration, and cognitive status); 5) pathological assessment methods (i.e. sampling sites, staining methods, and immunohistochemistry techniques); and, 6) neuropathological data (i.e. anatomical areas involved, types and extent of pathology). Data on cases with a mixed-motor onset (i.e., limb and bulbar symptoms at initial disease presentation) were excluded. Neuropathological data were extracted for all areas within the cerebral cortex, as well as the hippocampus, amygdala, basal ganglia, thalamus, and cerebellum. For the brainstem, only data pertaining to the selected motor nuclei that innervate bulbar structures (i.e., the trigeminal motor, facial, vagus, and hypoglossal nuclei) were extracted. Data on the spinal cord were beyond the scope of this study and thus not extracted. Partial data extraction (25%) was checked by a second rater (VK) and discrepancies were resolved by consensus.
3.4 Results

3.4.1 Literature Retrieval

The literature search and study selection flow diagram is shown in Figure 3-1. In addition to the 1377 studies identified through database searching, 5 were found by searching citation lists of included articles. Duplicate abstracts were removed leaving 1208 unique abstracts, of which 880 articles were excluded leaving 328 articles for full review. At this level, an additional 311 were excluded leaving 17 full articles (Averbuch-Heller et al., 1998; Bak et al., 2001a, 2001b; Bodansky et al., 2010; Ishihara et al., 2006; Kamo et al., 1987; Kato et al., 1994; Kato, Oda & Tanabe, 1993; Kuwahara et al., 2010; Miki et al., 2010; Mochizuki, Mizutani & Takasu, 1995; Nagy, Kato & Kushner, 1994; Nakano, Nakaso, Nakashima & Ohama, 2004; Ota et al., 2005; Sugiyama et al., 2013; Troost, Smitt, De Jong & Swaab, 1992; Tsuchiya et al., 2000; Tsuchiya et al., 2002). At abstract screening, the inter-rater agreement was 84.5% for the accept/reject criteria, with a 79% agreement for the reason of rejection. At full text screening, the accept/reject percent agreement was 89.5%, with an 81% agreement for reason of rejection.
Figure 3-1. Selection of included studies.

**Duplicates:**
- n = 174

**Abstract Screening**
- No Abstract: n = 106
- Tutorial, education report, literature/systematic review, book chapter: n = 152
- Animal study: n = 159
- Neuroimaging study (i.e., MRI): n = 3
- Familial ALS: n = 102
- <90% with typical ALS (i.e., Guamian ALS, juvenile ALS, etc.): n = 75
- <90% with ALS (i.e., Alzheimer's, Parkinson's): n = 157
- No histopathology methodology (i.e., blood serum, CSF analysis): n = 126
- **Total**: n = 880

**Full-text Screening**
- Tutorial, education report, literature/systematic review, book chapter: n = 5
- <90% with ALS (i.e., Alzheimer's, Parkinson's): n = 3
- Did not investigate the brain or brainstem: n = 180
- Did not indicate sites of onset: n = 60
- Did not allow for bulbar-onset data extraction (i.e., only aggregate data, only Spinal-onset cases): n = 63
- **Total**: n = 311

<table>
<thead>
<tr>
<th>Source</th>
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</tr>
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<tbody>
<tr>
<td>MEDLINE</td>
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<td>EMBASE</td>
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<tr>
<td>PsycInfo</td>
<td>33</td>
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<td>Other sources</td>
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</tr>
<tr>
<td><strong>Total</strong></td>
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</tr>
<tr>
<td>Accepted Abstracts</td>
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</tr>
<tr>
<td>Included Full Papers</td>
<td>17</td>
</tr>
<tr>
<td>Mixed-group studies:</td>
<td>11</td>
</tr>
<tr>
<td>bALS-only studies:</td>
<td>6</td>
</tr>
</tbody>
</table>
3.4.2 Study Characteristics

Of all 17 included articles, none directly investigated the neuropathological differences between sALS and bALS. Out of the included studies, however, 11 reported individual neuropathy results for a mixed group of patients—those with bALS or sALS (Averbuch-Heller et al., 1998; Bodansky et al., 2010; Kamo et al., 1987; Kato et al., 1994; Kato et al., 1993; Mochizuki et al., 1995; Nagy et al., 1994; Nakano et al., 2004; Ota et al., 2005; Sugiyama et al., 2013; Troost et al., 1992)—allowing for a comparison between the two groups (Table 3-1). The other 6 studies investigated the clinical and neuropathological abnormalities in bALS patients only (Bak et al., 2001b; Ishihara et al., 2006; Kuwahara et al., 2010; Miki et al., 2010; Tsuchiya et al., 2000; Tsuchiya et al., 2002) (Table 3-3). Below, we present these two groups of findings separately as the former set of studies allows the comparison of the two disease subtypes, and the latter set of studies provides a more in-depth clinicopathologic analysis of bulbar-onset cases only.

3.4.3 Studies allowing Direct Comparisons between bALS and sALS

3.4.3.1 Study Characteristics

All 11 included articles had a case series study design. The 11 studies included a total of 123 subjects, with a median of 8 subjects per study and range of 1 to 24. A total of 32 bALS cases and 91 sALS cases were examined. None of the studies reported any genetic mutations for any of the included subjects as the studies predated the discovery of most ALS genetic mutations. Four (Averbuch-Heller et al., 1998; Kato et al., 1994; Mochizuki et al., 1995; Ota et al., 2005) studies reported patients’ bulbar symptomology prior to death, which included severe dysphagia and dysarthria, along with tongue atrophy and fasciculation. The cognitive status of the patients was described in 7 studies (Averbuch-Heller et al., 1998; Kato et al., 1994; Kato et al., 1993; Mochizuki et al., 1995; Nagy et al., 1994; Nakano et al., 2004; Ota et al., 2005), specifically in 41% of the bALS cases and 40% of the sALS cases, and mostly reported symptomatically as the presence or absence of dementia.

3.4.3.2 Comparison of Clinical Outcomes between bALS and sALS

Patient demographics and clinical characteristics of the cases are presented in Table 3-1. The average disease duration was significantly shorter (p<.05) for bALS cases (M = 29.04 months, SD = 25.98) than sALS cases (M = 51.74 months, SD = 51.59). The two groups were similar in
age of onset, except for three studies: two (Kato et al., 1994; Ota et al., 2005) studies reported bALS patients that were older (>15 years) than the sALS patients at disease onset, and one study (Nakano et al., 2004) reported sALS older than bALS patients.
Table 3-1. Patient demographics and clinical characteristics for studies with bulbar-onset and spinal-onset ALS subjects.

<table>
<thead>
<tr>
<th>Included Studies</th>
<th>Bulbar –Onset Group</th>
<th>Spinal- Onset Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (M/F)</td>
<td>Nr, age at onset;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Disease Duration</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bulbar symptomology</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Number of cases</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Number of cases</td>
</tr>
<tr>
<td></td>
<td></td>
<td>with clinical signs</td>
</tr>
<tr>
<td></td>
<td></td>
<td>with artificial</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cognitive/</td>
</tr>
<tr>
<td></td>
<td></td>
<td>language</td>
</tr>
<tr>
<td></td>
<td></td>
<td>impairment (N, % of</td>
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<tr>
<td></td>
<td></td>
<td>group, Type)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Number of cases</td>
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<tr>
<td></td>
<td></td>
<td>with artificial</td>
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<tr>
<td></td>
<td></td>
<td>respirator support</td>
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<td></td>
<td></td>
<td>(N, % of group)</td>
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<td></td>
<td></td>
<td>Number of cases</td>
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<td></td>
<td></td>
<td>with artificial</td>
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<tr>
<td></td>
<td></td>
<td>respirator support</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(N, % of group)</td>
</tr>
<tr>
<td>Kamo et al., 1987</td>
<td>1M/3 F 66.5 (3.83)</td>
<td>3M/4F NR, 58.43</td>
</tr>
<tr>
<td>(Kamo et al., 1987)</td>
<td>21 NR</td>
<td>(35.78)</td>
</tr>
<tr>
<td>Troost et al., 1992</td>
<td>3M/6 F 62.88 (9.51)</td>
<td>16M/8 F 64.04</td>
</tr>
<tr>
<td>(Troost et al., 1992)</td>
<td>24.88 NR</td>
<td>(13.19)</td>
</tr>
<tr>
<td>Kato et al., 1993</td>
<td>1M/2 F 62.33 (6.56)</td>
<td>7M/5F NR, 65.58</td>
</tr>
<tr>
<td></td>
<td>15 NR</td>
<td>(85.74)</td>
</tr>
<tr>
<td></td>
<td>3 (100%), None</td>
<td>97.33 NR</td>
</tr>
<tr>
<td></td>
<td>NR</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>NR</td>
<td>5 (42%)</td>
</tr>
<tr>
<td>Study (Year)</td>
<td>Gender</td>
<td>Age</td>
</tr>
<tr>
<td>-------------</td>
<td>--------</td>
<td>-----</td>
</tr>
<tr>
<td>Kato et al., 1993</td>
<td>4M</td>
<td>66.75</td>
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<tr>
<td>Kato et al., 1994</td>
<td>1F</td>
<td>NR, 55</td>
</tr>
<tr>
<td>Nagy et al., 1994</td>
<td>1M</td>
<td>52, 53</td>
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<tr>
<td>Mochizuki et al., 1995</td>
<td>1F</td>
<td>NR, 55</td>
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</table>

NR indicates not reported.
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<tr>
<th>Year</th>
<th>Gender</th>
<th>Age (Mean, SD)</th>
<th>Diagnosis</th>
<th>Other Stigmata</th>
<th>Outcome</th>
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<tbody>
<tr>
<td>Averbuch -Heller et al., 1998 (Averbuch-Heller et al., 1998)</td>
<td>IF</td>
<td>54, 60</td>
<td>Complete anarthria, atrophic tongue with fasciculation</td>
<td>1 (100%), inappropiate behaviour/pseudobulbar affect</td>
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<tr>
<td></td>
<td>M/F</td>
<td>56</td>
<td></td>
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<tr>
<td>Nakano et al., 2004 (Nakano et al., 2004)</td>
<td>M/F</td>
<td>40, 137</td>
<td>None</td>
<td>1 (100%)</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>NR</td>
<td>NR</td>
<td></td>
<td></td>
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<tr>
<td>Ota et al., 2005 (Ota et al., 2005)</td>
<td>M/F</td>
<td>70.5, 17</td>
<td>Dysarthria, dysphagia, tongue atrophy and fasciculation</td>
<td>None</td>
<td>None</td>
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<tr>
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<td>NR</td>
<td>(2.12), (9.89)</td>
<td></td>
<td></td>
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<tr>
<td>Bodansky et al., 2010 (Bodansky et al., 2010)</td>
<td>M/F</td>
<td>77.8, 23.6</td>
<td>NR</td>
<td>NR</td>
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<td></td>
<td>NR, F</td>
<td>(3.96), (10.4)</td>
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<td>Bodansky et al., 2010 (Bodansky et al., 2010)</td>
<td>M/F</td>
<td>56.5, 29.5</td>
<td>Dysarthria, dysphagia, tongue atrophy and fasciculation</td>
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<td>None</td>
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<tr>
<td></td>
<td>(6.36), (13.43)</td>
<td>(30.36)</td>
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<tr>
<td>Sugiyama et al., 2013 (Sugiyama et al., 2013)</td>
<td>1M 72, 36 NR 1 (100%), non-invasive positive pressure ventilation</td>
<td>3M/4F 58.42 (14.28), 62.42 (12.17)</td>
<td></td>
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<td>-----------------------------------------------</td>
<td>----------------------------------------------------------------</td>
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<tr>
<td></td>
<td>74 (14.28), 62.42 (12.17)</td>
<td>2 (29%), Artificial ventilator</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

NR= Not Reported. CNE= Cranial Nerve Exam
Across all studies assessing cognitive abnormalities, it was reported in 54% of bALS cases and 14% of the sALS cases. Two patients with bALS (disease duration: M=86.5 months, SD=71.42) and 7 patients with sALS cases (disease duration: M=78.57 months, SD=39.25) received artificial respiratory support (ARS) for more than 1 year prior to death, while 10 bALS patients (disease duration: M = 24.75 months, SD= 18.31) and 21 sALS patients (disease duration: M = 98.93 months, SD = 90.85) did not. ARS status for the remaining 81 cases was not reported.

Patients from both groups, bALS and sALS, presented with spinal symptoms at time of death, including weakness and paralysis of their extremities and/or respiratory difficulties.

3.4.3.3 Critical Appraisal

Methodological quality of the studies with bulbar-onset and spinal-onset patients is addressed in Table 3-2. Almost all studies recruited ALS patients using appropriate eligibility criteria. There was no evidence of attrition bias, in that all cases that were tested were reported, and very little evidence of reporting bias, where all pre-specified outcomes, such as all brain regions and stains, were reported.

Of the 11 “mixed-group” studies, only 3 (Averbuch-Heller et al., 1998; Kato et al., 1994; Ota et al., 2005) had an equal number of patients with long-term artificial respiratory support (ARS) in the bulbar- and spinal-onset groups. Four studies (Kamo et al., 1987; Mochizuki et al., 1995; Nagy et al., 1994; Troost et al., 1992) failed to specify the prevalence and duration of ARS, and the remaining 4 (Bodansky et al., 2010; Kato et al., 1993; Nakano et al., 2004; Sugiyama et al., 2013) introduced bias in the neuropathology data by having an unequal proportion of ARS users for the two groups.

Seven of the 11 “mixed-group” studies (Averbuch-Heller et al., 1998; Kato et al., 1994; Kato et al., 1993; Mochizuki et al., 1995; Nagy et al., 1994; Nakano et al., 2004; Ota et al., 2005) reported antemortem cognitive signs of the patients; five of these studies only reported the presence or absence of dementia; another study (Averbuch-Heller et al., 1998) reported “inappropriate behaviour”, however, it was unclear whether these were signs of cognitive behavioural dysfunction or pseudobulbar affect; only one study reported specific cognitive changes on an extensive neuropsychological assessment battery (Kato et al., 1994). The
remaining four studies (Bodansky et al., 2010; Kamo et al., 1987; Sugiyama et al., 2013; Troost et al., 1992) did not mention the cognitive status of their patient population.

Most studies (Bodansky et al., 2010; Kamo et al., 1987; Kato et al., 1993; Nagy et al., 1994; Nakano et al., 2004; Sugiyama et al., 2013; Troost et al., 1992) did not report the presence or absence of bulbar signs (i.e., dysarthria, dysphagia) during the disease course for their patient population.

Five of the 11 “mixed-group” studies (Averbuch-Heller et al., 1998; Kato et al., 1994; Nagy et al., 1994; Nakano et al., 2004; Ota et al., 2005) had methodological biases concerning their neuropathology protocols; the studies either did not uniformly sample the brain regions across cases, or did not anatomically define the regions of interest. Furthermore, most studies used a semi-quantitative rating scale to evaluate the severity of neuronal loss and gliosis. This poses another limitation as subjective rating scales were not standardized across studies. The studies also did not indicate whether more than one rater was used to obtain these measures, suggesting a potential detection bias.

Only one of the studies screened for and documented the presence of co-existing FTLD through TDP-43 immunostaining (Bodansky et al., 2010). Most studies, albeit, were much older and predated the discovery of TDP-43 as the major component of the NCIs in ALS-FTD subtypes (Arai et al., 2006; Neumann et al., 2006). Four (36%) of the mixed-group studies (Kato et al., 1994; Nakano et al., 2004; Ota et al., 2005; Troost et al., 1992) stained for ubiquitin, which was the only identifiable component of the inclusions found in ALS-FTD pathology at the time. 10 studies (91%) screened for and documented coexisting neurodegenerative phenomena, such as Alzheimer’s Disease (AD) pathology, using various silver staining methods and/or anti-tau sera. None of the studies staged the AD-related changes using the Braak staging method (Braak & Braak, 1995).

