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Network Analysis in Motor Neuron Diseases: A Cortico-Muscular Coherence Study

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degree of Doctor of Philosophy (PhD)

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Declaration

I declare that this thesis has not been submitted as an exercise for a degree at this or any other university and, unless stated otherwise, it is entirely my own work. Where any of the content presented is the result of input or data from related collaborative research this is acknowledged in the text.

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Finally, full and informed consent was obtained from all study participants.

Signed: 

Amina Coffey

To My Three Little Musketeers

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Abstract

Neurodegenerative conditions are associated with widespread changes in the motor networks. There is preliminary evidence from (f)MRI studies that the structural changes extend beyond the primary motor areas in cortical regions and spinal networks, but it is unclear how the function of these networks is affected in the disease.

The recording of joint multi-channel electroencephalogram (EEG) and electromyogram (EMG) for time-series analysis has been found to be instrumental for assessing the communication between cortical brain regions and the periphery by quantifying the oscillatory motor drives to muscles during specified motor tasks; hence, providing direct neuro-electric signatures of network disruption.

The overarching aim of the study was to develop corticomuscular coherence-based biomarkers as potential tools for assessing network disruption in selected motor subsystems in MND patient subgroups during functional isometric motor tasks.

More specifically, I hypothesized that cortico-muscular coherence (CMC) between EEG-EMG can interrogate disease-specific alterations in the brain's motor networks within and beyond the primary motor cortex in MND.

I used high-density 128-channel EEG and 8 bipolar surface electromyographic recordings from extrinsic and intrinsic hand muscles to obtain measures from patients with dominant upper (PLS), lower (Polio/SMA) and mixed upper/lower (ALS) motor neuron degeneration as well as from healthy controls, during isometric precision grip tasks.

My findings showed distinct pathological changes in the patient groups compared to controls, which indicate a pathological increase of cortico-muscular coherence over frontal and parietal brain regions. These include abnormal frequency band changes over parietal regions in PLS, abnormal alpha band coherence in ALS, as well as abnormal gamma-band coherence patterns between APB/FPB muscles and the frontal/parietal regions in the Polio/SMA

groups. This work reveals a previously unrecognised functional change in the motor networks.

My results suggest that the EEG-EMG coherence during functional motor tasks mark pathological functional changes in the central-peripheral communication, due to adaptive or compensatory mechanism. I argue that due to disruption in the main corticospinal pathways, alternative communications pathways re-establish with the networks not typically used which may have been “pruned” in early life. The findings from my experiments illustrate that highly connected areas operate as a network that can be identified using modern neurophysiological methods, and regardless of the point of origin of a lesion (UMN/LMN), my findings support the idea that neuronal circuitry adapts, and communication pathways are re-modelled as individual neurons within the motor circuit degenerate. This remodelling occurs both “upstream” and “downstream” of the neuronal injury.

These network-level changes have the potential to be used as biomarkers of disease, as well as prospective tools for patient stratifications in the clinical trial settings.

Peer-reviewed publications from this thesis

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Table of Contents

1. Chapter 1: Introduction.....	3
1.1. Motor Neuron Disease.....	3
1.1.1. MND Clinical Features.....	4
1.1.2. Clinical Management of ALS	19
1.1.3. Moving Towards Precision Medicine.....	19
1.1.4. A window into ALS complexity.....	20
1.1.5. Anatomy of ALS	21
1.1.6. “Experimental Models”	30
1.2. PLS: A model for a pure UMN disorder.....	31
1.2.1. Clinical Features	31
1.3. Polio: A model for a pure LMN disorder	35
1.3.1. Clinical features	35
1.3.2. Management.....	36
1.3.3. Aetiology	39
1.4. SMA: An infant onset pure lower motor neuron disorder of genetic origin	40
1.4.1. Clinical Features	40
1.5. Conclusion	43
2. Chapter 2: Biomarkers in ALS.....	45
2.1. Diagnostic Biomarkers.....	45
2.2. Prognostic Biomarkers.....	45
2.3. Pharmacodynamic Biomarkers.....	46
2.4. Examples of Biomarkers.....	47
2.4.1. Neurofilament Proteins	47
2.4.2. Blood Biomarkers.....	47
2.4.3. Imaging Biomarkers.....	47
2.5. Electrophysiology.....	48
2.5.1. Current tools of U/LMN assessment	48
2.5.2. H-reflex.....	49
2.5.3. F-Waves.....	52
2.5.4. MUNE.....	54
2.5.5. MUNIX.....	55
2.6. Research Based Electrophysiological Methods.....	58