None of the studies conducted the appropriate genetic testing to validate the observed neuropathological abnormalities, even though two studies (Bodansky et al., 2010; Nagy et al., 1994) discussed SOD1 and TARDBP mutations as potential explanations to the observed pathological findings.
Table 3-2. Summary of methodological quality assessment for studies with bulbar-onset and spinal-onset patients. +=Yes, -=No; ? = not reported/ unknown/ unclear.

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Selection Bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kamo et al.</td>
<td>1987</td>
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</tr>
<tr>
<td>Troost et al. (Kamo et al., 1987)</td>
<td>1992</td>
<td>+</td>
</tr>
<tr>
<td>Kato et al. (Kamo et al., 1987)</td>
<td>1993</td>
<td>+</td>
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<td>Mochizuki et al. (Mochizuki et al., 1995)</td>
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Selection Bias

1. Did the study develop and apply appropriate eligibility criteria for the ALS patients and a control population? +

2. Does the analysis account for important confounding and modifying variables through matching, stratification, or other approaches?
   a) Were the number of patients with long-term artificial respiratory support?

   ?                  ?                  -               +               ?               ?
b) Were antemortem cognitive/language signs of the patients reported?  
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c) Was appropriate genetic testing reported for all cases?  
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d) Was the presence of bulbar signs during the disease course reported?  
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### Attrition Bias

1. Were all the cases that were tested, reported?  
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### Detection Bias

1. Were the histopathological...  
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outcomes assessed using valid and reliable measures?

2. Was there more than one rater for the qualitative outcome measures?  


3. Was the histopathology methodology, such as sites of tissue sampling and staining, similar for all cases?

| + | + | - | ? | - | + |

4. Did the study screen for, and document coexisting FTLD?

| - | + | - | + | - | - |

5. Did the study screen for, document, and stage coexisting neurodegenerative phenomena (i.e., AD, mesial temporal sclerosis)?

| + | + | + | + | + | + | + |

---

**Reporting Bias**
1. Were all pre-specified outcomes, such as areas of the brain and stains, reported?

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<thead>
<tr>
<th></th>
<th>Averbuch-Heller et al., 1998</th>
<th>Nakano et al., 2004</th>
<th>Ota et al., 2005</th>
<th>Bodansky et al, 2010</th>
<th>Sugiyama et al., 2013</th>
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<tbody>
<tr>
<td>Reproducibility</td>
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Averbuch-Heller et al., 1998 (Averbuch-Heller et al., 1998)
Nakano et al., 2004 (Nakano et al., 2004)
Ota et al., 2005 (Ota et al., 2005)
Bodansky et al, 2010 (Bodansky et al., 2010)
Sugiyama et al., 2013 (Sugiyama et al., 2013)

Selection Bias
1. Did the study develop and apply appropriate eligibility criteria for the ALS patients and a

|                | ?                             | +                   | +                | +                    | +                   |

Averbuch-Heller et al., 1998 (Averbuch-Heller et al., 1998)
Nakano et al., 2004 (Nakano et al., 2004)
Ota et al., 2005 (Ota et al., 2005)
Bodansky et al, 2010 (Bodansky et al., 2010)
Sugiyama et al., 2013 (Sugiyama et al., 2013)
control population?

2. Does the analysis account for important confounding and modifying variables through matching, stratification, or other approaches?
   a) Were the number of patients with long-term artificial respiratory support equal for both groups?

   b) Were antemortem cognitive/language signs of the patients reported?

   c) Was appropriate genetic testing reported for all cases?
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<th>d) Was the presence of bulbar signs during the disease course reported?</th>
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**Attrition Bias**

1. Were all the cases that were tested, reported?

   | +  +  +  +  +  +  |

**Detection Bias**

1. Were the histopathological outcomes assessed using valid and reliable measures?

   | +  +  ?  +  - |

2. Was there more than one rater for the qualitative outcome measures?

   | NA  NA  ?  NA  ? |

3. Was the histopathology methodology, such as sites of tissue sampling and staining, similar for all cases?

   | ?  +  -  +  +  + |
4. Did the study screen for, and document coexisting FTLD? | - | + | + | + | + | + |

5. Did the study screen for, document, and stage coexisting neurodegenerative phenomena (i.e., AD, mesial temporal sclerosis)? | + | + | + | - | + | + |

**Reporting Bias**

1. Were all prespecified outcomes, such as areas of the brain, reported? | + | + | - | + | + | + |

**Reproducibility**

1. Did the study clearly define each region of interest anatomically? | - | - | - | + | + | + |

2. Was the site of onset well-defined? | + | + | - | + | + | + |
3.4.3.4 Comparison of Neuropathology between bALS and sALS

Regional analyses: The results of the regional analysis between bALS and sALS cases are summarized in Figure 3-2. Four studies suggested differences in the regional profiles of involvement for bALS as compared to sALS patients (Kato et al., 1994; Kato et al., 1993; Nakano et al., 2004; Sugiyama et al., 2013) (Figure 3-2A). These studies suggested an increased prevalence of co-existing FTLD in bALS cases, compared to sALS cases. The unique regional distribution of pathological findings for bALS patients involved, primarily, the extramotor cortical regions, including the frontal, temporal, cingulate, and insular cortices, and frontotemporal white matter (WM). One study also reported pathology in the hippocampus and amygdala in bALS but not in sALS cases (Kato et al., 1993). Most of these cortical and subcortical areas, however, may not be differentially involved in bALS as 4 other studies in this subgroup of articles reported pathology in these areas for both subtypes (Nagy et al., 1994; Nakano et al., 2004; Ota et al., 2005; Sugiyama et al., 2013). Of particular interest is the one study that reported differential involvement of the Broca and Wernicke areas – regions that are highly associated with speech and language processing – in bALS cases, which were not involved in the sALS cases (Kato et al., 1994). While lobular assessment of the frontal and temporal cortices was conducted in other studies, these smaller regions were not individually examined in any of the other included 11 mixed-group studies.

In contrast, all the mixed-group studies (n=11) reported an overlap in the regional distribution of pathology between bALS and sALS cases—including the frontotemporal cortices and other extra motor cortical regions—and to a similar extent, suggesting that both subtypes were within the same spectrum of disease (see Figure 3-2B). Some studies that reported these overlapping regions of involvement, however, noted differences in the degree of pathological processes, defined as the number of inclusions, or severity of neuronal loss or gliosis ranked on a standardized scale. For example, the primary motor cortex was involved in both subtypes, but more notably affected in sALS than bALS in three studies (Kamo et al., 1987; Mochizuki et al., 1995; Sugiyama et al., 2013), and another study (Averbuch-Heller et al., 1998) reported a greater degree of neuronal loss in the facial and vagus nuclei for the bALS cases compared to the sALS cases.
Figure 3-2. Summary of anatomic regions of involvement between bALS and sALS cases. Summary in panel A (above the solid line) lists studies reporting differences in the regional involvement between ALS variants. Summary in panel B (below the solid line) shows studies reporting shared regions of the brain for both variants. Some studies are shown in both panels as they report results of both types (i.e., differences in some regions but similarities in other regions). CST = corticospinal tract; CC = corpus callosum; Fasc. = fasciculus; Nuc. = nucleus.
Type of pathology: Figure 3-3 displays differences in the types of pathology, cumulative across the whole brain, for the two subtypes. Figure 3-3A lists four studies that reported unique pathological features for the two disease variants. Specifically, one study reported gliosis, spongiosis, and ubiquitinated inclusions in the whole brain for bALS cases, but not sALS cases (Kato et al., 1994). An ALS diagnosis was confirmed for these sALS cases based on the loss of Betz cells in the primary motor cortex. Another study reported the presence of senile plaques in the motor cortex of bALS cases, that were absent from sALS cases (Kamo et al., 1987). Two other studies reported skein-like inclusions and ubiquitinated inclusions that were immunoreactive to TDP-43 in sALS cases, which were absent in bALS cases (Bodansky et al., 2010; Sugiyama et al., 2013).

All 11 studies, however, identified similar types of pathology for both subtypes (Figure 3-3B). The compiled data suggested that, on a neurological examination, both bALS and sALS cases displayed an equal extent of neuronal loss, gliosis, and myelin pallor, as well as a comparable amount of bunina bodies, phosphorylated neurofilaments, senile plaques, and skein-like or round intraneuronal inclusions that were positive for ubiquitin and TDP-43.
Figure 3-3. Summary of pathology types compared between bALS and sALS cases. Summary in panel A (above the solid line) lists studies that report unique pathological characteristics for one subtype, but not the other. Summary in panel B (below the solid line) reports studies that indicate similar pathology types for both subtypes. Some studies are shown in both panels as they report results of both types (i.e., differences in some pathology types but similarities in other pathology types). Ubi+ = ubiquitin-positive; NCI = neuronal cytoplasmic inclusions. Incl. = inclusions.

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* = Inclusions were immunopositive for both TDP-43 and Ubiquitin. Inclusions were also present in the glial cells.  
† = both groups presented with mild gliosis, but with more widespread cortical gliosis in the bulbar-onset cases.  
& = Patchy reactive astrocytes were found in both groups, but fibrous-patchy reactive astrocyte clusters were found only in the spinal-onset cases, not the bulbar-onset cases.
3.4.4  Neuropathology in bALS-Only Studies

3.4.4.1  Study Characteristics

All 6 included articles had a case series study design. Across studies, the total number of subjects was 11. All studies reported bulbar symptomology and cognitive status for the patients.

3.4.4.2  Clinical Characteristics

Nine out of 11 cases were diagnosed with ALS-FTD as disease progressed (See Table 3-3). All the bALS patients presented with spinal symptoms at time of death, characterized by weakness and paralysis of their extremities and/or respiratory difficulties. Only one case received ARS for more than one year prior to death. None of the studies reported genetic mutations.

Table 3-3. Patient demographics and clinical characteristics in studies with only a bulbar-onset population.

<table>
<thead>
<tr>
<th>Included Studies</th>
<th>N (M/F)</th>
<th>Age at onset; Age at death (years, SD)</th>
<th>Disease Duration (months, SD)</th>
<th>Bulbar symptomology</th>
<th>Number of cases with clinical signs of cognitive/language impairments (N, % of group, Type)</th>
<th>Number of cases with artificial respiratory support (N, % of group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tsuchiya et al., 2000 (Tsuchiya et al., 2000)</td>
<td>1F</td>
<td>74; 75</td>
<td>12</td>
<td>Severe; dysphagia (tube feeding) and dysarthria; atrophy and fasciculation of the tongue</td>
<td>1 (100%), Primary Progressive Aphasia – non-fluent variant</td>
<td>NR, (but died of respiratory disturbances)</td>
</tr>
<tr>
<td>Bak et al., 2001 (Bak et al., 2001b)</td>
<td>4M/1F</td>
<td>58.5 (9.56); NR</td>
<td>26 (4.89)</td>
<td>Severe dysarthria</td>
<td>5 (100%), Personality and behaviour change with</td>
<td>NR</td>
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</table>
## 3.4.4.3 Critical Appraisal

Methodological quality of the articles with only bALS cases is depicted in Table 3-4. Studies were evaluated using similar quality assessment criteria as the studies with mixed patients, but when appropriate, questions were modified for studies with only bALS patients.
Three out of six studies (Bak et al., 2001b; Ishihara et al., 2006; Tsuchiya et al., 2000) did not specify the presence and duration of ARS, and none of the studies characterized the genetic makeup of the patients or operationally defined the regions of interest using anatomical landmarks.

All of the bALS studies reported bulbar symptomology at some point of the disease course (Bak et al., 2001b; Ishihara et al., 2006; Kuwahara et al., 2010; Miki et al., 2010; Tsuchiya et al., 2000; Tsuchiya et al., 2002), and all studies reported antemortem cognitive and language signs of the patients. All the bALS studies screened for and documented both co-existing FTLD, by staining against ubiquitin and/or TDP-43, as well as co-existing neurodegenerative phenomena, by staining for AD pathology. Three out of the 6 studies (Bak et al., 2001b; Kuwahara et al., 2010; Tsuchiya et al., 2002) staged the observed AD-related neurofibrillary changes in the brain using Braak’s staging methodology.
Table 3-4. Summary of methodological quality assessment for studies with bulbar-onset patients only. +=Yes, -=No; ? =not reported/unknown/ unclear.

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<th>Study</th>
<th>Selection Bias</th>
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<td>Tsuchiya et al., 2000</td>
<td>1. Did the study develop and apply appropriate eligibility criteria for the ALS patients and a control population? +</td>
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<tr>
<td>Bak et al., 2001</td>
<td>2. Does the analysis account for important confounding and modifying variables through matching, stratification, or other approaches?</td>
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<td>Tsuchiya et al., 2002</td>
<td>a) Was long-term artificial respiratory support reported for all cases? -</td>
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<tr>
<td>Ishihara et al., 2006</td>
<td>b) Were antemortem cognitive/language signs of the patients reported? +</td>
</tr>
<tr>
<td>Kuwahara et al., 2010</td>
<td>c) Was appropriate genetic testing reported for all cases? -</td>
</tr>
<tr>
<td>Miki et al., 2010</td>
<td>d) Was the progression of bulbar signs during the disease course reported? -</td>
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Attrition Bias
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<th>Were all the cases that were tested, reported?</th>
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<td>3. Was the histopathology methodology, such as sites of tissue sampling and staining, similar for all cases?</td>
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<td>4. Did the study screen for, and document coexisting FTLD?</td>
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<td>5. Did the study screen for, document, and stage coexisting neurodegenerative phenomena (i.e., AD, mesial lateral sclerosis)?</td>
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3.4.4.4 Neuropathology of bALS

Regional Analysis and Type of Pathology: The neuropathological findings for the studies that investigated exclusively the bALS cases (n=6) are summarized by the regions of involvement (Figure 3-4), and pathology types (Figure 3-5). Overall, these studies reported similar regions of involvement and types of pathology to the bALS subjects from the mixed-group studies shown in Figures 3-2 and 3-3, including involvement in the Broca area. The findings from this group, however, also noted the presence of neurofibrillary tangles (NFTs) in the hippocampus and amgydala of bALS brains that were reported by 5 studies in which most patients developed FTD with disease progression (see Table 3-4). NFTs were not detected in any of the bALS cases within the mixed-group studies in Figure 3-3. Furthermore, one of the studies (Ishihara et al., 2006) identified basophilic inclusions that did not stain against ubiquitin, tau, or alpha-synuclein antibodies in the frontotemporal regions in the bALS cases. P62, neurofilament or FUS staining was not performed on these inclusions.
Figure 3-4. Summary of neuropathology by anatomical regions in bALS-only studies. CST = corticospinal tract; Nuc. = nucleus.

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* = pathology in the Substantia Nigra, but no pathology in the caudate or putamen

- Presence of pathology
- Absence of pathology
- Not reported
Figure 3-5. Summary of pathology types in bALS-only studies. Ubi+ = ubiquitin-positive; NCI = neuronal cytoplasmic inclusions. Incl. = inclusions.