2.6.1.	EEG	58
2.6.2.	Corticomotoneural Pathway Assessment.....	59
2.7.	Discussion	63
3.	Chapter 3: Aims and Objectives	66
3.1	Aims.....	66
3.1.	Objectives.....	67
3.1.1.	Objective A	67
3.1.2.	Objective B	67
3.1.3.	Objective C	68
4.	Chapter 4: General Materials and Methods	70
4.1.	Ethical Approval and Informed Consent	70
4.2.	Recruitment of Patients and Healthy Controls.....	70
4.2.2.	ALS and PLS patient recruitment	71
4.2.3.	SMA patient recruitment.....	71
4.2.4.	Polio patient recruitment	72
4.2.5.	Healthy Control recruitment	72
4.3.	Clinical, cognitive and behavioural measures	72
4.3.1.	ALS functional rating scale revised (ALSFRS-R)	73
4.3.2.	Date of Onset	73
4.3.3.	Cognitive testing.....	73
4.4.	Experimental Procedure.....	73
4.4.1.	General Procedure Set-Up of surface EEG Acquisition.....	73
4.4.2.	Surface EMG Acquisition	74
4.4.3.	Resting state Recording.....	75
4.5.	Experiments for Corticomuscular Coherence.....	75
4.6.	Data Acquisition	77
4.6.1.	Hardware	77
4.6.2.	Software	78
4.7.	Data Analysis	78
4.7.1.	Pre-processing	78
4.7.2.	CMC pre-processing	79
4.7.3.	Channel Selection and Re-referencing.....	79
4.7.4.	Resting-state Pre-processing.....	80

4.8.	Signal processing.....	81
4.8.1.	Sensor Space- CMC.....	81
4.8.2.	Source Space- CMC.....	81
4.8.3.	Source Space -Resting State	82
4.9.	Statistics.....	82
5.	Chapter 5: ALS Results.....	84
5.1.	Corticomuscular Coherence in ALS	84
5.1.1.	Introduction	84
5.1.2.	Methods	86
5.1.3.	Results	89
5.1.4.	Discussion.....	94
5.2.	Resting State in ALS.....	95
5.2.1.	Introduction	96
5.2.2.	Materials and Methods	97
5.2.3.	Results	98
5.2.4.	Discussion.....	101
5.2.5.	Limitations.....	104
5.2.6.	Conclusions	105
6.	Chapter 6: Poliomyelitis Results	107
6.1.	Corticomuscular Coherence in Polio	107
6.1.1.	Introduction	107
6.1.2.	Methods	111
6.1.3.	Results- CMC in Poliomyelitis	114
6.1.4.	Discussion.....	121
6.2.	Resting State in poliomyelitis.....	123
6.2.1.	Introduction	123
6.2.2.	Methods	124
6.2.3.	Results	125
6.2.4.	Discussion.....	128
6.2.5.	Limitations.....	129
6.2.6.	Conclusion	129
7.	Chapter 7: SMA Results.....	131
7.1.	Corticomuscular Coherence in SMA	131

7.1.1.	Introduction.....	131
7.1.2.	Methods.....	132
7.1.3.	Results.....	135
7.1.4.	Discussion	140
7.1.5.	Limitations	141
7.1.6.	Conclusion.....	141
8.	Chapter 8: PLS Results	143
8.1.	Introduction.....	143
8.2.	Methods.....	144
8.3.	Results.....	147
8.3.1.	Corticomuscular Coherence	149
8.3.2.	Resting State	151
8.3.3.	Source analysis CMC.....	154
8.4.	Discussion	157
8.5.	Limitations	159
8.6.	Conclusion.....	159
9.	Chapter 9: Discussion and Conclusion	160
9.1.	Summary of Results.....	167
9.1.1.	ALS Corticomuscular Coherence	167
9.1.2.	Resting State Beta-band Measures in EEG and ALS Motor Symptoms	168
9.1.3.	Motor β -band power and Clinical Scores.....	169
9.1.4.	Polio Corticomuscular Coherence	169
9.1.5.	Polio - Resting State Study	171
9.1.6.	SMA Corticomuscular Coherence	172
9.1.7.	PLS Corticomuscular Coherence	173
9.1.8.	Resting State in PLS	174
9.2.	Proposed Neuronal Selection model in Motor Neuron Disease.....	175
9.3.	Advantages of Using Neurophysiological Measures	179
9.3.1.	CMC- A Means to unlocking Neurodegeneration.....	179
9.3.2.	Relevance of a Networked Based Approach.....	179
9.4.	Impact and Clinical Applications of CMC.....	180
9.4.1.	CMC as a Therapeutic Outcome Measure in Neurodegenerative Diseases	180

9.5.	Limitations	182
9.6.	Future Work	182
9.6.1.	Longitudinal CMC Studies	182
9.7.	Overall Conclusion.....	183
10.	Bibliography	185
11.	Appendices	213
11.1.	Appendix 4.1	213
11.2.	Appendix 4.2	214
11.3.	Appendix 4.3	216
11.4.	Appendix 6.1	223