<table>
<thead>
<tr>
<th>Routinely-stained pathology</th>
<th>Immunohistopathology</th>
<th>Inclusion shape</th>
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<tr>
<td>Neuronal Loos</td>
<td>Gliosis</td>
<td>Spongiosis</td>
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<tr>
<td>Myelin Pallor</td>
<td>Macrophage Aggregates</td>
<td>Ubi+/P62+NCIs</td>
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<tr>
<td>TDP-43+NCIs</td>
<td>Bunina Bodies</td>
<td>Lewy body-like incl.</td>
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<tr>
<td>Senile Plaques</td>
<td>Neurofibril. tangles</td>
<td>Basophilic inclusions</td>
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<td>Pick bodies</td>
<td>Skein-like incl.</td>
<td>Round Incl.</td>
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- Tsuchiya et al., 2000
- Bak et al., 2001
- Tsuchiya et al., 2002
- Ishihara et al., 2006
- Kuwahara et al., 2010
- Miki et al., 2010

* = inclusions did not stain against Ubi, tau, or alpha-synuclein. † = TDP-43+ inclusions also seen in glial cells, & = neurofibrillary pretangles were observed.
3.5 Discussion

The overall goal of this study was to compare and contrast the neuropathologic features - both morphology and anatomic distribution - of bALS to sALS by synthesizing the findings from existing literature. This systematic review summarized clinical and neuropathological data from 17 studies. The studies were analysed with respect to two contrasting hypotheses (Abrahams, 2013; Ichikawa et al., 2012; Schreiber et al., 2005). The first hypothesis proposed categorical pathological differences between bALS and sALS, with the former presenting with substantial extramotor (FTLD) signs as compared to the latter. The second hypothesis proposed that the two subtypes lay within the same spectrum of pathology. The results suggested that both subtypes, defined by the site of symptom onset, presented with comparable FTLD as well as the involvement of the brainstem and deep cortical structures. However, the involvement of regions that have been highly associated with speech and language functions (i.e., Broca and Wernicke areas) may be unique to bALS. When assessing the morphological properties of the pathology, both subtypes presented with neuronal loss and myelin pallor in the cortex and brainstem, along with P62/ubiquitin and/or TDP-43 positive inclusion bodies. In addition, there was evidence of secondary reactive changes, such as gliosis, spongiosis, and microglial activation in both subtypes. Yet, some bALS cases presented with atypical pathology, neurofibrillary tangles and basophilic inclusions, which were not found in any sALS cases.

3.5.1 Differential Involvement of Speech/Language Regions in bALS

Two studies reported differential involvement of specific regions of interest within the frontal and temporal cortices in bALS patients that were spared in sALS patients. Specifically, small regions related to speech and language processing—Broca and Wernicke areas—were differentially affected in bALS. The antemortem assessment of these patients indicated that these bALS cases initially presented with bulbar motor decline, which later evolved into primary progressive aphasia – a subtype of FTD with language changes. None of the sALS cases across the remaining studies were reported to have changes in these regions. Recent genetic and subsequent neuropathological studies have linked the co-existence of motor neuron disease and FTLD to C9ORF72 repeat expansions (Bigio, 2011; Murray et al., 2011). Extensive cortical and subcortical frontotemporal involvement may be associated with the C9ORF72 mutation, including reports of involvement of the Broca area (Bede, Bokde, Byrne, et al., 2013). Genetic testing for C9ORF72 mutations for the subjects in the two included studies reporting Broca area
involvement was not possible as the studies predated the discovery of the gene (Renton et al., 2011). The data, however, suggest a potential linkage between site of symptom onset, specifically bALS, and particular genotypes, specifically C9ORF72, reinforcing the concept that the pathogenesis of ALS may involve multiple distinct pathways that mediate disease onset and progression (Rothstein, 2009). Linking neuropathology with genetic information remains high priority for future studies.

The involvement of speech and language processing areas in bulbar ALS has been previously suggested by studies that reported language impairments, in ALS that were related to either bulbar-onset disease (Ichikawa et al., 2011; Ichikawa et al., 2008; Lomen-Hoerth et al., 2003), or to the degree of bulbar motor decline (Ichikawa et al., 2012; Massman et al., 1996; Phukan et al., 2012; Sterling et al., 2010; Yoshizawa et al., 2014), independent of dementia and motor disabilities. These observations, along with our analysis, suggest a potential co-development of aphasia and bulbar motor symptoms, which, from a pathophysiological perspective, may be directly related to the pattern of disease propagation within the brain. Currently, the factors that govern dissemination of pathology between segregated regions of the brain are unknown. A predominant theory postulates that propagation occurs within neuronal networks that form functional and structural connectomes, along the axonal wiring structure of the brain (Agosta, Weiler & Filippi, 2015; Evans et al., 2015; Schmidt et al., 2016; Seeley et al., 2009; Verstraete, Van Den Berg, Van Den Heuvel et al., 2014). A recent large-scale brain connectivity study in ALS has shown that abnormal proteins propagate to anatomic regions that are more closely interconnected by WM tracts than regions that are proximally closer, but with lesser connectivity (Schmidt et al., 2016). A co-development of aphasia following bulbar changes seems supportive of the connectome hypothesis, in which the pathways connecting the speech motor areas (i.e., the ventral region of the primary motor cortex) to speech/language processing areas may be degenerating in ALS. The speech-language connectome, however, has never been investigated in functional and structural connectivity studies in ALS. This may partially be due to the fact that tracts related to bulbar motor (i.e., corticobulbar tract) and language (i.e., arcuate fasciculus) functions were, until recently, difficult to isolate with existing imaging techniques (Li et al., 2013) and were not anatomically well-defined (Catani & De Schotten, 2008; De Schotten et al., 2011). Recent methodological advances have opened opportunities for this type of investigation.
While the bALS cases in the included studies presented with obvious bulbar changes during the
disease course, all sALS cases also presented with bulbar changes towards the end stage of the
disease, characterized by dysarthria and dysphagia. This may explain why some extra-motor
cortical regions that are involved in the speech-language connectome, such as the insular and
cingulate cortices (Fuertinger et al., 2015), were pathologically involved in both bALS and
sALS cases (Nagy et al., 1994; Nakano et al., 2004). Future studies would need to examine the
pathological profiles of those with fully preserved bulbar function at the time of death
(Fujimura-Kiyono et al., 2011), and compare them with those with bulbar disease, in order to
understand the link between bulbar disease, irrespective of onset site, and extramotor
involvement.

The comparison is further complicated by the fact that ALS, by definition, is a disease with
upper motor neuron (UMN) and lower motor neuron (LMN) pathology. The associated bulbar
changes are often characterised clinically by mixed spastic and flaccid dysarthria. The examined
studies did not define bulbar signs from the perspective of this distinction. Existing cognitive
behavioural studies reported a more prominent cognitive impairment in patients with UMN
involvement as compared to those with primarily LMN involvement (Abrahams et al., 1997;
David & Gillham, 1986; Gallassi et al., 1985; Gallassi et al., 1989), suggesting that
abnormalities of cognitive/language function may be indicators of a subgroup of patients with
corticobulbar (UMN) neuronal damage. However, such abnormalities do not seem to be
exclusive to the pseudobulbar palsy subtype (Abrahams et al., 1997) and the relation between
extramotor dysfunction and LMN bulbar symptoms should be addressed in future studies.

3.5.2 Atypical Pathological Features in some bALS Cases

The diagnostic tools and classification for ALS have evolved rapidly in recent years. The classic
descriptions of ALS focused on the degeneration of the motor neurons, suggesting a motor
neuron selective disorder (Hirano, 1991; Lawyer & Netsky, 1953). However, with the recent
discovery of TDP-43 protein (Neumann et al., 2006) and TARDBP mutation (Kabashi et al.,
2008; Sreedharan et al., 2008) linking ALS and FTD, a wave of new clinical, genetic,
neuropathological and epidemiological studies have suggested that ALS and FTD represent a
continuum of disease with shared clinical and pathological features (Lillo et al., 2012; Lomen-
Hoerth, Anderson, & Miller, 2002; Ringholz et al., 2005) including the presence of ubiquitin-
positive, TDP-43 positive, tau- and α-synuclein-negative inclusions throughout the central
nervous system (Lillo & Hodges, 2009; Neumann et al., 2006). For the cases included in this review, both bALS and sALS equally presented with typical ALS pathology, including TDP-43 proteinopathy.

Interestingly, however, 5 out of 16 studies that stained for co-existing AD pathology reported the presence of tau-positive neurofibrillary tangles in the hippocampus (Bak et al., 2001a; Kuwahara et al., 2010; Miki et al., 2010; Tsuchiya et al., 2000; Tsuchiya et al., 2002) and amygdala (Tsuchiya et al., 2002) for bALS cases, which were not reported for any of the sALS cases. One study also reported basophilic inclusions in the frontotemporal cortices and cerebellum (Ishihara et al., 2006) for bALS patients. FUS or neurofilament staining was not performed on these inclusions. Both NFTs and basophilic inclusions are not typically seen in classic ALS/FTD subtypes, but more common in other neurodegenerative phenomena (e.g. AD) and atypical forms of ALS, such as Guamanian ALS (Kokubo & Kuzuhara, 2004; Shankar et al., 1989) and Juvenile ALS (Bäumer et al., 2010; Tateishi et al., 2010). These atypical forms of the disease do not show a greater prevalence of bulbar-onset cases, however (Bäumer et al., 2010; Elizan et al., 1966). Furthermore, the anatomical distribution of pathology for the bALS patients with these atypical features was not distinctively different when compared with bALS cases with typical pathology; they all presented with a multi-system degeneration, including involvement of the amygdala, basal ganglia, thalamus, and cerebellum (Averbuch-Heller et al., 1998; Kato et al., 1993; Nakano et al., 2004; Ota et al., 2005). Although the medical history of these cases indicated a seemingly typical ALS, the clinical disease profiles were not sufficiently defined (e.g., cognitive evaluation was not reported), making it difficult to understand the clinical significance of these atypical pathological features. Noteworthy, these atypical inclusions were not observed in any of the sALS patients across all studies with similar staining protocols, even for the cases with similar disease durations, suggesting that these bALS patients may represent a unique subtype that is neuropathologically distinct from typical bALS and sALS.

3.5.3 Quality Assessment: Limitations of Existing Studies

Critical appraisal of the individual studies identified a number of methodological challenges of the published studies. They include: 1) a lack of matching between patient groups for their disease characteristics (i.e., use of ARS) and demographics, resulting in large variability in disease durations and severities; 2) insufficient clinical (i.e., both motor and cognitive)
description of cases; 3) insufficient documentation of co-existing FTLD and other neurodegenerative phenomena; 4) large variability in the neuropathology methodology across cases within and between studies, and 5) a lack of genetic information. Each of these methodological violations can place a study at substantial risk of bias, affecting the external validity of the findings. For example, the use and duration of ARS may be a confounding factor to the development of extra-motor impairments in ALS (Gordon et al., 2010; Kim et al., 2007; Massman et al., 1996), as it can lead to a more widespread pattern of cortical atrophy due to longer disease durations, and consequently, greater disease progression (Whitwell, Jack, Senjem, & Josephs, 2006). Secondly, antemortem bulbar signs were rarely reported and were limited to the mention of dysarthria (Cedarbaum et al., 1999). The perceptual judgement of the presence of speech changes may not be sensitive to detect more subtle abnormalities in bulbar physiology (Green et al., 2013). As a result, a clear distinction between cases with bulbar disease and those with pure spinal symptoms may be challenging. Thirdly, studies under documented co-existing FTLD in the ALS cases. This may be because most studies predated the discovery of the TDP-43 protein (Neumann et al., 2006), and the encoding TARDBP gene (Kabashi et al., 2008; Sreedharan et al., 2008) that were first to link ALS and FTD. Furthermore, the large variability in neuropathology protocols between subjects may have introduced a detection bias, where the observed pathological differences may be a result of differences in staining methods and sampling sites between subjects, and not a consequence of the disease. Lastly, some pathological abnormalities, especially the atypical features that were reported for some bALS cases, may be directly related to a known genetic mutation, which would better account for the pathological differences between groups.

3.5.4 Suggestions for Future Studies

Synthesizing findings across studies for the purposes of this review was difficult, primarily due to a lack of standardization across the neuropathology protocols. Overall, the existing studies offer limited information for determining if bALS has a unique neurodegenerative profile relative to sALS. In order to adequately distinguish the neuropathology for subtypes in ALS, future studies need to:

1. Expand and standardize clinical assessments to include antemortem clinical signs and symptoms, with an emphasis on bulbar changes and cognitive testing;
2. Include genetic testing in order to validate the neuropathological findings;

3. Expand and standardize neuropathology protocols regionally to include whole-brain analyses—with regions related to both motor and extramotor functions within the cortex, brainstem, deep cortical structures, and cerebellum—as well as include smaller, more specific, regions of interest within the cortex and WM tracts;

4. Expand and standardize staining methodology to include standardized screening and staging of co-existing neurodegenerative pathology such as Alzheimer’s disease, FTLD, and mesial temporal sclerosis;

5. Match patient groups or control for confounding factors, such as specific disease characteristics (i.e., duration of bulbar disease, and duration of ARS) and demographics.

3.5.5 Conclusions

The distinction between bulbar- versus spinal-onset patient groups is common in ALS literature and has implications for clinical management (i.e., predicting disease course and planning symptom management), and allocation to clinical trials. Yet, there is limited knowledge regarding the neuropathological differences between these subtypes. Neuroimaging and behavioural studies have suggested that the two subtypes may be distinct, with greater extramotor involvement in bALS (Lomen-Hoerth et al., 2003; Ogawa et al., 2009; Strong et al., 1999). However the literature remains inconclusive. This systematic review approached this knowledge gap by comparing neuropathology of the two subtypes across a number of existing studies. The findings revealed a great deal of overlap in the regions of involvement and types of pathology between the two subtypes. However a handful of studies suggested unique distribution and nature of pathology in bALS with a subsequent progression to primary progressive aphasia. Specifically, smaller cortical regions of interest related to speech and language processing seemed differentially involved in this subtype of bulbar-onset ALS. Critical appraisal of the literature gleaned that further work is needed as existing studies revealed multiple methodological limitations. In summary, determining if and how subtypes of ALS differ will require future studies designed to have standardized neuropathology protocols with clinical and genetic patient profiles.
Chapter 4
Neuropathology of the Speech Network Distinguishes Bulbar from Non-Bulbar Amyotrophic Lateral Sclerosis

4.1 Abstract

Bulbar ALS has significant implications for survival and quality of life and is associated with greater cognitive-linguistic deficits affecting brain structures outside of the motor system. The aim of this study was to compare neuropathology of the motor and extramotor cortical regions associated with the Speech Network (SpN) in three cases of bulbar-onset ALS (bALS), three cases each of spinal-onset ALS with and without antemortem bulbar dysfunction (sALSwB and sALSnoB) and three controls. All three bALS cases showed widespread neuronal loss and secondary changes across SpN regions; these changes were largely not observed in the sALSwB and sALSnoB groups. TDP-43 grading revealed marked pathology across all SpN regions in the three bALS cases; mild and focal pathology in two of the sALSwB cases; and no TDP-43 pathology in the sALSnoB cases. Two bALS cases showed widespread atypical TDP-43 morphologies and co-existing neurofibrillary tangles. The findings suggested that bulbar-onset ALS may have a distinct neuropathological signature with severe and widespread SpN damage and atypical proteinopathy. Milder SpN changes in sALSwB cases suggested that the extent of SpN damage may be related to bulbar motor disease. Findings support a clinicopathologic link between bulbar ALS and pathology in the SpN regions with significant clinical and research implications.

4.2 Introduction

Amyotrophic Lateral Sclerosis (ALS) is a multisystem neurodegenerative disease encompassing both motor and extramotor systems (Strong et al., 2017). The disease is fatal and typically fast progressing with a median survival of 3-5 years from disease onset (Haverkamp, Appel & Appel 1995). Among the motor manifestations of the disease are muscle spasticity, hyperreflexia, weakness, and atrophy, leading to eventual paralysis due to the progressive loss of upper (UMN) and lower motor neurons (LMN). Extramotor manifestations include behavioural and cognitive-linguistic deficits of frontotemporal dysfunction; 10% present with overt frontotemporal dementia (i.e., ALS-FTD) (Raaphorst et al., 2010), while up to 50% present with detectable
cognitive-linguistic deficits on neuropsychological assessment (Montuschi et al., 2015; Phukan et al., 2012).

Bulbar ALS is a clinical phenotypic subtype of ALS affecting speech and swallowing musculature (Langmore & Lehman, 2014). 30% of patients present with bulbar-onset ALS (bALS) which is characterized by initial symptoms in these muscles. The remaining patients present with spinal-onset ALS (sALS) initially affecting limbs and trunk muscles, yet, nearly 80% of sALS patients develop bulbar symptoms with disease progression (Haverkamp, Appel & Appel, 1995). Motor speech difficulties, or dysarthria, are the most common bulbar symptom affecting 93% of patients with bulbar ALS (Carpenter 3rd, McDonald & Howard Jr., 1978).