List of Figures

Figure 1-1 Gene Frequencies in ALS. From Alsultan et al. 2016 ⁵⁶	13
Figure 1-2 Pathogenetic mechanisms involved in amyotrophic lateral sclerosis (ALS). From Bonafede et al. (2017) ⁵⁸	16
Figure 1-3 Schematic representations corticospinal From Lemon et al (2008) ¹¹²	23
Figure 1-4 Characteristics of motor neuron sub-classes from Kanning et al(2010) ¹²¹ .25	
Figure 1-5 Morphological features of Motor neurons from Kanning et al (2010) ¹²¹	26
Figure 2-1 Outline of Biomarker categorization. From Verber et al. 2019 ²⁰³	46
Figure 2-2 Illustration of H reflex and M wave from Wynne et al. 2006 ²⁴¹	52
Figure 4-1 Pincer grip in isometric task	77
Figure 5-1 ALS Patients show abnormal group level CMC in non-primary motor areas.	92
Figure 5-2 Significant pathological increases in CMC seen at individual as well as group level in ALS.	93
Figure 5-3 The normalized β -band power in motor network shows a significant correlation with the fine motor functions sub-score	99
Figure 5-4 The normalized β -band power in motor network shows a significant correlation with the fine motor functions	100
Figure 5-5 The normalized β -band power in motor network shows a significant correlation with LMN score.....	100
Figure 5-6 The β -synchrony shows a significant correlation with the LMN score	101
Figure 6-1 Illustration of methodological steps carried out to calculate banded cortico- muscular coherence (pCoh), and its classical counterpart	114
Figure 6-2 The cortico-muscular coherence spectra in the healthy control group, showing the significant values at individual and group level.	118
Figure 6-3 Patients with Post-Polio Syndrome (PPS) show abnormal group-level Cortico- Muscular (EEG-EMG) Coherence in non-primary motor area.	119
Figure 6-4 The cortico-muscular coherence spectra in the polio patient group, showing the significant values at individual and group-level.	120
Figure 6-5 Patient subgroups with onsets in infancy (<24 months) vs. childhood (>24 months) show different abnormal Cortico-Muscular (EEG-EMG) Coherence patterns.	121
Figure 6-6 In Polio patients, spectral power is significantly increased between θ and β frequency bands, especially in the parietal, occipital and subcortical regions.....	126
Figure 6-7 Observed widespread co-modulation decrease between healthy controls and Polio patients in α , β and γ h frequency bands.	127
Figure 6-8 Comparisons between co-modulation and spectral power.....	128
Figure 7-1 Patients with SMA show abnormal group level CMC in non primary motor areas.....	138
Figure 7-2 Significant pathological increases in CMC seen at individual as well as group level.	139

Figure 8-1 Patients with PLS show abnormal Cortico-Muscular (EEG-EMG) Coherence in non-primary motor areas.	149
Figure 8-2 CMC shows a partial positive correlation with UMN score between APB muscle and Pz region	150
Figure 8-3 CMC shows a negative partial correlation with UMN Score between APB muscle and C3 region.....	150
Figure 8-4 CMC shows a negative partial correlation with UMN Score between APB muscle and C3 region.....	151
Figure 8-5 CMC shows a partial negative correlation with UMN Score between APB muscle and C4 region.....	151
Figure 8-6 Functional network changes on resting state EEG in PLS.	153
Figure 8-7 Cortico-muscular coherence (CMC) plots of PLS patients and healthy controls	154
Figure 8-8 Cortico-muscular coherence (CMC) plot of PLS patients and healthy control group.....	155
Figure 9-1 The establishment of the developmental process from infancy to adulthood of ‘neuronal selection’.....	177

List of Tables

Table 1-1 Motor Neuron Diseases	4
Table 1-2 Revised El Escorial Criteria. From Brooks et al (2000) ¹²	6
Table 1-3 Gold Coast Criteria for the diagnosis of ALS. From Shefner et al. (2020) ¹⁹	8
Table 1-4 Diagnostic classification of frontotemporal syndromes in ALS. From Strong et al. (2017) ²¹	9
Table 1-5 Pathways involved in UMN lesions in ALS.....	22
Table 1-6 Differential Diagnosis for PLS. From Turner et al.(2020) ¹⁶³	33
Table 1-7 Consensus Diagnostic Criteria for PLS. From Turner et al.(2020) ¹⁶³	34
Table 1-8 Differential diagnosis for acute Polio	36
Table 1-9 March of Dimes Diagnostic Criteria from Li Hi Shing et al 2019 ¹⁷⁹	39
Table 1-10 SMA Classification from Farooq et al 2013. ¹⁹⁶	42
Table 2-1. Current neurophysiological tools available in either clinical or research based settings and their corresponding pathophysiological measures.	54
Table 2-2 CMC frequency ranges and its associated physiological correlates	61
Table 5-1 Clinical and demographic data of analysed patients.	90
Table 5-2 Summary of correlation analysis results between EEG and clinical scores..	98
Table 6-1 Frequency bands and their physiological correlates described in literature in healthy controls	110
Table 6-2 Clinical and demographic data of analysed patients.	115
Table 6-3 Clinical and demographic data of patient sub-groups.....	115
Table 6-4 Demographic Characteristics of Patients	116
Table 7-1 Clinical and demographic data of analysed SMA patients.	136
Table 7-2 Clinical and demographic data of SMA patient sub-groups.....	136
Table 7-3 Individual clinical LMN scores of analysed patients.....	136
Table 8-1 Clinical and demographic data of analysed patients.	147

Abbreviations

AD - Alzheimer's disease

ADM- Abductor digiti minimi

ALS – Amyotrophic lateral sclerosis

ALSbi – ALS with behavioural impairment

ALSci – ALS with cognitive impairment

ALSFRS - Amyotrophic lateral sclerosis functional rating scale

ALSFRS-R - Amyotrophic lateral sclerosis functional rating scale revised

AP – Anteroposterior

APB – Abductor pollicis brevis

APL- Abductor pollicis longus

AUROC – Area under the receivership operating characteristics curve

bvFTD – Behavioural variant frontotemporal dementia

CMAP – Compound muscle action potential

CMC- Corticomuscular Coherence

CMS – Common sense

CST- Corticospinal tract

DFT- Discrete Fourier transformation

ECAS - Edinburgh Cognitive and Behavioural ALS Screen

EDC- Extensor digitorum communis

EEG – Electroencephalography

eLORETA – Exact low resolution electrical tomography

EMG – Electromyography

EPB- Extensor pollicis brevis

ERD – Event related desynchronization

ERP – Event related potential

FALS- Familial ALS

FDI- First dorsal interosseous

FDR – False discovery rate

FDS- Flexor digitorum superficialis

FDMB- Flexor digiti minimi brevis

fMRI – Functional magnetic resonance imaging

FPB- Flexor pollicis brevis

FTD – Frontotemporal dementia

GABA – Gamma aminobutyric acid

H-reflex- Hoffman reflex
LCMV – Linearly constrained minimum variance
LMN – Lower motor neuron
LORETA – Low resolution electrical tomography
M1 – Primary motor cortex
MUNE- Motor unit number estimation
MUNIX- Motor unit index
MRI – Magnetic resonance imaging
MRC- Medical research council
MVC- Maximum voluntary contraction
Nd – Negative difference
NFL – Neurofilament light chain
NMJ- Neuromuscular junction
PA – Posteroanterior
PD – Parkinson's disease
PLS- Primary lateral sclerosis
PPS- Post polio syndrome
SALS- Sporadic ALS
SMA- Spinal muscular atrophy
TDP-43 - TAR DNA binding protein 43
TMS – Transcranial magnetic stimulation
UMN- Upper motor neuron

1. Chapter 1: Introduction

Clash of the titans- pathology vs physiology: what we know in motor neuron disorders

“Symptoms, then, are in reality nothing but the cry from suffering organs”- Jean Martin Charcot

In 1865 Charcot was able to formalise into a specific disease his first description of a patient with muscle spasms. What was originally thought to be related to hysteria, is what turned out to be later described as amyotrophic lateral sclerosis. Charcot noted at autopsy that there was involvement of the lateral tracts "la sclerose laterale" that connects the cortical structures with spinal cord circuitry, This clinicopathological correlation separated ALS from the other sclerosis-namely Multiple sclerosis ("Sclerose en plaques") and the involvement of the posterior fasciculi of the spinal cord ("sclerose combine")¹.

1.1. Motor Neuron Disease

Motor neuron disease (MND) is a progressive neurodegenerative disorder affecting both upper (UMN) and lower (LMN) motor neurons. Motor neuron diseases are the result of dysfunction of upper motor neurons in the precentral gyrus of the frontal lobe and/or lower motor neurons in the ventral horn of the spinal cord.

The most common motor neuron disease is amyotrophic lateral sclerosis, other motor neuron diseases include hereditary spastic paraparesis, spinobulbar muscular atrophy, and infectious motor neuron diseases including polio. Spinal muscular atrophy is a genetic motor neuron disease with recent advances in therapy, including the antisense oligonucleotide, nusinersen approved in 2016. While late childhood onset spinal muscular atrophy exists, it more often affects infants and toddlers. For the purposes of this thesis I have focused on adult SMA type 2 and 3. See Table 1-1 for a list of the most common motor neuron diseases.

Amyotrophic Lateral Sclerosis (ALS) is the most common motor neurone disease, accounting for over 75% of all cases ². In many parts of the world, this term is used synonymously with MND. In this thesis, the specific term ALS is used rather than the broader term MND. Where the term MND is used, it refers to the full clinical spectrum of associated diseases.

ALS of all the MND syndromes has the worst prognosis, with the average life expectancy 2-3 years after onset of symptoms ³. Despite its grim prognosis there remains a lack of effective treatment. While no treatment is available currently, gene therapy for some types of MND may be on the horizon. By stratifying ALS into multiple subtypes and genetic features this would improve the homogeneity of trial cohorts, in effect giving rise to precision medicine within MND.

Table 1-1 Motor Neuron Diseases

DISEASE	MOTOR NEURON AFFECTED	CAUSES
ALS MULTIFOCAL MOTOR NEUROPATHY	Mixed U/LMN LMN	Multifactorial/Unknown Unknown/Immune mediated
SPINAL MUSCULAR ATROPHY	LMN	Genetic
KENNEDY DISEASE	LMN	Genetic
MONOMELIC AMYOTROPHY	LMN	Unknown
POLIOMYELITIS	LMN	Viral Infection
WEST NILE VIRUS	LMN	Viral Infection
PARANEOPLASTIC MOTOR NEURON DISEASE	LMN/undefined	Immune Mediated Complication of Cancer

1.1.1. MND Clinical Features

1.1.1.1. ALS

The main feature of ALS is motor neuron dysfunction. Symptoms usually begin in one region of the spinal cord usually affecting upper or lower limb (spinal onset ALS); however, it may also initially affect cranial nerve nuclei, termed bulbar onset ALS. Spinal onset occurs in 80% with the remainder 20% classified as bulbar onset ⁴.