Bulbar ALS is arguably one of the most devastating subtypes of ALS – onset of bulbar signs and symptoms are associated with a faster rate of functional decline resulting in a shorter survival time (<2 years) (Haverkamp, Appel & Appel, 1995; Jablecki, Berry & Leach, 1989; Norris et al., 1993). Greater bulbar symptoms are associated with an increased risk of aspiration pneumonia (Hardiman, 2011) which is one of the main causes of death in ALS (Corcia et al., 2008). Further, patients with ALS report the most devastating effects of dysarthria and dysphagia on their quality of life (Bourke et al., 2006; Worwood & Leigh, 1998; Simmons et al., 2013) with a loss of communication leading to social isolation (Watts & Vanryckeghem, 2001; Yorkston, 2007) and swallowing difficulties leading to lifestyle changes and complications of choking and/or malnutrition (Muscaritoli et al., 2012). In addition to the devastating motor consequences of this subtype, neuropsychological investigations have linked bulbar symptoms to an increased burden of extramotor cognitive-linguistic deficits (Sterling et al., 2010; Ichikawa et al., 2010; Abrahams, Goldstein, Pickering et al., 1997), the presence of which may be an independent adverse prognostic factor in ALS (Elamin et al., 2011). The extent of the link between bulbar motor and extramotor dysfunctions is not yet known.

Autopsy continues to play an important role in the understanding and diagnosis of ALS as post mortem examination confirms the clinical diagnosis and identifies specific subtypes of ALS, some of which are hereditary and have implications for surviving family members (Renton et al., 2011; Rosen et al., 1993). It also allows the presence of co-existing neurodegenerative phenomena to be documented and staged and provides data for tissue-based research into the pathogenesis of this devastating disease. Macroscopic neuropathological abnormalities associated with classical ALS include atrophy of the anterior nerve roots and the precentral
Microscopically, ALS cases exhibit neuronal loss with concomitant gliosis and spongiosis. Additionally, the accumulation of ubiquitin/P62-immunoreactive intracellular misfolded protein aggregates primarily composed of transactive response (TAR) DNA-binding protein of 43 kDa (TDP43) are found within the primary motor cortex, anterior horns, and motor cranial nerve nuclei in the surviving neurons or glial cells. Degeneration of descending corticospinal tracts is also prominent (Ince et al., 2003). Co-existing frontotemporal lobar degeneration (FTLD) is seen in most ALS-FTD cases and in 25 to 50% of “pure” ALS cases (Mackenzie & Feldman, 2003; Okamoto et al., 1991) and is characterized by variable atrophy of frontal and temporal regions with TDP-43-immunoreactive dystrophic neurites and neuronal inclusions (FTLD-TDP43) (Arai et al., 2006). Four different histological types of FTLD-TDP43 have been recognized (A-D) based on unique conformations of TDP-43 aggregates and clinicopathologically linked to FTD phenotypes, including a Harmonized Type B associated with ALS-FTD phenotype (Mackenzie et al., 2011). As such, a clinical and neuropathological spectrum between ALS and FTD has been supported, with differences between phenotypes partially explained by pathological variations in the neuronal type (i.e., motor vs. extramotor neurons), and the morphology and density of TDP-43 pathology (Arai et al., 2006; Mackenzie et al., 2011). Neuropathological differences in motor and extramotor regions between bulbar and spinal phenotypes have not yet been empirically examined.

The neuroanatomy of speech function has been well established based on fMRI, electrical stimulation, and lesion studies in humans (Hickok 2012, 2001; Hagoort & Levelt 2009; Guenther, Ghosh & Tourville, 2006b; Golfinopoulos, Tourville & Guenther, 2010). One of the most influential and well-tested computational models of speech production is the DIVA model by Guenther and colleagues (2006a). The DIVA model maps a feedforward control system that plans and initiates speech movements and a feedback control scheme that guides the movements using somatosensory and auditory feedback onto cortical and subcortical neuroanatomy. The established neural substrates underlying this model include: the ventral (oral) portion of the primary motor cortex (bulbar PMC); premotor and supplementary motor areas in the prefrontal cortex related to planning speech sound targets and initiating a chosen movement; ventral somatosensory cortices (i.e., bulbar PSC) receiving real-time somatosensory feedback used to correct motor commands; auditory cortices (i.e., primary acoustic cortex (PAC) in the transverse temporal gyrus) receiving real-time auditory feedback used to correct motor commands; inferior
frontal gyrus (IFG) contributing to sequence planning and timing release of planned speech movements (Golfinopoulos, Tourville & Guenther, 2010); posterior superior temporal gyrus (pSTG) a “higher-order” auditory cortical area integrating auditory feedback and target; and cerebellum and basal ganglia, contributing to precisely timed commands, together forming a large-scale speech network (SpN) (Guenther, Ghosh, & Tourville, 2006b; Golfinopoulos, Tourville & Guenther, 2010). The SpN encompasses both motor and extramotor regions, some of which are also involved in cognitive-linguistic processing. Specifically, the IFG and pSTG are known to be involved in mapping sound-to-meaning and in syntactic structuring and processing of sentences (A D Friederici, 2003; St Heim, Opitz & Friederici, 2003; Whitney et al., 2011; Lau, Phillips & Poeppel, 2008). The high occurrence of speech dysfunction caused by bulbar motor disease and the possible link between bulbar symptoms and cognitive-linguistic deficits suggest that an impairment of SpN in bulbar ALS is highly likely. However, the extent and distribution of pathology within the SpN in ALS remains speculative.

Our recent systematic review (Shellikeri et al., 2017) was designed to identify studies that compared the neuropathology of bALS and sALS and found that the relevant studies were very few. The review found that selected frontal and temporal extramotor regions may be affected exclusively in bALS (Bak et al., 2001; Kato et al., 1994). Specifically, published studies reported neuronal loss and TDP-43 inclusions in the IFG and pSTG in selected bALS cases but not in any sALS cases. Differences in proteinopathy between bALS and sALS were also reported – some bALS cases had basophilic inclusions and/or tau immunoreactive neurofibrillary tangles (NFTs) in the frontal and temporal lobes of the cortex (Ishihara et al., 2006; Tsuchiya et al., 2002; Bak et al., 2001; Kuwahara et al., 2010; Miki et al., 2010). Together, the findings suggested that bALS might be distinct from sALS with neuroanatomic and compositional differences in the underlying pathology. However, the neuropathological studies to date are not designed to compare ALS subtypes, and as such the reported cases were not matched for demographics or disease-related variables. The presence of antemortem bulbar motor impairments, particularly for sALS cases, was not indicated in the majority of studies to allow comparisons between bulbar variants (i.e., bALS versus sALS patients that develop bulbar dysfunction with disease progression). Reports of region-based results were also rare. In addition, most blocking protocols that neuropathologists employ for ALS cases do not routinely include sampling of the SpN (other than the motor cranial nerve nuclei), likely contributing to
our poor understanding of the pathogenesis of bulbar symptoms in ALS. There is a need to study hypothesis-specific regions in the brain that may be vulnerable to bulbar ALS.

The main aim of the present study was to further our understanding of the differences between bulbar and non-bulbar subtypes of ALS by examining post-mortem neuropathological changes in specific frontal and temporal regions of the SpN. Specific objectives of this study were to compare the neuroanatomic regional distribution, severity, and composition of neuropathology in the SpN regions between bALS cases, sALS cases with antemortem bulbar motor disease (sALSwB), and “pure spinal” sALS cases without antemortem bulbar motor disease (sALSnoB). We hypothesized that the severity and neuroanatomic distribution of pathology in SpN regions would be greatest in cases with bALS compared to sALSwB and sALSnoB cases.

4.3 Materials and Methods

4.3.1 Study Cohort

This study was based on the clinical and autopsy findings of nine individuals who had a clinical and autopsy-confirmed diagnosis of sporadic adult-onset ALS (Brooks, Miller, Swash, & Munsat, 2000) at the ALS/MND Clinic at Sunnybrook Health Sciences Centre, Toronto, Canada between the years of 1998-2019. As of January 2019, the complete autopsy database comprised of 86 ALS subjects. Autopsy cases predating 2009 followed a different autopsy sampling protocol and so were excluded to minimize detection bias, leaving 44 cases for inclusion. A reviewer (Author SS) blinded to the contents of the autopsy reports (e.g., presence or absence of co-existing neurodegenerative phenomena) performed retrospective chart reviews to pseudo-randomly select nine ALS cases for inclusion in the study. Specifically, the first three cases in each of the three ALS groups that met the a priori inclusion criteria and with accessible archived formalin-fixed cadaveric brain tissue were included in this study: three cases of bulbar-onset ALS (bALS), three cases of spinal-onset ALS (sALS) with reported antemortem bulbar dysfunction (sALSwB), and three sALS cases with intact antemortem bulbar function (sALSnoB) were chosen. Operational definition of antemortem bulbar dysfunction is described in the section below. In addition, three sex- and age-matched control cases without significant neuropathology were included in this study.
The tissue archives used in this study followed approved procedures for the donation and storage of clinical information in accordance with Sunnybrook Research Ethics Board guidelines and the Declaration of Helsinki.

4.3.2 Clinical and Medical Autopsy Reports

All patients were clinically assessed by an experienced neuromuscular neuropathologist (Author LZ) at the ALS/MND clinic at Sunnybrook Health Sciences Centre, Toronto, Canada. Clinical information and demographics were extracted from retrospective review of the patient clinical chart and included: age and sex, date of disease onset, date of bulbar symptom onset, site and laterality of onset, and antemortem cognitive status. Antemortem bulbar dysfunction was operationally-defined as any finding of bulbar signs and/or symptoms on: neurological examination indicating UMN or LMN signs in orofacial musculature; patient-reported changes on the speech, swallowing, or salivation domains on the ALS Functional Rating Scale – Revised (Cedarbaum et al., 1999); or patient reports of dysarthria, dysphagia or related functional changes (e.g., choking, drooling, soft voice, etc.) at any disease stage. Cognitive dysfunction were operationally defined as a score <26 on the last-recorded Montreal Cognitive Assessment (MoCA, (Nasreddine et al., 2005)) or a symptom report of behavioural and/or cognitive changes.

The neuropathologic autopsy reports for each case were also reviewed (by Author SS), and the final neurodegenerative diagnoses and stages were recorded.

4.3.3 Pathological Evaluations

4.3.3.1 Tissue Sampling and SpN Regions-of-Interest

All cases had previously undergone standard neuropathologic diagnostic protocols at the time of autopsy. For the ALS cases, this included the right hemisphere, hemi-cerebellum, hemi-brainstem and three levels of spinal cord, snap frozen. The remaining left cerebral hemisphere, left hemi-brainstem and hemi-cerebellum, and levels of spinal cord were fixed in formalin for at least two weeks. After formalin fixation, the left cerebral hemisphere was sectioned into approximately 1 cm thick coronal sections; the hemi-brainstem and spinal cord tissue were sectioned into approximately 3 mm thick axial sections, and the cerebellum was sectioned sagittally. A standard ALS blocking protocol was followed which included 23 blocks submitted from the sensorimotor cortex, frontal and temporal cortex, hippocampus, subcortical grey matter
structures, three levels of brainstem, cerebellum, and cervical, thoracic and lumbar spinal cord. These tissue blocks were then processed, embedded in paraffin, cut into 7 micrometer thick sections, mounted on glass slides, and stained with H&E/LFB and immunohistochemistry for p62, TDP-43, beta-amyloid, alpha-synuclein, and/or tau (AT8). These slides were examined by an experienced neuropathologist and enabled a neuropathological diagnosis of ALS with or without co-existing FTLD, and/or Alzheimer’s type neuropathologic change, and/or Lewy bodies to be made. A diagnostic neuropathologic autopsy report was then issued.

For the purposes of this study, original blocks containing the pons and medulla were retrieved, cut into additional 7 micrometer thick sections, and stained to assess pathology in brainstem LMN nuclei. For cortical regions and additional brainstem components of the SpN, the archived formalin-fixed brain tissue was retrieved and our study regions-of-interest that were not part of the original blocking protocol were identified and blocked using predefined anatomical landmarks (see Table 4-1).

Eight blocks corresponding to 11 regions were evaluated in total. Table 1 lists the blocks, corresponding regions, and landmarking details used for tissue sampling. Figure 1 shows the anatomical mapping of the regions that were sampled and studied. Regions were chosen based on previous literature indicating their role in the SpN (Fuertinger, Horwitz, & Simonyan, 2015; Guenther & Perkell, 2004; L K Tyler & Marslen-Wilson, 2008; Vassal et al., 2016); cortical regions were chosen due to the frequent involvement of cerebral changes in ALS patients. In addition, brainstem LMN nuclei that innervate bulbar (orofacial) musculature (i.e., tongue, lips, and jaw) were assessed in order to characterize the relative contribution of LMN loss to bulbar presentation. For brainstem regions, the pons and medulla were sampled in total. Five of the nine blocks contained two adjacent gyri; in order to help with orientation under the microscope, one of the two gyri was nicked and noted during tissue sampling. PMC regions were anatomically confirmed through microscopic identification of motor neurons within the tissue section.
Table 4-1. A list of the included blocks with the corresponding SpN and brainstem regions and anatomic sampling locations. CN = Cranial nerve.

<table>
<thead>
<tr>
<th>Block #</th>
<th>Region(s)-of-interest</th>
<th>Anatomic sampling location</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cerebral Cortex</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| 1 | (3) Bulbar PMC  
(4) Bulbar PSC | Pre- and post-central gyri taken at the level of the occipital horn, one slice anterior to Calcarine cortex, corresponding to the “oral” region on the motor and sensory homunculi |
| 2 | (5) Supplementary motor area 
(SMA)  
(6) Premotor area | Superior (sfg) and middle frontal gyri (mfg) taken at the level of the hypothalamus |
| 3 | (7) IFG | Inferior frontal gyrus taken at the level of the caudate head roughly at either the pars triangularis or pars opercularis |
| 4 | (8) pSTG | Posterior superior temporal gyrus taken at the level of the pulvinar |
| 5 | (9) pSTG deep white matter 
(WM) | White matter underlying the pSTG taken at the level of the pulvinar |
| 6 | (10) Primary Acoustic Cortex 
(PAC) | Transverse temporal gyrus (TT) taken at the level of the subthalamic nucleus |
| **Brainstem** | | |
| 7 | (11) Hypoglossal nucleus 
(CNXII) | Lower medulla |
| 8 | (12) Trigeminal motor nucleus 
(CNVmo)  
(13) Facial nucleus (CNVII) | Superior pons |
Figure 4-1. The neuroanatomical mapping of the SpN cortical regions and brainstem LMN regions that were assessed in this study.
In addition, the original stained slides from each case were retrieved. The original tau (AT8) and beta-amyloid stained slides were re-reviewed by an experienced neuropathologist (Author JK) and the presence of Alzheimer’s type neuropathologic change and Lewy body pathology were re-assessed and staged.

4.3.3.2 Stains and Histological Features

The eight blocks created for each study patient were cut and stained with H&E/LFB and immunohistochemistry for p62, TDP-43 and/or tau (AT8) using the methods described in Appendix C: Neuropathology Supplementary Material.

Cortical regions were assessed for neuronal loss, gliosis and spongiosis; brainstem LMN nuclei were assessed for neuronal loss and gliosis; and white matter tracts were assessed for pallor of myelin staining, all on H&E/LFB staining. These features were chosen for analysis, along with the presence and density of TDP-43 and p62 inclusions, because they represent the classic neuropathologic findings in ALS and FTLD-TDP43 (Esiri, Squier, and Perl, 2006; Mackenzie et al., 2011). Tau (AT8) was used to screen for neurofibrillary tangles (NFTs) based on previous findings of tauopathy in bALS cases (Strong et al., 2006; Strong & Yang, 2011; Shellikeri et al., 2017).