While extra motor involvement is increasing recognised, spinal onset is the most commonly recognized presentation of ALS. Patients often present with painless progressive limb weakness, which may be often focal, distal and asymmetrical at the early stages of disease. Upper limb involvement often results in poor hand dexterity and grip, associated with wasting of the intrinsic muscles of the hand. When proximal weakness occurs, patients are more likely to report fasciculations in the large muscle groups. If the lower limb is affected, the patient may report walking difficulties, foot drop, difficulty rising from chair/climbing stairs

or stiffness in one or both legs. Muscle wasting (especially of the tibialis anterior), fasciculations and /or spasticity may be seen on clinical examination ³

Bulbar onset is associated with symptoms of dysphagia (affecting liquids more than solids), dysarthria and sialorrhea. Clinical examination features include tongue weakness with spasticity or wasting and fasciculations. UMN involvement is suggested by the pathological presence of a brisk jaw jerk.

Rarely respiratory onset (2 % of cases) can present in patients with early dyspnoea, orthopnoea or hypercapnic features from hypoventilation such as hypersomnolence, early morning headaches and reduced exercise tolerance. Signs of neck weakness and thoracic paraspinals are also be reported.

ALS is no longer considered purely a disorder of the motor system alone but one that affects cognition. It is well recognised that there is a pathological, genetic and clinical overlap between ALS and frontotemporal dementia (FTD) ⁵. Acknowledging and detecting early behavioural and cognitive changes in ALS is both important to ALS caregivers as well as the patient themselves, as there is an association with a rapid decline as well as increased caregiver burden ⁶. Up to 50% of ALS patients develop executive dysfunction, along with features associated with FTD ^{7, 8}. Studies have shown that up to 15% of ALS patients presenting with advanced behavioural changes meet the criteria of behavioural variant FTD (bvFTD) ⁷.

The most common cognitive domain impaired in ALS patients is executive function, with an increasing recognition of language dysfunction occurring independent of executive impairments ⁹. Associated risk factors for cognitive involvement, all be it inconsistently reported, in ALS have been noted to include older age, male sex, and bulbar onset of disease ¹⁰.

1.1.1.2. Diagnostic Criteria

The El Escorial consensus criteria for the diagnosis of ALS were first proposed in 1994 ¹¹ and revised in 1999 to increase its sensitivity¹². In the revised criteria, patients are categorised along a spectrum of probability from “possible” to “definite” ALS based on the number and specific bodily regions affected, the involvement of upper and/or lower motor neurones and the presence or absence of supportive neurophysiological findings (Table 1-2). These criteria were proposed to provide a formal framework while also allowing for increased diagnostic certainty in the diagnosis of ALS. By re-defining core clinical features away from the complex

presentations, has allowed for the standardisation of patients to be included in multi-centre clinical trials ¹³. Recognition of patients with “possible” ALS allows for the inclusion of those with only region affected (if both UMN and LMN signs are present). This permits those in the earliest clinically detectable stages of disease to partake in clinical trials without increasing the risk of including those with erroneous diagnoses ¹³. Yet, critics of the framework would argue that it is unclear whether the objectives of improving diagnostic accuracy and reducing diagnostic delay have been achieved ¹³. Furthermore, they highlight the limitations of the framework in regard that the exclusion of cognitive MND sub-phenotypes, in turn limits the inclusion of these sub-phenotypes into clinical trials.

Table 1-2 Revised El Escorial Criteria. From Brooks et al (2000)¹²

Definite ALS	Presence of UMN, as well as LMN signs, in the bulbar region and at least two spinal regions <u>or</u> Presence of UMN and LMN signs in three spinal regions
Probable ALS	Presence of UMN and LMN signs in at least two regions with some UMN signs necessarily rostral to (above) the LMN signs.
Probable ALS – Laboratory supported	Presence of UMN and LMN signs in only one region, <u>or</u> Presence of UMN signs alone in one region, and LMN signs defined by EMG criteria are present in at least two regions
Possible ALS	Presence of UMN and LMN signs in only one region <u>or</u> Presence of UMN signs in two or more regions <u>or</u> Presence of LMN signs rostral to UMN signs and the diagnosis of Probable ALS – Laboratory supported cannot be proven

The revised El Escorial (rEEC) criteria, which remains clinically based, outlined 4 levels of diagnostic certainty, (definite, probable, possible, and suspected ALS), which is centred on the degree of UMN and LMN dysfunction ¹⁴. To allow for improvements in diagnostic sensitivity, the rEEC introduced a “laboratory supported probable ALS category,” which allowed EMG results to support clinical findings ^{11, 12}. While the criteria were specific, issues were raised around its sensitivity in early diagnosis and the possible delay implications this had on clinical trial recruitment ^{15, 16}.

The Awaji criteria, which based itself more around neurophysiological results, was developed to increase diagnostic utility especially in the early stages of ALS ¹⁷. EMG changes showing LMN dysfunction was now deemed equivalent to LMN signs, along with the addition of fasciculations as a LMN sign.

Both rEEG and Awaji criteria were often seen as complex with poor inter-rater variability reported in some studies ¹⁸. The Gold coast criteria was developed to address the potential issues and limitations of the previous diagnostic criteria ¹⁹ (Table 1-3). One study showed that the Gold Coast criteria was comparable to previous criteria, in fact sensitivity was maintained despite functional impairment or disease duration ¹⁴. It was noted that the sensitivity of the Gold Coast criteria was superior when detecting definite/probable ALS ¹⁴.

In all cases the ALS criteria for the identification of UMN signs remains clinical. In ALS patients this can be difficult to detect and as such potentially reduces the sensitivity of clinical diagnostic criteria. This raises the need for reliable neurophysiological biomarkers to increase diagnostic sensitivity ²⁰.

Table 1-3 Gold Coast Criteria for the diagnosis of ALS. From Shefner et al. (2020)¹⁹

1. Progressive motor impairment documented by history or repeated clinical assessment, preceded by normal motor function, and
2. Presence of upper¹ and lower² motor neuron dysfunction in at least 1 body region³, (with upper and lower motor neuron dysfunction noted in the same body region if only one body region is involved) or lower motor neuron dysfunction in at least 2 body regions, and
3. Investigations⁴ excluding other disease processes

Footnotes

¹Upper motor neuron dysfunction implies at least one of the following:

1. Increased deep tendon reflexes, including the presence of a reflex in a clinically weak and wasted muscle, or spread to adjacent muscles
2. Presence of pathological reflexes, including Hoffman sign, Babinski sign, crossed adductor reflex, or snout reflex.
3. Increase in velocity-dependent tone (spasticity)
4. Slowed, poorly coordinated voluntary movement, not attributable to weakness of lower motor neuron origin or Parkinsonian features

²Lower motor neuron dysfunction in a given muscle requires either:

Clinical examination evidence of

Muscle weakness, and

Muscle wasting

Or

EMG abnormalities that must include:

Both evidence of chronic neurogenic change, defined by large motor unit potentials of increased duration and/or increased amplitude, with polyphasia and motor unit instability regarded as supportive but not obligatory evidence.

And evidence of ongoing denervation including

Fibrillation potentials or positive sharp waves, or fasciculation potentials

³Body regions are defined as bulbar, cervical, thoracic and lumbosacral. To be classified as an involved region with respect to lower motor neuron involvement, there must be abnormalities in two limb muscles innervated by different roots and nerves, or one bulbar muscle, or one thoracic muscle either by clinical examination or by EMG.

⁴The appropriate investigations depend on the clinical presentation, and may include nerve conduction studies and needle EMG, MRI or other imaging, fluid studies of blood or CSF, or other modalities as clinically necessary.

Criteria proposed by Strong et al in 2017 ²¹ allowed for the classification of milder cognitive changes associated with ALS. These criteria expand on a previously published version (International Behavioural Variant FTD Criteria Consortium (FTDC) for the diagnosis of behavioural variant FTD ²²) to include language impairment and primary progressive aphasia as a sub-category of ALS- FTD. The Strong criteria below refers to the second axis of the guidelines, describing diagnostic criteria for behavioural and cognitive syndromes in ALS.

Table 1-4 Diagnostic classification of frontotemporal syndromes in ALS. From Strong et al. (2017)²¹

ALS with behavioural impairment (ALSbi)	
<i>diagnosis requires either</i>	
Identification of apathy with or without other behaviour change	
Meeting at least two non-overlapping supportive diagnostic features from the Rascovsky criteria ²²	
ALS with cognitive impairment (ALSci)	
<i>requires evidence of either/both</i>	
Executive dysfunction, including social cognition (requires either)	
i	Impaired verbal fluency (letter)
ii	Impairment on two other non-overlapping measures of executive functions (which may include social cognition)
Language dysfunction	
i	Impairment on two non-overlapping tests and in which language impairment is not solely explained by verbal fluency deficits
ALS with combined cognitive and behavioural impairment (ALS-cbi)	
Meet the criteria for both ALSci and ALSbi	
ALS-FTD	
<i>requires both</i>	
Evidence of progressive deterioration of behaviour and/or cognition by observation or history	
Any of	
i	The presence of at least 3 of the behavioural/cognitive symptoms outlined by Rascovsky criteria ²²
ii	The presence of at least 2 of those behavioural/cognitive symptoms, together with loss of insight and/or psychotic symptoms
iii ^a	The presence of language impairment meeting criteria for semantic dementia/ semantic variant PPA or non-fluent variant PPA.

1.1.1.3. *Epidemiology*

Most of the population-based epidemiology studies in ALS have been carried out on individuals of European origin ²³⁻²⁶ and the findings are relatively consistent. ALS is more common in men than women with an incidence in European populations of 2–3 people per year per 100,000 of the general population ²⁷.