All slides were assessed at a multi-header microscope by two raters, an experienced neuropathologist (Author JK) who was blinded to the case ID and clinical diagnosis, and a trainee (Author SS). The two raters achieved consensus on all assessments. Slides were scored semi-quantitatively on a 5-point Likert scale with 0 indicating a complete absence of pathology and 3+ indicating severe or marked pathology. Figure 4-2 shows examples of ratings for TDP-43 scores in the cortex.

In order to establish intra-rater reliability on the ratings, a subset of cases (n=4) were evaluated a second time after 6 months. Scores were compared with the initial ratings for each histological feature in each region and showed excellent reliability with an intra-rater variance of <3.5%, calculated as the percentage of regions with an altered score. For those with an altered score, the first rating was replaced with the second rating. Furthermore, to ensure consistency in ratings for regions with complicated neuroanatomy (e.g., the CNVmo, arcuate fasciculus, and transverse temporal gyrus), the slides for all cases were grouped by region and re-evaluated a second time.
Selected slides were digitally scanned using a Leica Biosystems Aperio AT Turbo model scanner and digital photomicrographs were taken using Aperio software.
Figure 4-2. Photomicrographs demonstrating semi-quantitative ratings of: mild (1+, A), moderate (2+, B), and severe (3+, C) TDP-43 pathology (NCIs) in the cortex (shown for MND12: pSTG, SFG, and IFG regions, respectively).
4.4 Results

4.4.1 Clinical Descriptors and Diagnostic Neuropathologic Autopsy Summaries

Table 4-2 summarizes the demographics, clinical information, and neuropathologic autopsy reports for each examined ALS case. Disease durations were calculated from date of reported symptom onset to death. None of the subjects were treated with invasive ventilation, but all subjects had used non-invasive ventilation (i.e., BiPAP) during the course of the illness.

The control cases consisted of 2 males and 1 female with an average age of 63 years (SD = 7). Causes of death were epiglottitis for one control subject and myocardial infarction for the other two.
Table 4-2. Summary of clinical and diagnostic neuropathological data obtained from the medical charts and autopsy reports for each ALS case. bALS = bulbar-onset ALS; sALSwB = spinal-onset ALS with antemortem bulbar disease; sALSnoB = spinal-onset ALS without antemortem bulbar disease; R = right side; L = left side; bi = bilateral; NA = not applicable; NR = not reported; M = Male; F = Female; UE = Upper extremities; LE = lower extremities; LMN-p = Lower motor neuron predominant; hd = Head Drop; Y = Yes; Bulbar signs: + = UMN and/or LMN signs in the tongue (fasciculations, needle EMG abnormalities); ++ = functional oromotor changes (dysarthria, dysphagia); +++ = anarthria. Cognitive dysfunction: MoCA scores (Nasreddine et al., 2005) in addition to symptom reports.

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>bALS</th>
<th>sALSwB</th>
<th>sALSnoB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case ID</td>
<td>MND12 MND10 MND11</td>
<td>MND05 MND03 MND19</td>
<td>MND18 MND17 MND08</td>
</tr>
<tr>
<td>Sex</td>
<td>M</td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>Site of onset</td>
<td>Bulbar</td>
<td>Bulbar</td>
<td>Bulbar</td>
</tr>
<tr>
<td>Age at death</td>
<td>73</td>
<td>67</td>
<td>52</td>
</tr>
<tr>
<td>Disease duration (months)</td>
<td>7</td>
<td>18</td>
<td>23</td>
</tr>
<tr>
<td>Bulbar disease duration (months)</td>
<td>7</td>
<td>18</td>
<td>23</td>
</tr>
</tbody>
</table>

**Clinical Signs at Onset**

- **Bulbar signs**: ++ ++ ++ ++
- **UE signs**: - + - + (LMN-p) - - ++ ++ (++hd) ++ (++hd)
- **LE signs**: - - - ++ (R) ++ (LMN-p) ++ + -
- **Cognitive Dysfunction**: - - -
- **Other**: Flail-arm variant Flail arm variant

**Clinical Signs with progression**

- **Bulbar signs**: +++ +++ +++ ++ ++ + - - -
- **UE signs**: + ++ ++ ++ - - ++ +++ ++ +++
<table>
<thead>
<tr>
<th>LE signs</th>
<th>+</th>
<th>+</th>
<th>++</th>
<th>+</th>
<th>+++</th>
<th>+++</th>
<th>+++</th>
<th>+</th>
<th>++ (L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cognitive Dysfunction</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(pseudobulbar affect)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Diagnostic Neuropathologic findings**

<table>
<thead>
<tr>
<th>ALS</th>
<th>Y</th>
<th>Y</th>
<th>Y</th>
<th>Y</th>
<th>Y</th>
<th>Y</th>
<th>Y</th>
<th>Y</th>
<th>Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>FTLD-TDP43</td>
<td>Y</td>
<td>Y</td>
<td>-</td>
<td>Y</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Braak NFT Stage (I-VI)</td>
<td>II</td>
<td>-</td>
<td>III</td>
<td>-</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CERAD plaque stage (A-C)</td>
<td>B</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>B</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lewy bodies (distribution)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Y</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

(Notes: ALS, FTLD-TDP43, Braak NFT Stage, CERAD plaque stage, Lewy bodies, (pseudobulbar affect), (brainstem))
4.4.2 Neuropathological Findings

4.4.2.1 Patterns of Neuronal Loss, Gliosis and Superficial Spongiosis across Case Groups

Table 4-3 shows the ratings for neuronal loss (in shades of blue), gliosis (red), and superficial spongiosis (green) for each region and case as assessed on HE/LFB staining. Data points that were missing or not assessed are indicated in grey.

Nearly all SpN regions were most severely and widely affected in the three bALS cases. The motor and extramotor SpN regions showed neuronal loss and secondary changes only in these cases. Both sALS phenotypes were mostly unremarkable on routine staining. Interestingly, WM pallor underlying the pSTG was noted in one case per each phenotype (see Figure 2). For brainstem motor regions, particularly of CNXII, changes were observed across nearly all cases, regardless of phenotype. None of the three control cases had neuronal loss or degenerative phenomena in any of the examined regions.
Table 4-3. Ratings for neuronal loss (NL, in shades of blue), gliosis (Gli., pink), and superficial spongiosis (Spon., green) across cortical SpN and brainstem LMN regions for each case, as seen on the HE/LFB stain. Grey cells indicate missing data or not applicable. + = mild, ++ = moderate, +++ = severe, - = none; b = moderate burden of Bunina bodies were seen in the LMN. ^ = white matter region evaluated for axonal pallor; bALS = bulbar-onset ALS; sALSwB = spinal-onset ALS with antemortem bulbar dysfunction; sALSnB = spinal-onset ALS without antemortem bulbar dysfunction.

<table>
<thead>
<tr>
<th>Region/Feature</th>
<th>bALS</th>
<th>sALSwB</th>
<th>sALSnB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MND12</td>
<td>MND10</td>
<td>MND11</td>
</tr>
<tr>
<td>Cortex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulbar PMC</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Bulbar PSC</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>SFG (SMA)</td>
<td>-</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>MFG (PreMot or)</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IFG</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>pSTG</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>pSTG deep WM</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>PAC (TT)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Brainstem</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNVmo</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>CNVII</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>CNXII</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>
Figure 4-3. Photomicrographs of white matter myelin pallor scores of 0 in MND18 (A, scale bar: 2mm; C, scale bar: 200um) and 2+ in MND11 (B, scale bar: 2mm, D, scale bar: 200um) for the white matter underlying pSTG, HE/LFB.
4.4.2.2  Patterns of TDP-43 and Tau Immunopathology across Case Groups

Figure 4-4 shows the distribution and severity of TDP-43 (in shades of red/orange/yellow) and tau (in shades of blue) proteinopathy for each examined region and case as seen on the TDP-43 and AT8 immunohistochemical stains. All observed TDP-43 and tau immunopositive inclusions were also immunoreactive for p62, which did not highlight any additional neurodegenerative type inclusions. None of the three control cases had proteinopathy in any of the examined regions.
Figure 4-4. Severity ratings of TDP-43 (shades of red/orange/yellow) and tau (shades of blue) proteinopathy for each cortical SpN and brainstem LMN region and case, as seen on the TDP-43 and AT8 immunohistochemical stains. bALS = bulbar-onset ALS; sALSwB = spinal-onset ALS with antemortem bulbar dysfunction; sALSnoB = spinal-onset ALS without antemortem bulbar dysfunction; HC = neurotypical controls; TDP-43 -NCI = TDP-43 –immunoreactive neuronal cytoplasmic inclusions that have “skein-like” or “circumferential” morphology; TDP-43 -DN = TDP-43- immunoreactive dystrophic neurites; NFT = neurofibrillary tangles; g = inclusions also found in glial cytoplasm; ~ = tau-positive DN were also seen in superficial (II and III) and deep (>IV) cortical layers; O = TDP-43 inclusions were seen in superficial and deep cortical layers; w = inclusions also seen in subcortical white matter.

<table>
<thead>
<tr>
<th>Cortex</th>
<th>bALS</th>
<th>sALSwB</th>
<th>sALSnoB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulbar PMC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulbar PSC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SFG (SMA)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MFG (PreMotor)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pSTG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pSTG deep WM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brainstem</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
**TDP-43 pathology**: The 3 bALS cases differed from the other two phenotypes, sALSwB and sALSnoB, by a greater density (up to 3+) and a more widespread spatial distribution of TDP-43 aggregates across the SpN cortical regions. In contrast, the TDP-43 burden was less (up to 2+) and localized to only selected SpN regions in 2 out of 3 sALSwB cases, and absent from the SpN in sALSnoB cases (except for MND08 with aggregates in the bulbar sensorimotor cortex). TDP-43 inclusions were found in the LMN of the brainstem for most cases across all phenotypes, particularly in the hypoglossal nucleus (CNXII).

Figure 4-5A-B show the two predominant morphologies of TDP-43 protein aggregates seen in the cortical and brainstem neurons of the cases—(A) “circumferential” and (B) “skein-like” neuronal cytoplasmic inclusions (Tan et al., 2017). A third common inclusion type was TDP-43 positive dystrophic neurites (DN), shown in Figure 4D (outlined arrows). One bALS case (MND12) had round, dot-like neuronal cytoplasmic inclusions, shown in Figure 4C-D (filled arrows), as well as granular TDP-43 positive cytoplasmic aggregates, in many of the examined SpN regions. The same case also had an isolated TDP-43 and P62-positive glial cytoplasmic inclusion (GCIs) in the pSTG WM, shown in Figure 4-4E.

**Tau pathology within the SpN**: One bALS case (MND11) with an abundance of TDP-43 dystrophic neurites also had widespread and up to 3+ burden of neurofibrillary tangles in the SpN regions. Another bALS case (MND12) that had atypical morphologies of TDP-43 inclusions also had an isolated NFT in the pSTG. The sALSwB and sALSnoB cases did not show tauopathy in any examined regions.

The predominant morphology of tau inclusions was neurofibrillary tangles (NFTs), shown in Figure 4-4F (filled arrows). One bALS case (MND11) also had 3+ tau-positive inclusions in the glial cells (i.e., GCIs) and tau-positive DN across multiple SpN regions (Figure 4-4F, outlined arrows). This case had no other abnormalities reminiscent of an FTLD-tau.
Figure 4-4. Photomicrographs of TDP-43 and tau immunopositive inclusions. “Circumferential” TDP-43 neuronal cytoplasmic inclusions (NCIs) in the IFG of MND10 (A, TDP-43). “Skein-like” TDP-43 positive NCIs in the CNVmo of MND07 (B, TDP-43). “Dot-like” TDP-43- and P62-immunoreactive NCIs in the bulbar PMC of MND12 (C, TDP-43; D, P62, filled arrows). TDP-43 dystrophic neurites (DNs) in the bulbar PMC of MND12 (D, outlined arrow, P62). Isolated TDP-43 and P62-positive glial cytoplasmic inclusion in the arcuate white matter of MND12 (E, P62). Tau-positive NFTs (filled arrows) and DNs (outlined arrows) in the IFG of MND11 (F, AT8). Scale bars: 100 um.
4.5 Discussion

4.5.1 Summary of Findings

This study characterized the neuropathology of the Speech Network (SpN) regions in bulbar and non-bulbar clinical variants of ALS in order to assess the neuropathological features associated with bulbar symptoms in ALS. The findings suggested neuropathological differences in distribution and types of pathology between bulbar-onset ALS (bALS) and spinal-onset ALS (sALS). Specifically, we found widespread neuronal loss with secondary reactive changes and a high density of TDP-43 inclusions across most SpN cortical regions in all three bALS cases. By contrast, the sALS cases (both sALSwB and sALSnoB) showed no neuronal loss but mild and variable TDP-43 pathology in focal SpN regions, particularly in the sALSwB cases. Compared to the cortical regions, brainstem LMN regions were comparably affected across all cases and phenotypes. Examination of compositional differences in proteinopathy showed that two of the three bALS cases had widespread atypical TDP-43 morphologies; these two cases also had co-existing NFTs in the SpN regions. Furthermore, pathology distribution differences between bALS, sALSwB and sALSnoB suggested a potential link between the severity of antemortem bulbar dysfunction and degree of SpN damage. The findings may have potentially significant clinical and research implications.

4.5.2 SpN motor changes across ALS phenotypes

As expected, all three bALS cases presented with neuronal loss and severe burden of TDP-43 pathology in the bulbar PMC. This region was relatively spared in the other two phenotypes, including in the sALSwB cases with reported bulbar symptoms. An exception to this was one sALSwB case with up to 2+ TDP-43 NCIs in the bulbar PMC region- this individual presented with the longest bulbar disease duration compared to all other examined cases. The two remaining sALSwB cases without this pathology were clinically described as LMN-predominant at onset, presumably contributing to the lack of UMN loss seen in their bulbar PMCs. Limb sub-regions on the PMC may show neuropathological changes in these cases, but were not included in our tissue sampling. ALS cases with confirmed clinical UMN signs but no evidence of neuronal loss in the PMC have also been described previously (Ellison et al., 2012).

Bulbar PMC was spared in all sALSnoB cases except for one case showing mild density of atypically localized TDP-43 NCIs and DNs. The clinical consequences of this atypical
pathology are not clear; descriptions of antemortem bulbar dysfunction were limited to the clinical assessment of bulbar dysfunction by the neurologist or symptom reports (i.e., ALSFRS-R). This case did not differ from the other two pure spinal cases in terms of demographics or other clinical characteristics. Deeper phenotyping with a more comprehensive instrumental assessment of speech may be required to further understand the motor underpinnings of bulbar ALS (Green et al., 2013; Yunusova, Green, Wang, Pattee, & Zinman, 2011).

In contrast to cortical UMN changes, brainstem LMN regions showed comparable neuronal loss and TDP-43 inclusions across all examined cases and phenotypes, including in cases without antemortem bulbar symptoms. This finding is consistent with previous ALS literature indicating a universal vulnerability of brainstem motor nuclei, specifically of the hypoglossal nucleus innervating tongue musculature and controlling respiration (DePaul, Abbs, Caligiuri, Gracco, & Brooks, 1988; Halliday et al., 2016; Langmore & Lehman, 2014).

4.5.3 SpN extramotor regional changes across ALS phenotypes

Extramotor regions part of the SpN that were affected in our cohort included secondary motor areas such as the SMA and premotor cortex in the planning and programming of speech; “lower-order” stimuli processing regions such as the bulbar PSC and PAC that are involved in processing somatosensory and auditory feedback during speech; and “higher-order” cognitive-linguistic regions such as the IFG and pSTG that are involved in integrating information from multiple regions (Guenther et al., 2006). Previous studies in ALS have identified changes in similar regions: specifically, cortical thinning and reduced fractional anisotropy of WM in the SMA, IFG and pSTG has been consistently reported, particularly in bALS individuals (Graaff et al., 2011; Kim et al., 2017; Mezzapesa et al., 2013; Sala et al., 2019). Reports of changes in stimuli processing areas, however, are more rare (but see Grosskreutz et al., 2006; Mohammadi et al., 2009; Shellikeri et al., 2019). In our study, neuronal loss and TDP-43 inclusions in the bulbar PSC were observed for all cases with pathology in the adjacent bulbar PMC; neuropathology of the PAC was observed only in the three bALS cases. The findings from our study widen the current understanding of the extramotor disease effects in ALS (Brettschneider et al., 2014; Geser et al., 2009; Mackenzie et al., 2011) to include primary stimuli processing areas within the SpN.
4.5.3.1  Neuropathological differences between bulbar onset and spinal onset cases

The three bALS cases showed a distinct neuropathological signature compared to the six sALS cases. Overall, bALS was characterized by widespread neuronal loss and a severe burden of TDP-43 proteinopathy across all regions of the cortical SpN. The finding suggests that bALS may show preferential involvement of frontotemporal regions, as was seen here within the SpN. This would be consistent with existing studies reporting greater overall cortical involvement in bALS compared to sALS cases (Piao et al., 2003; Shellikeri et al., 2017). Greater cortical atrophy could not be attributed to longer disease durations in the three bALS cases (Iwanaga et al., 1997; Nishihira et al., 2009). Instead, bulbar onset disease may be pathogenically unique with a longer prodromal phase, enabling greater cortical atrophy before onset of symptoms. This idea is supported by large population-based studies that reported a more “aggressive” presentation in bALS with a faster rate of functional decline and a shorter survival (Haverkamp, Appel, & Appel, 1995; Jablecki et al., 1989; Norris et al., 1993). It is worthy to note that the rate of bulbar motor decline between bALS and sALS individuals are similar (Haverkamp et al., 1995; Mills, 2003) and so, does not explain the worse prognosis of bALS subtype. Prodromal widespread extramotor involvement may be contributing to the aggressiveness of the bALS phenotype however the underlying factors mediating these changes remain largely unexplained. Potential differences in disease pathogenesis between bALS and sALS subtypes need to be further explored. Additionally, serial examination of the brain in required in order to ascertain that the observed changes extend to other frontotemporal regions beyond the SpN.

Regional differences in the distribution of neuropathology between onset types revealed that the IFG and pSTG were among the regions consistently affected in bALS but largely spared in sALS (except for in one case with the longest antemortem bulbar symptoms). These regions have been associated with cognitive and language functions including verbal fluency and syntactic and semantic processing of language (Friederici, 2003; Hickok & Poeppel, 2007; Lau et al., 2008; Obleser, Wise, Alex Dresner, & Scott, 2007; Rauschecker & Tian, 2000; Tyler et al., 2011). Our finding provides neuroanatomic support for the previously reported clinical link between bulbar-onset ALS and greater cognitive-linguistic deficits (Abrahams, Goldstein, Al-Chalabi, et al., 1997; Bak & Chandran, 2012; Raaphorst et al., 2010; Sterling et al., 2010). The finding informs the need for cognitive and language testing in patients with bulbar presentation.
at disease diagnosis (Gladman, Cudkowicz, & Zinman, 2012; Mitumoto, Brooks, & Silani, 2014).

The presence of co-existing neurodegenerative phenomena did not differentiate between onset types (Piao et al., 2003). In our cohort, FTLD-TDP43 was noted in three cases; AD-like neuropathological changes in two cases (Montine et al., 2012); Primary Age-Related Tauopathy (PART) (Crary et al., 2014) in three cases; and Lewy bodies in the brainstem in one case. However, compositional properties of proteinopathy within the SpN appeared to be distinct between the two onset types. Specifically, two bALS cases showed atypical TDP-43 morphologies and co-existing atypical tauopathy in SpN regions, while all the examined sALS cases only showed typical skein-like or circumferential TDP-43 NCI s. One bALS case (MND11) showed a prominent appearance of TDP-43 dystrophic neurites and no neuronal cytoplasmic inclusions, which was reminiscent of FTLD-TDP43 Harmonized Type C associated with semantic and behavioural variants of FTD (Mackenzie et al., 2011; Takeuchi et al., 2016). Our case did not have a clinical diagnosis of FTD and cognitive screening appeared normal. This case also had evidence of PART (Braak stage III), which is typically restricted to the entorhinal cortex and infrequently seen in the frontal cortex (Yang, Ang, & Strong, 2005). This particular case however also showed severe, widespread, and atypically-localized (transcortical) NFTs across frontal and temporal SpN regions. Co-existing tauopathy has been previously highlighted as a component of some ALS cases with mild cognitive impairments (i.e., ALSci subtype) (Strong et al., 2006; Strong & Yang, 2011). The clinical consequence of the distinctive TDP-43 aggregate morphology and co-existing tauopathy in our case is not clear as this individual was the youngest of the cohort and did not differ considerably in antemortem disease duration, motor symptomology, or cognitive status compared to the other bALS cases. The second bALS case (MND12) showed atypical filamentous and granular morphologies of TDP-43 inclusions in SpN regions. This case also had an isolated NFT in the pSTG. The findings suggest a clinicopathologic link between bulbar-onset disease and atypical proteinopathies, which requires further investigation in a larger cohort of bALS cases.

4.5.4 Network-based degeneration of the SpN

Recent neuropathological and neuroimaging findings have proposed a network-based model of disease spread in ALS that may be related to the axonal connectivity between affected regions (Brettschneider et al., 2013; Schmidt, de Reus, Scholtens, van den Berg, & van den Heuvel,
2016; Verstraete, Veldink, van den Berg, & van den Heuvel, 2014). A recent neuropathology study that was validated with human diffusion data reported early TDP-43 spread from the PMC to densely connected areas in the prefrontal and somatosensory cortices (Brettschneider et al., 2013; Schmidt et al., 2016). Similarly, structural connectivity studies reported an expanding disconnection of a motor-prefrontal-subcortical network in ALS with areas that are densely interconnected (Buchanan, Pettit, Storkey, Abrahams, & Bastin, 2015; Verstraete et al., 2014). Our study provides indirect evidence for an “expanding” SpN with greater bulbar motor severity. The SpN was affected in both bALS and sALSwB, albeit to a much lesser degree in sALSwB. The clinical bulbar severity differences between the two groups were also similar: bALS cases were completely anarthric by end-stage of disease while sALSwB cases presented with dysarthria and dysphagia but still maintained some speech and swallowing function by end-stage. The parallel findings between clinical and neuroanatomical differences suggests a link between the degree of bulbar motor disease and the extent of SpN damage.

Similar to previous reports (Brettschneider et al., 2013; Schmidt et al., 2016), “early” changes in the SpN appeared to be in the bulbar sensorimotor cortex and secondary motor areas of the prefrontal cortex- TDP-43 pathology in the bulbar PSC were observed in all cases with damage to the bulbar PMC; SMA and premotor areas showed neuropathological changes in all bALS and almost all bALSwB cases. On the other hand, changes in the PAC, IFG, and pSTG were affected only in bALS cases and spared in most sALSwB cases, suggesting that changes to these SpN regions occur at “later” (more severe) stages of bulbar impairment. This implied pattern of “expanding” changes within the SpN is supported by the axonal architecture of this network: the PMC, which is presumably the initial lesion site of ALS in the cortex, has direct axonal connections to secondary motor areas (Dum & Strick, 1996; Muakkassa & Strick, 1979; Schmahmann et al., 2007), but only indirect connections through the premotor cortex to other SpN regions such as the IFG, pSTG, and PAC (Catani & Ffytche, 2005). The findings from our study indirectly supported a network-based model of disease spread in which the anatomical axonal connections may serve as a conduit for spreading misfolded proteins across areas that are synaptically connected (Ayers, Fromholt, O’Neal, Diamond, & Borchelt, 2016; Ravits, 2014; Schmidt et al., 2016).
4.5.5 Study Limitations and Future Directions

The primary limitation of this work is in the number of cases examined, which was relatively small (n=3 in each group); the work needs to be replicated in the larger number of cases. Additionally, the tissue sampling was limited to the cortical SpN regions. This work should be expanded to include the full extent of SpN regions, including subcortical grey matter structures and cerebellum, and go beyond the SpN to examine the full extent of frontotemporal regional pathology. Neuronal loss, gliosis, spongiosis, white matter pallor, and proteinopathy were all assessed using a semi-quantitative scale. Although excellent inter- and intra-rater reliability was established, the method relied on subjective ratings of severity. The findings need to be replicated using objective measures, such as digital microscopy based formal quantification of neuronal density and protein inclusions. Additionally, tissue sampling was conducted from the remnants of previously dissected archived cadaveric brain tissue resulting in missing data points for certain regions, and a subtle variance in the level of sampling for the various anatomical regions. Lastly, antemortem bulbar motor involvement was characterized by the presence of any bulbar signs or symptoms, as reported in clinical records. The nature and severity of bulbar symptoms need to be further characterized in the future using objective measures of bulbar dysfunction.

4.5.6 Conclusions

Uniquely, this study examined neuropathological difference in well-defined clinical phenotypes, showing notable differences between bulbar onset and spinal onset cases. The findings suggested that bulbar-onset disease may have a distinct neuropathological profile with widespread and severe neuropathological changes in motor and extramotor cortical regions of the SpN. Further, the two onset types may differ in terms of protein compositions; bALS may present with unique TDP-43 morphologies and co-existing tauopathy in affected regions. Additionally, findings suggested a potential link between severity of bulbar symptoms and pathology in the SpN regions.
Chapter 5
Discussion

5.1 Overarching Conclusions

This work aimed to further our understanding of the neuroanatomical underpinnings of bulbar ALS with a particular focus on the cortical speech network (SpN). Bulbar ALS was studied due to the particularly debilitating nature of this disease subtype; the onset of bulbar signs and symptoms are associated with a faster rate of decline compared to those with purely spinal symptoms, resulting in a shorter survival time (Haverkamp, Appel & Appel, 1995; Jablecki, Berry & Leach, 1989; Mandrioli et al., 2006; Norris et al., 1993). Bulbar symptoms also have a detrimental effect on overall quality of life as a result of social isolation from the loss of communication and dietary and lifestyle restrictions (Bourke et al., 2006; Worwood & Leigh, 1998; Simmons et al., 2013). Furthermore, bulbar ALS may be linked to a greater burden of cognitive-linguistic deficits (Massman et al., 1996b; Sterling et al., 2010), which needs further investigation.

The cortical SpN was investigated due to the high occurrence of speech production deficits in ALS as a result of bulbar dysfunction and the frequently reported involvement of cerebral networks in ALS (Trojsi et al., 2018b; Buchanan et al., 2015). Further, the reported link between bulbar symptoms and cognitive-linguistic impairments (Strong et al., 1999; Schreiber et al., 2005; Massman et al., 1996a; Sterling et al., 2010) may be mechanistically explained by changes in the SpN; Certain cognitive-linguistic functions have been mapped to language regions that are also shared by the SpN (Hickok and Poeppel 2007a; Rauschecker & Tian, 2000; Hagoort, 2014; Lieberman, 2002; Hickok & Poeppel, 2007). The work has significant impact on ALS subtyping which is crucial to understanding disease pathogenesis, and clinical implications to diagnosis, prognosis, and for recruitment into clinical trials.

To examine the extent of SpN involvement in bulbar ALS, three studies were conducted. Study 1 of this dissertation compared MRI metrics of the SpN regions between ALS patients with varying bulbar dysfunction and neurotypical controls and in relation to severity of bulbar dysfunction, using in vivo structural neuroimaging. Study 2 was a systematic review of the existing neuropathology literature comparing brain and brainstem distributions and types of pathology between bulbar-onset ALS (bALS) and spinal-onset ALS (sALS). The findings from
Study 2 informed the empirical neuropathology investigation in Study 3 which compared the spatial distribution and histological characteristics between bALS, sALS with antemortem bulbar symptoms (sALSwB), and sALS without bulbar symptoms (sALSnoB) in cortical SpN and brainstem regions. Cumulative findings across studies suggested that bulbar-onset ALS may be neuropathologically distinct from spinal-onset ALS with more severe and widespread cortical changes and atypical proteinopathy in select bALS cases. Further, the findings suggested a potential link between bulbar disease stage and extent of SpN changes across onset types.

The key specific findings across the three studies included the following:

(1) Group comparisons between ALS and controls showed *in vivo* structural differences in the right motor cortex and left extramotor SpN regions in a cohort of mixed bulbar-onset and spinal-onset patients. Abnormalities in the right bulbar PMC, left pSTG and bilateral PAC correlated with measures of bulbar motor dysfunction, but not with limb dysfunction or disease severity. A portion of the IFG, which is deemed a “general-domain” region that has high functional diversity (i.e., not solely specialized to speech and language functions), were associated with disease progression rates.

(2) As suggested by the results of the systematic review, neuropathology in SpN regions was greater and more expansive in bALS cases as compared to the sALS cases and included the IFG, pSTG, and PAC. Histopathological characteristics were similar between bALS and sALS cases with the exception of atypical proteinopathy: alterations in TDP-43 configurations were found in two bALS cases; these cases exclusively showed co-existing tauopathy in SpN regions, as also found in the systematic review. Alzheimer’s Disease pathology did not differentiate between onset types.

(3) SpN involvement was not unique to bALS - sALSwB also showed TDP-43 inclusions in SpN regions, however pathology was milder and in more focal areas of the cortex. SpN pathology were absent in the “pure spinal” sALSnoB cases, with the exception of one case with TDP-43 pathology in the sensorimotor cortex.

Overall, the findings suggested that bulbar-onset ALS has a distinct neuropathological signature that is characterized by severe neuronal loss and TDP-inclusions in motor and extramotor regions of the SpN as well as atypical morphologies of TDP-43 inclusions and co-existing
tauopathy in these regions. Milder and more focal SpN pathology were seen in sALSwB cases which supported an association between the severity of bulbar motor disease and SpN damage.

5.2 Speech Network Damage in Bulbar ALS

Historical models of speech control identified the posterior two thirds of the IFG as a speech production region (also known as “Broca’s Area”), and the pSTG as a comprehension region (also known as “Wernicke’s Area”), linked by the arcuate fasciculus (often synonymous with the superior longitudinal fasciculus (SLF)) (Fedorenko, Duncan & Kanwisher, 2012; Lichtheim, 2009; Broca, 1861; Wernicke & Eggert, 1874). With advances in functional imaging and brain stimulation techniques, new empirical research has uncovered the sophisticated neural system that underlies speech production, processing, and comprehension that extends much beyond these two regions (Gracco, Tremblay & Pike, 2005; Tremblay & Dick, 2016; Tremblay, Shiller & Ostry, 2003; Tremblay & Gracco, 2009; Dick & Tremblay, 2012). Rather than local functional segregation, modern theoretical perspectives propose that speech production and perception might be more accurately characterized by a network of interactive brain regions (Pulvermuller, 2005; Hickok & Poeppel, 2000; Friederici & Alter, 2004; Indefrey & Levelt, 2004; Tremblay, 2019).

The prominent Directions into Velocities of Articulators (DIVA) is a neurocomputational model that integrates electrical stimulation, lesion-based, and functional MRI data on speech acquisition and production to identify a set of functionally specialized cortical and subcortical regions involved in speech production (Golfinopoulos, Tourville & Guenther, 2010). The DIVA model’s input is approximately equivalent to the output of the phonological encoding stage proposed by Levelt (1989) in his model of word production. The DIVA model is composed of several interconnected components that encompass two types of motor control schemes: a feedforward control subsystem to plan and initiate speech movements, and a feedback control subsystem that uses auditory and somatosensory feedback against the expected sensory reference frames to guide and modulate these movements. The neural substrates underlying this integrated control scheme include the following: ventral primary motor cortex (i.e., bulbar PMC) where the articulator map resides; premotor and supplementary motor areas in the prefrontal cortex containing the speech sound map that is used for planning the speech sound target (corresponding to the “mental syllabary” described in Levelt and colleagues (1999)) and the initiation map responsible for activation of a chosen movement; inferior frontal gyrus (IFG)
also containing cells of the speech sound map; ventral somatosensory cortex (i.e., bulbar PSC) housing the somatosensory state and target maps used to compare the somatosensory response to the expected target; auditory cortex (i.e., primary acoustic cortex (PAC) or Heschl’s gyrus in the transverse temporal) and posterior superior temporal gyrus (pSTG) housing cells of the auditory state and target maps where the intended acoustic target is compared to the auditory feedback; as well as the cerebellum and basal ganglia which contribute to precisely timed commands. Together, these regions form the Speech Network (SpN).