ALS rates outside of Caucasian/European descent are not well-characterized, with few detailed prospective population-based studies undertaken ²⁸. The most detailed population-based studies from South America have been confined to Uruguay and Cuba. Uruguay is primarily of European descent, with ALS incidence similar to those of southern European countries ²⁹. In countries with populations with greater degree of admixture there is evidence to suggest that the rate of ALS may be lower. Cuba, a country with mixed heritage has been reported to have lower rates than its neighbouring country (Uruguay) ^{30, 31}. There is not enough information available to determine the incidence of ALS in large parts of the world, including Africa, Russia and India ³². One systematic analysis for global burden of disease showed the prevalence of ALS increasing especially after 50 years of age, with a peak noted at 85 years³³. They hypothesised that the sharp decline in incidence of ALS in the >85 years age group may be due to the complex clinical presentations and multiple co-morbidities, with these patients less likely to be referred to tertiary neurology clinics and less likely to get a correct diagnosis ³³.

Cognitive and behavioural impairment in ALS has been recognised for some time. An Irish population-based incidence study reporting 13% of cases have ALS–FTD and a further 40% have evidence of cognitive impairment ^{5, 34}. Carriers of the C9orf72 repeat expansion (11% of the Irish cohort) are more likely to show cognitive changes ³⁵.

Differing clinical phenotypes have been associated with the C9orf72 repeat expansion featuring increased incidence of neuropsychiatric disease such as, suicide and psychotic illnesses, suggesting that in some populations ALS can possibly share genetic susceptibilities with neuropsychiatric illnesses ^{36, 37}.

1.1.1.4. *Diagnosis and Staging in ALS*

Currently, a clinical diagnosis of ALS is made by the identification of dysfunction in both upper and lower motor neurons, this is encompassed for research purposes by

the revised El Escorial diagnostic criteria, which requires the presence of progressive loss of upper and lower motor neuron function, and the absence of another explanation.

However, it is now recognized that while the neurodegenerative disease that is ALS is primarily characterized by progressive loss of motor function, there is evidence of extensive extra-motor involvement in some patients ³⁸.

The progressive nature of the disease often means that patients are faced with a rapid decline in motor function and many present at the later stages of the disease. In many cases diagnostic certainty is delayed by up to 1 year from the onset of symptoms ³⁹. However clinical signs of UMN degeneration may not be easily apparent in a limb with concurrent LMN involvement⁴⁰. It has been reported that anywhere from 30-75% of patients with “pure LMN” disease at autopsy have UMN involvement without apparent clinical signs^{41, 42}. Our current reliance on symptoms and clinical examination is framed through an end stage clinicopathological prism and underpinned by insights from animal models. This perspective means that in humans, it is the case that the disease has often progressed to such an extent that degeneration renders it irreversible. This in turn leads to unavoidable delays in diagnosis, with ensuring limitations in the institution of potential disease modifying therapeutics.

ALS staging offers the opportunity to monitor disease progression inline to defined clinical milestones chosen to reflect disease severity and prognosis^{43, 44}, while measures like the ALSFRS-R rating scale assess functional decline independently of these milestones. At differing stages of disease patients can have differing needs, with earlier stages requiring diagnostics and various therapeutic supports, whilst later stages needing respiratory and nutritional interventions and a more palliative approach to care⁴³⁻⁴⁵. Staging criteria are important in guiding therapeutic decision making, resource allocation and patient selection and outcome assessment in clinical trials^{43, 44}. ALS-specific staging systems have been shown to correlate with functional measures, quality of life scores, disease biomarkers, health utility and healthcare and socioeconomic costs⁴⁶.

The current staging systems used in ALS to measure disease progression are : The King's staging system⁴⁴ and the Milano–Torino (MiToS) Staging system⁴³. The King's staging system has five different stages, categorised from 1 to 5 and is based on the neuroanatomical distribution of disease as well as disease burden measured by significant nutritional or

respiratory failure⁴⁵. The MiToS system has six stages, graded from 0 to 5 and is established from one's functional ability as measured by the ALSFRS-R⁴⁵. In both systems, stage 5 equates with death^{43, 44}. A retrospective study comparing both staging systems found greater resolution in early to mid-disease with the King's staging system compared with higher resolution in late disease for the MiToS staging system⁴⁵. This reflects the necessity that functional decline stems from anatomical involvement⁴⁵. The utilization of both systems is recommended for complementary and comprehensive data collection⁴⁵.