In this dissertation, findings from all three studies gleaned a link between bulbar ALS and cortical changes in regions part of the SpN. The MRI neuroimaging study examined GM and WM abnormalities of the bulbar PMC, IFG (ParsT and ParsO), pSTG, and PAC and found that a number of these regions were affected in a mixed cohort of bulbar-onset and spinal-onset cases with bulbar disease. Specifically, compared to age and sex matched healthy controls, patients with bulbar ALS showed cortical thinning of the right PMC and left ParsT as well as reduced diffusivity underlying left PAC and left pSTG. Further, the structural changes observed for the bulbar PMC and auditory processing regions of the SpN (i.e., left pSTG and bilateral PAC) were associated with performance on a speech-based measure of bulbar motor dysfunction. The neuropathology systematic review identified pathology in the IFG and pSTG exclusively in bulbar-onset patients. The post-mortem analysis was extended to other SpN regions in the neuropathology study and revealed pathology in the same regions as the imaging study, as well as in the bulbar PSC, SMA, and premotor cortex exclusively in cases with bulbar ALS (e.g., bulbar-onset and spinal-onset with reports of bulbar dysfunction). SpN regions were largely spared in the “pure spinal” ALS cases. Together, the findings suggest an increased vulnerability of the cortical SpN in bulbar ALS and a relative sparing of the same regions in cases without bulbar disease. This double disassociation implies a unique neurodegenerative cortical signature for bulbar ALS subtype, which may be characterized by greater and more widespread brain damage in the affected individuals.

Degeneration of the PMC was as expected in ALS and has been consistently observed in previous neuroimaging and neuropathology investigations of ALS (Grolez et al., 2016; Brettschneider et al., 2016; Piao et al., 2003). Previous studies also frequently report structural and/or functional changes of the IFG and pSTG, particularly but not exclusively in individuals with bulbar-onset disease (Kiernan & Hudson, 1991; Chang et al., 2005a; Lillo et al., 2012;
Reported abnormalities of sensory processing areas such as the PSC and PAC are less common. Regional metabolic changes in the PAC were previously reported in a cohort of 16 patients with ALS (Elman et al., 2013). Two recent fMRI studies also identified abnormalities underlying the PSC in patients with ALS (Lule et al., 2010; Zhou et al., 2014). The findings from our study contribute to these limited data and widen the current understanding of the extramotor disease effects in ALS to include primary sensory processing areas (e.g., PAC and PSC). Clinical effects of these regional differences need to be examined using somatosensory and auditory testing, especially in patients with bulbar signs and symptoms.

5.3 Network-based Disease Spread

The mechanisms underlying disease spread to the SpN exclusively in bulbar ALS are not yet clear. Studies examining the pathogenesis of ALS have shown that while the site of initial onset in the central nervous system can be triggered stochastically (Ravits, 2014), the extension of disease to other regions of the brain and spinal cord does not appear to occur randomly. A “prion-like” mechanism of disease propagation has been hypothesized with self-mediated seeding and spread of misfolded neurotoxic TDP-43 proteins from one neuron to another (Smethurst, Sidle & Hardy, 2015; Ravits, 2014; Ayers et al., 2016; Pradat, Kabashi & Desnuelle, 2015). Two types of “prion-like” disease propagation mechanisms have been theorized in ALS: (1) a proximity-based spread and (2) a network-based spread. The first mechanism involves a contiguous spread of disease advancing soma-to-soma (i.e., “laterally”) through the extracellular matrix and independent of synaptic connection (Kanouchi, Ohkubo & Yokota, 2012; Ravits & La Spada, 2009). The support for the contiguous spread is based on clinical observations of progressive worsening of symptoms in the initial region of onset and the sequential dysfunction to contiguous body parts (i.e., to contralateral side of the body) (Körner et al., 2011; Ravits 2014). It has been also supported by recent cultured cell line and animal model studies reporting a “prion-like” mechanism of SOD1 and TDP-43 self-propagating within neuronal cells and transmitting to adjacent neighboring cells (Nonaka et al., 2013; Sábado et al., 2014; Münch, O’Brien & Bertolotti, 2011).

The second more recently supported mechanism is the network-based model of propagation, advancing end-to-end through functionally and anatomically connected networks and thus dependent on synaptic connectivity (Seeley et al., 2009; Ravits, 2014; Buchanan et al., 2015;
Trojsi et al., 2018a). The evidence for network propagation in ALS comes primarily from recent MRI studies. Structural DTI and network-based statistical modeling demonstrated an impaired corticomotor subnetwork comprised of PMC, SMA, cingulate gyrus, and the basal ganglia (Buchanan et al., 2015; Verstraete et al., 2011, 2010). The findings showed that ALS affects not only primary motor connections, but also connected regions in the prefrontal cortex and subcortical structures. One longitudinal DTI study showed an expanding loss of network structure in patients with ALS. Initial structural connectivity changes were noted in the PMC, PSC, superior frontal (i.e., SMA), posterior cingulate, thalamus, basal ganglia, and brainstem; structural changes expanded over time involving more frontal, temporal, and parietal connections and regions, including ParsT and ParsO of the IFG, middle temporal gyrus, pSTG, and insula (Verstraete et al., 2014). The finding showed that network degeneration expands with disease progression, rather than progressively affecting a fixed set of impaired connections.

The network propagation view is also supported in existing neuropathological studies: Brettschneider et al., (2013) recently proposed a four time-sequential staging of neuropathology in ALS, demonstrating a neuroanatomical spread of TDP-43 pathology based on what appears to be an orderly anatomical gradation of pathological changes centered around the PMC (Braak et al., 2013; Brettschneider et al., 2013). “Early” TDP-43 changes were seen in the PMC, brainstem LMN, and spinal cord alpha-motoneurons, followed by increased burden of TDP-43 in the prefrontal cortex (middle frontal, i.e., portion of the premotor cortex), brainstem reticular formation, precerebellar nuclei, and red nucleus (stage 2). Severe stages were characterized by TDP-43 burden in the PSC, striatum, and hippocampus. Staging was based on burden of pathology, not clinical severity. A follow-up study cross-referenced Brettschneider’s stages of TDP-43 pathology with in vivo diffusion imaging data and simulated disease spread in an ordering of regions that strongly overlapped with the empirically observed ordering of TDP-43 involvement in post-mortem brains (Schmidt et al., 2016b). The study also revealed that Brettschneider’s TDP-43 regions formed a densely interconnected anatomical subnetwork and that the network topological distance between TDP-43 regions were greater for pairs spanning more stages. The findings support the notion that the anatomical axonal connections may direct disease spread in the brain by serving as a conduit for spreading misfolded proteins across areas that are synaptically connected (Müller et al., 2016; Ayers et al., 2016). In support of this disease mechanism, cell-culture studies showed both anterograde and retrograde spreading of TDP-43 aggregates. A predominance of TDP-43 inclusions were observed in regions receiving
strong afferents from pyramidal neurons suggesting a corticofugal axonal spread (Braak et al., 2013); A retrograde spreading was also demonstrated through TDP-43 reuptake by axon terminals and transport to neuronal somata (Alami et al., 2014) via the axonal wiring connections (Feiler et al., 2015). Overall, an increased vulnerability of cortical networks is highly supported in ALS, particularly for networks that are interconnected with the PMC which presumably is the primary site of UMN degeneration in ALS (Waragai, 1997; Verstraete et al., 2010, 2014).

The network-based hypothesis of disease spread may explain the observed link between the SpN damage and bulbar ALS: areas part of the SpN may be preferentially vulnerable in bulbar ALS due to their increased connectivity to the ventral “bulbar” portion of the PMC mapped to tongue, lips, and larynx functions, compared to the medial regions mapping upper and lower limb movements. Our imaging and neuropathology studies provided multiple lines of evidence indirectly supporting the network degenerative hypothesis within the SpN in bulbar ALS. First, group differences from the imaging study revealed right PMC changes but left lateralized extramotor changes of SpN regions in ALS. A left-lateralized network for speech and language processing has been traditionally accepted in the speech literature (Hickok & Poeppel, 2007a; Rauschecker & Scott, 2009). The finding from our study suggested preferential vulnerability of functionally-related areas which is in line with the evidence from recent fMRI studies showing a breakdown in functional connectomes (Lulé et al., 2007; Geevasinga et al., 2017; Schmidt et al., 2014). Second, converging findings from the imaging and neuropathology studies suggested an association between the extent of SpN damage and bulbar ALS severity. MRI metrics of bulbar PMC, PAC, and pSTG were associated with severity of bulbar motor dysfunction and not with limb or overall disease severity. Neuropathology in SpN regions were absent in “pure spinal” cases, mild and focal in sALS cases with dysarthria and dysphagia (sALSwB), and greatest and most widespread in bALS cases that were anarthric.

Third, the observed regional differences in pathology between bALS and sALSwB suggest a sequential order of disease spread within the SpN that is consistent with the axonal connectivity of the network. Beyond the sensorimotor cortex, TDP-43 pathology was most commonly seen in the SMA and premotor areas in both sALSwB and bALS groups, suggesting that early SpN involvement occurs in secondary motor areas of the prefrontal cortex. Indeed, non-human primate and human diffusion data have revealed direct axonal fiber pathways between the bulbar
PMC and the premotor cortex, SMA, and bulbar PSC (Dum & Strick, 1996; Muakkassa & Strick, 1979; Schmahmann et al., 2007). These regions were affected in both bulbar phenotypes presumably due to their direct synaptic connectivity to the bulbar PMC. By contrast, the IFG, pSTG, and TT (containing the PAC) showed TDP-43 pathology exclusively in the bALS cases, suggesting their involvement only in very advanced stages of bulbar motor disease. These regions do not have direct connections to the PMC, but appear to be interconnected to each other (Bernal & Altman, 2010; Glasser & Rilling, 2008) and indirectly to the PMC through the premotor cortex (Catani & Ffytche, 2005). The observed topographical distribution pattern within the SpN provides support for anatomical axonal connections serving as a conduit for spreading misfolded proteins across areas that are synaptically connected (Ayers et al., 2016; Ravits, 2014; Schmidt et al., 2016b).

5.4 SpN Regional Specificity

The neuroanatomy of speech production and language comprehension are not distinct and instead recruit shared cortical regions that subserve both functions (Hickok, 2001). Therefore, the link between SpN damage and bulbar ALS that is proposed in our findings can mechanistically explain the existing behavioural reports of greater cognitive-linguistic deficits in patients with bulbar-onset ALS and/or dysarthria compared to non-bulbar forms of the disease (Schreiber et al., 2005; Abrahams et al., 1997; Beeldman et al., 2016). Neuropsychological studies reported greater verbal dysfluency in bulbar-onset ALS and in patients with dysarthria (Lomen-Hoerth et al., 2003; Schreiber et al., 2005; Ringholz et al., 2005; Crockford et al., 2018; Massman et al., 1996) which has been mapped to abnormalities of the IFG and pSTG (Horshorn & Thompson-Schill, 2006; Schlosser et al., 1998). Studies also report sentence comprehension deficits (Yoshizawa et al., 2014; Crockford et al., 2018) which may be attributed to either auditory comprehension dysfunction, mapped to the PAC and pSTG (Ahissar et al., 2001; Friederici et al., 2003), or phonological and syntactical processing deficits which involves the IFG among other areas (Amici et al., 2007). Greater errors in spelling have also been reported in patients with dysarthria (Ichikawa et al., 2008; Crockford et al., 2018), which is associated with changes in left IFG and supramarginal gyrus (Shim et al., 2012). The findings propose a co-development of motor speech and language dysfunction in ALS, especially when the SpN damage extends beyond the PMC.
Certain regions of the SpN are considered “domain-specific” because they presumably selectively respond to speech production and processing tasks, for example the PAC (Geranmayeh et al., 2014). They are compared with “domain-general” regions that are active during a range of cognitive-linguistic and motor processes (Fedorenko & Thompson-Schill, 2014). Among others (e.g., insula and cingulate cortex), the IFG is considered a domain-general region: it contributes to phonological planning and speech motor sequencing within the SpN (Bohland & Guenther, 2006b; Indefrey & Levelt, 2004), but is also involved in domain-general functions such as hierarchical structure building, aspects of action processing, working memory, and cognitive control (Fedorenko, Duncan & Kanwisher, 2013). The “multi-purpose” role of the IFG is also demonstrated in architectonic investigations identifying up to eight anatomically-defined subdivisions of “Broca’s area”, each presumably sub-serving a different cognitive-linguistic function (Heim, Eickhoff & Amunts, 2008). Graph-theoretical analyses in healthy subjects have illustrated that domain-general regions may undergo “nodal stress” due to their active role in multiple functional connectomes resulting in higher total connectional flow (i.e., “high-traffic” nature) (Zhou et al., 2012; Verstraete et al., 2010). Our neuroimaging study found prominent thinning of this region and reduction of white matter diffusivity of IFG in ALS, which were associated with disease progression rates. Similarly, our neuropathology study suggested a relationship between disease decline rate and IFG by identifying greater TDP-43 pathology in IFG for cases with shorter disease durations (i.e., the three bALS cases). The findings suggest that domain-general regions may have prognostic value for predicting survival and may potentially serve as a therapeutic target for future interventions.

5.5 Neuropathological Differences Bulbar-onset and Spinal-onset ALS

5.5.1 Greater frontotemporal involvement in bALS

Our systematic review and neuropathology study compared the distribution and types of pathology between bulbar-onset (bALS) and spinal-onset (sALS) cases. Findings from both studies suggested that bALS may have a neuropathologically distinct profile compared to sALS with greater extramotor frontotemporal pathology. The systematic review reported differences in selected extramotor regions exclusively in bulbar-onset ALS (bALS) – IFG and pSTG. Our neuropathology study, however, identified extramotor pathology including IFG and pSTG in both bALS and sALSsWb patients, suggesting that extramotor damage was not exclusive to bALS. Interestingly, the extent and nature of extramotor neuropathology differed substantially
between bALS and sALS cases: bALS had a distinct neuropathological signature compared to sALS and was characterized by widespread neuronal loss and severe burden of TDP-43 proteinopathy across all examined regions of the cortical SpN, compared to sALS cases that showed lesser pathology in these regions. The findings suggested that bALS may show preferential involvement of the frontotemporal regions; this would be consistent with other studies reporting greater overall cortical involvement in bALS compared to sALS cases (Piao et al., 2003; Shelliketi et al., 2017). One explanation is that bulbar-onset disease may be pathogenically unique from spinal-onset ALS with a longer prodromal phase enabling greater cortical atrophy before onset of symptoms. This idea is supported in large population-based studies reporting a more “aggressive” presentation in bALS with a faster rate of overall decline and shorter survival post-diagnosis (Haverkamp, Appel & Appel, 1995; Jablecki, Berry & Leach, 1989; Norris et al., 1993) that cannot be explained by a faster rate of bulbar decline (Haverkamp, Appel & Appel 1995; Mills, 2003). Widespread extramotor involvement may be contributing to the aggressiveness of the bALS phenotype.

5.5.2 Unique TDP-43 Configurations in bALS

The systematic review comparing onset types revealed atypical proteinopathy in a subset of bALS cases; tau NFTs and basophilic inclusions were seen in some bALS cases and were absent in all reported sALS cases. Our neuropathology study revealed unique morphologies of TDP-43 proteins in our bALS cases. Specifically, a combination of dot-like, filamentous, and granular TDP-43 proteinopathy was seen in one bALS case presenting with the most “aggressive” disease presentation (i.e., shortest disease duration and most expansive SpN damage). Another bALS case presented with cortically widespread TDP-43 dystrophic neurites and no neuronal cytoplasmic inclusions, which is reminiscent of FTLD-TDP43 Harmonized Type C associated with semantic and behavioural variants of FTD (Takeuchi et al., 2016; Mackenzie et al., 2011). Our examined case did not have a clinical diagnosis of FTD and cognitive testing was normal, however. Both described cases were also accompanied by atypical tauopathy in the form of NFTs, isolated to the pSTG in one case and widespread in frontal and temporal SpN regions in the other. Co-existing tauopathy has been previously highlighted as a component of some ALS cases with mild cognitive impairments (i.e., ALSci subtype) (Strong et al., 2006; Strong & Yang, 2011). The clinical consequence of the distinctive TDP-43 aggregate morphology and co-existing tauopathy in our case is not clear as the one individual with widespread tauopathy was
the youngest of the cohort and did not differ considerably in antemortem disease duration, motor symptomology, or cognitive status compared to the other bALS cases.

Studies in tau disease have highlighted clinicopathologic links between protein configurations and unique clinical phenotypes (Sanders et al., 2014). Similarly, the presence of a wide variety of different clinical ALS phenotypes could suggest that different morphologies of misfolded TDP-43 may contribute to variations in phenotypes. Clinicopathologic links for TDP-43 morphologies have been previously described, for example when ALS is clinically limited to LMNs in PMA, a diffuse TDP-43 proteinopathy is seen (Geser et al., 2011). Similarly, TDP-43 configurations in FTLD have been defined into four distinct histopathological subtypes (A-D) associated with behavioural and semantic variants of FTD and ALS-FTD (Mackenzie et al., 2011). Our findings suggest a clinicopathologic link between atypical TDP-43 morphologies and co-existing tauopathy and bulbar-onset disease, suggesting a unique TDP-43-mediated neuropathological profile in this subtype. Clinical Implications

The findings of these studies have significant implications to the understanding and potentially clinical management of bulbar ALS. Characterizing the neural underpinnings of bulbar ALS subtype can aid in the development of sensitive markers for early identification of bulbar disease (Green et al., 2013). Ascertaining the early development of bulbar motor deficits in patients has a potential to expedite ALS diagnosis (Kraemer, Buerger & Berlit, 2010), which is crucial for disease subtyping and then to ensure timely and appropriate clinical care. Further with the emergence of potentially effective neuroprotective drugs in ALS, there is a clear need for the development of robust biomarkers for disease diagnosis and monitoring, particularly for bulbar dysfunction (Smith et al., 2017; Yunusova et al., 2019).

While brain imaging is currently not being used to establish the diagnosis of ALS, structural MRI studies have demonstrated its sensitivity to early UMN changes in ALS (Agosta, Spinelli & Filippi, 2018; Mitsumoto et al., 2007; Kalra & Arnold, 2003) and its ability to detect and characterize extramotor cortical changes (Chang et al., 2005a; Bede, Bokde, Elamin, et al., 2013). Our imaging study demonstrated motor and extramotor neural correlates in the SpN for bulbar ALS, which were not associated with motor limb and overall disease severity. Extramotor neuropathology staging suggested early involvement of prefrontal secondary motor areas in bulbar ALS, however, these regions were absent from our imaging analyses due to methodological issues in delineating anatomical landmarks needed for the region-of-interest
analysis. MRI changes in these regions need to be characterized in future investigations, as they may serve as a potential early diagnostic marker for bulbar disease. The findings provide neuroanatomical targets for characterizing bulbar ALS changes in the brain and support the use of MRI metrics as a future biomarker in bulbar ALS subtype.

Detecting the extent of the disease propagation across the SpN may allow staging of severity and presence of extramotor (cognitive-linguistic) deficits, which is another important area of clinical management. Cognitive dysfunction is a negative prognostic factor in ALS as it interferes with personal care and judgement for end-of-life decisions (Rusina et al., 2010; Elamin et al., 2011). The presence of cognitive dysfunction can be detrimental to the outcomes of clinical trials, especially if outcome measures rely on intact cognitive-linguistic abilities (Traynor et al., 2004; Paganoni, Cudkowicz & Berry, 2014). Overall, improving subtyping in ALS has significant diagnostic and prognostic implications, and implications to the structure and outcome of clinical trials, which are all crucial to the development of a successful treatment for this incurable disease (Leigh et al., 2004; Smith et al., 2017).

5.6 Limitations and Future Directions

Although various limitations were addressed within each chapter, there are a few key constraints which remained throughout the dissertation and point to areas for future research. The first pertains to the overall small sample across the observational studies. This is a common issue in ALS research due to the rapidly debilitating nature of the disease restricting participation and contributing to attrition (Atassi et al., 2013). Recent data-sharing approaches and multicenter collaborative initiatives (e.g., CALSNIC, NiSALS) have maximized the contribution of research subjects and allowed larger datasets for cross-sectional and longitudinal analyses. These analyses will be forthcoming (Kalra et al., 2019).

The DTI protocol employed in this study was limited in its number of directions and did not allow tractography analyses. Individual fiber tract tracing may be especially important in investigating SpN tracts, especially the smaller and more variable WM pathways such as the corticobulbar tract and uncinate fasciculus. Even though a multi-modal MRI approach was used, analyses were limited to structural abnormalities. Combined functional and structural neuroimaging may better characterize the cortical changes associated with this condition (Filippini et al., 2011).
Another limitation is in the selectiveness of the SpN regions that were examined. As a first step to characterizing SpN involvement in ALS, both observational studies of the dissertation examined a subset of SpN regions and did not include the insula, cingulate gyrus, area SPT, subcortical GM structures, and cerebellum (Guenther, Ghosh & Tourville, 2006b). Furthermore, IFG was limited to two subregions - ParsT and ParsO - examined separately only in the imaging study. A recent paper illustrates the complex structural organization of the IFG, which includes more than eight subdivisions speculated to sub-serve individual cognitive-linguistic functions (Heim, Eickhoff & Amunts, 2008; Amunts & Zilles, 2012). Additional work is needed to characterize the full extent of SpN and cognitive-linguistic networks involvement in ALS.

Our neuropathology study assessed histological features using a semi-quantitative scale. Although excellent inter- and intra-rater reliability was established, the method relied on subjective ratings of severity. The findings need to be replicated using objective measures, such as digital count of neurons and protein inclusions. Further, tissue sampling was conducted from the remnants of pre-sampled archived cadaveric brain tissue certain parts of the brain were missing from the archived tissue, resulting in missing data points for certain regions. Despite the authors’ best attempts to standardize the collected samples, previous dissection of this cadaveric tissue likely introduced subtle variance in the level of sampling for the various anatomical regions. Lastly, antemortem bulbar motor involvement was characterized by the presence of any bulbar signs or symptoms, as reported on the clinical records. The nature and severity of bulbar symptoms need to be further characterized in the future using objective measures of bulbar dysfunction.

The results of the observational studies pointed to network-based degeneration of the SpN in bulbar ALS. However, we could not directly examine the anatomical connections between brain regions in living humans and our diffusion analyses were limited. A true understanding of the SpN in ALS requires a multimodal approach, such as fMRI and structural MRI studies combined with network-based analyses (i.e., graph-theoretical analyses of nodes and edges and their dynamics) (Bede and Hardiman 2014).

5.7 Conclusions

The findings from this work support a clinicopathologic link between bulbar ALS and neuroanatomical damage in cortical SpN regions. In vivo structural differences in SpN regions
are seen in patients with bulbar ALS and in relation to clinical measures of bulbar motor dysfunction. Neuropathology of SpN shows greater severity and more expansive damage in bALS cases compared to sALS with and without bulbar disease, including regions that also subserve cognitive-linguistic functions. Further, bulbar-onset ALS may be associated with unique morphology and co-existing atypical proteinopathy. The work has significant clinical and research implications – understanding bulbar subtype is crucial for predicting disease progression and the development of cognitive-linguistic dysfunction, delivering targeted clinical care, and improving patient stratification for recruitment into clinical trials. Future studies require detailed clinical, neuroimaging, and neuropathologic examination in a larger cohort of ALS patients in order to determine the true breadth of the relationship between bulbar ALS and SpN.
References


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Appendix A: Neuroimaging Supplementary Material

Segregating the PMC into oral and limb ROIs

For the purposes of this study, the precentral gyrus was divided into ventrolateral and dorsomedial ROIs, corresponding to the oral and limb regions of the PMC, respectively. Freesurfer’s pre-existing PMC ROI which does not support this partitioning (Desikan et al., 2006) (Figure 1, left panel) was divided into 100 equal divisions along the longitudinal axis using Freesurfer’s automatic label division toolkit (middle panel). Following, the ‘hand omega’ (Yousry et al., 1997) was manually identified in the axial orientation and then confirmed through its identification on the sagittal plane (See Figure 2). The division slice that corresponded with the most ventral and lateral portion of the ‘hand omega’ (see Figure 2) was combined with all dorsomedial slices to form the limb ROI. Likewise, slices ventrolateral to the ‘hand omega’ were combined to form a second ROI corresponding to the oral region of the PMC (Figure A-1, right panel).
Supplementary Figure A-1. A 3D volume rendered image of the left hemisphere showing the novel semi-automatic PMC partitioning: left, the pre-existing PMC ROI from Desikan et al., 2006; Middle, 100 equal divisions of the ROI; Right, the 2 new ROIs corresponding to the oral (yellow) and limb (red) areas of the PMC.
Supplementary Figure A-2. A 6-panel figure showing the identification of the ventrolateral-most slice of the ‘hand omega’ (indicated by the arrow), used as an anatomical landmark to delineate oral from limb areas on the primary motor cortex. The individual panels represent axial slices of the T1-weighted brain volume in the superior (top left)-to-inferior (bottom right) direction.
Inter-rater reliability of oral and limb PMC partitioning

Intra-class correlation coefficient (ICC) estimates (Koch, 1982) were obtained from two raters (authors SS and MM) in 14 randomly selected brains (7 ALS). Both raters independently identified the inferolateral-most division of the ‘hand omega’ to form the limb and oral ROIs. ICC estimates were computed for all MRI metrics for both hemispheres using a two-way mixed-effects single-rating model for absolute agreement. A high level of agreement between the two raters was evident across all MRI measures in both ALS and HC brains (see Supplementary Table A-1).
Supplementary Table A-1. ICC estimates and 95% confidence intervals of two independent raters for absolute agreement for MRI measures of the oral PMC ROI.

<table>
<thead>
<tr>
<th>Group</th>
<th>Surface Area (mm(^2))</th>
<th>GM volume (mm(^3))</th>
<th>Cortical thickness (mm)</th>
<th>Fractional Anisotropy</th>
<th>Surface Area (mm(^2))</th>
<th>GM Volume (mm(^3))</th>
<th>Cortical thickness (mm)</th>
<th>Fractional Anisotropy</th>
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<td>ALS</td>
<td>0.998</td>
<td>0.997</td>
<td>0.998</td>
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<td>0.993</td>
<td>1</td>
<td>0.997</td>
<td>0.980</td>
</tr>
<tr>
<td></td>
<td>(0.993 - 0.999)</td>
<td>(0.994 - 0.999)</td>
<td>(0.993 - 0.999)</td>
<td>(0.998 - 1)</td>
<td>(0.980 - 1)</td>
<td>(0.999 - 1)</td>
<td>(0.990 - 0.999)</td>
<td>(0.941 - 0.994)</td>
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<td>0.997</td>
<td>0.998</td>
<td>0.999</td>
<td>0.999</td>
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<td>0.998</td>
<td>0.994</td>
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<tr>
<td></td>
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<td>(0.993 - 0.999)</td>
<td>(0.996 - 1)</td>
<td>(0.998 - 1)</td>
<td>(0.992 - 0.998)</td>
<td>(0.998 - 1)</td>
<td>(0.993 - 0.998)</td>
<td>(0.990 - 0.996)</td>
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</table>
Validity of oral and limb PMC partitioning

Validity of the PMC partitioning was established using univariate linear regression models predicting MRI measures of the oral PMC ROI based on DDK rate, as well as MRI measures of the limb PMC ROIs based on left and right grip strength (kg/BMI) in participants with ALS. Left grip strength was modeled with the right limb PMC ROI, and vice versa. Sex and age both served as fixed covariates in all models. Additionally, estimated total intracranial volume (eTIV) served as a fixed covariate for all volumetric and surface area models.

Significant regression equations were found for predicting the FA and RD of the right corticobulbar tract (WM region underlying the oral PMC) based on DDK rate, $F(2,7) = 14.629, p = .007, \text{Adjusted } R^2 = .596$; $F(2,7) = 5.968, p = .045, \text{Adjusted } R^2 = .366$, respectively. Regression models predicting all other MRI measures of the oral PMC and underlying WM based on DDK rate were non-significant.

Left grip strength significantly predicted AD of the right corticospinal tract (WM subcortical to the right limb PMC) ROI, $F(2,8) = 15.568, p = .008, \text{Adjusted } R^2 = .638$. AD of the WM region decreased by .055 units for each kg/BMI decrease in left grip strength. All other models for left and right grip strength were non-significant.

These results indicate that the PMC partitioning method appropriately segregated the PMC into bulbar and limb motor areas, which were clinically validated with measures of motor dysfunction. The findings support the use of this method for evaluating bulbar and limb UMN involvement separately in healthy and atrophied brains.
Appendix B: Systematic Review Supplementary Material

Electronic Search Strategies.

Original searches were conducted on Sept. 1, 2015 (shown) and updated on July 12, 2016.

Table B-1. Original search strategy for MEDLINE.

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<td>Advanced</td>
</tr>
<tr>
<td>2</td>
<td>Motor Neuron Disease/</td>
<td>3738</td>
<td>Advanced</td>
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<tr>
<td>3</td>
<td>(Lou Gehrig* adj5 disease).mp,kw.</td>
<td>124</td>
<td>Advanced</td>
</tr>
<tr>
<td>4</td>
<td>immunohistochemistry/ or histological techniques/</td>
<td>275474</td>
<td>Advanced</td>
</tr>
<tr>
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<td>Immunocytochemistry/</td>
<td>258980</td>
<td>Advanced</td>
</tr>
<tr>
<td>6</td>
<td>exp Histology/</td>
<td>346909</td>
<td>Advanced</td>
</tr>
<tr>
<td>7</td>
<td>or/1-3</td>
<td>17475</td>
<td>Advanced</td>
</tr>
<tr>
<td>8</td>
<td>or/4-6</td>
<td>275474</td>
<td>Advanced</td>
</tr>
<tr>
<td>9</td>
<td>7 and 8</td>
<td>708</td>
<td>Advanced</td>
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<tr>
<td>10</td>
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<td>Advanced</td>
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<td>11</td>
<td>9 not 10</td>
<td>557</td>
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Table B-2. Original search strategy for Embase.

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<td>histochemistry/</td>
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<td>765</td>
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Table B-3. Original search strategy for PsycINFO.

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Appendix C: Systematic Review Supplementary Material

Table C-1. Special and immunohistochemical stains used for analysis and the corresponding histological features.

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<th>Clone</th>
<th>Vendor</th>
<th>Dilution</th>
<th>Heat retrieval</th>
<th>Antibody incubation time</th>
<th>Detection system</th>
<th>Histological features</th>
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<tbody>
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<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>Severity of neuronal loss, gliosis, superficial spongiosis, and myelin pallor</td>
</tr>
<tr>
<td>p62</td>
<td>3/p62lck</td>
<td>BD</td>
<td>1/200</td>
<td>High PH, 96°C, 30 min</td>
<td>30 min</td>
<td>Envision Flex Dako detection</td>
<td>Density and morphology of extracellular, intranuclear, or cytoplasmic protein aggregates</td>
</tr>
<tr>
<td>TDP-43</td>
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<td>Cedarlane</td>
<td>1/2000</td>
<td>High PH, 96°C, 30 min</td>
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<td>Envision Flex Dako detection</td>
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</tr>
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<td>Mach 4</td>
<td>Density of neurofibrillary tangles</td>
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