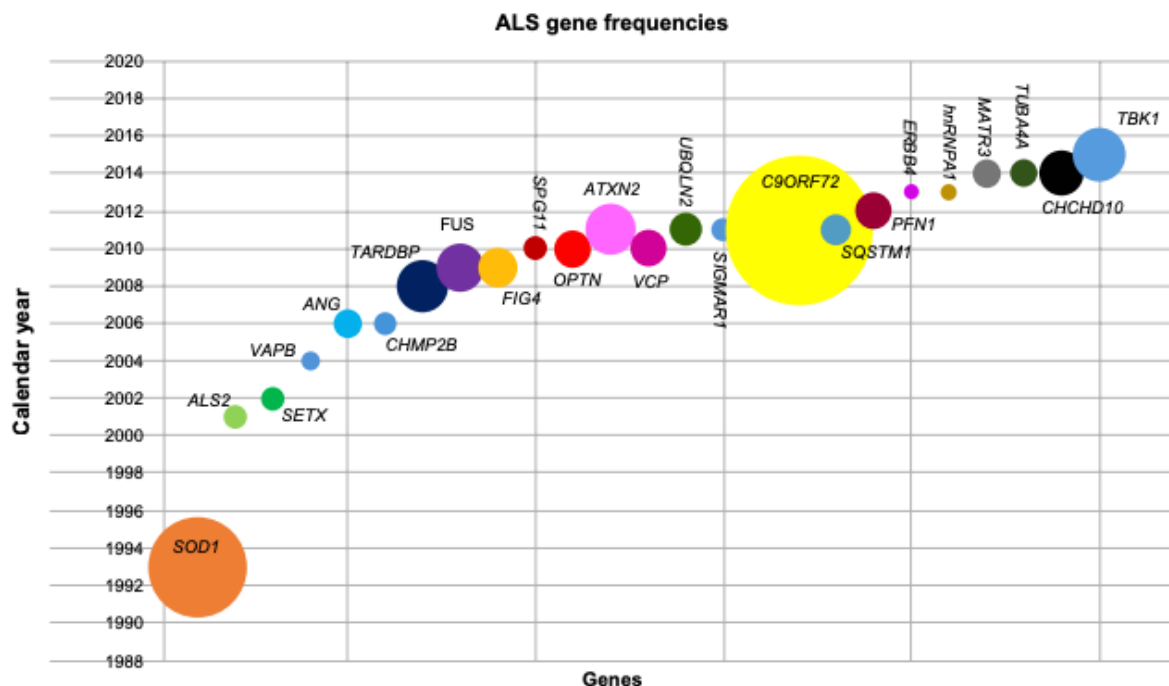
Both staging systems, however, are limited by their failure to include a measure of cognitive involvement, a known negative prognostic indicator⁴⁷ which requires its own specific approach to management. As cognitive impairment may develop at any time in the disease course, it is therefore difficult to translate cognitive involvement to a clear clinical milestone within an expected temporal sequence⁴⁴. While future ALS staging systems may include some measure of cognitive and/or behavioural impairment, at present the use of criteria grading the severity of cognitive and behavioural impairment in ALS²¹ is recommended in parallel to current ALS disease staging systems⁴⁶.

1.1.1.5. Genetics

ALS is a progressive neurodegenerative disease, affecting multiple systems, resulting from a mix of environmental and time factors that interact with pre-existing genetic characteristics²³. Evidence of the genetic origin of ALS can be seen in familial aggregation of the disorder with the description of over 30 genetic mutations that co-segregate with the clinical phenotype⁴⁸. However, 4 gene variants account for 55% of familial ALS cases in Europe⁴⁹.

While most cases are sporadic (SALS), around 5–10% of patients have a family history with ALS (FALS). Although more than 30 genes have been found to be implicated in the pathogenesis of ALS since 1993,⁵⁰ (Figure 1-1) a number of studies have highlighted C9orf72, SOD1, TARDBP and FUS as the most common mutated genes in European and Asian ALS populations⁵¹⁻⁵⁴. However, these mutation frequencies dramatically varied between studies.

A recent genome wide association study in ALS picked up 15 risk loci contributing to ALS risk⁵⁵. Patterns of shared genetic risk across specific locus was noted in other neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and progressive supranuclear palsy with ALS⁵⁵.



"Each gene is plotted against the year it was found; the size of the circles signifies the frequency of mutations in FALS or ALS as cited in the literature. Where gene frequencies were not available, these have been given a circle size equivalent to 1% for illustrative purposes."

Figure 1-1 Gene Frequencies in ALS. From Alsultan et al. 2016 ⁵⁶.

1.1.1.6. Pathogenesis

A number of theories in the pathogenesis of ALS have been explored. One of many was the identification of the role of excitatory toxicity on neuronal cells. The existence of the heterogeneous ALS phenotype maybe come down to a final common pathway that converges on a number of genetic, environmental and age dependent substrates ⁵⁷.

In sporadic forms of ALS(sALS), the onset of disease is thought to be due to a gene-environment inter-play; sALS has been described to be associated with "susceptibility" genes, that can trigger a cascade of neurodegeneration while interacting with environmental risk factors ⁵⁸. These susceptibility genes may play a role in familial ALS (fALS) ⁵⁹. Mutations in these susceptible genes could contribute to the development of disease only in the presence of other genetic or environmental risk factors ⁵⁸.

Studies have shown that patients presenting with fALS often develop symptoms at an earlier age when compared to the sporadic form, with penetrance, severity, progression and duration of disease varying in different gene mutations as well as different mutations in the same gene ^{48, 60}.

Advances in identifying the genetic root of ALS ^{61, 62} in animal models such as mice, zebrafish, and flies have demonstrated how mutations cause motor neuron degeneration, while modelling the biological processes that are often thought to be instrumental in the disease process itself. However, these models have their limitations, and none fully represents human disease. This is partly because most models are centred on gene overexpression (with the human variant inserted into a transgenic model) and because of the substantially different human neural network from that of lower animal models ⁶³. Notwithstanding, animal model findings can inform our understanding of cell biology and its underlying neurodegeneration. The reality of cellular dysfunction in ALS is a likely result of many different interacting mechanisms that culminate in larger network disruption ⁶³.

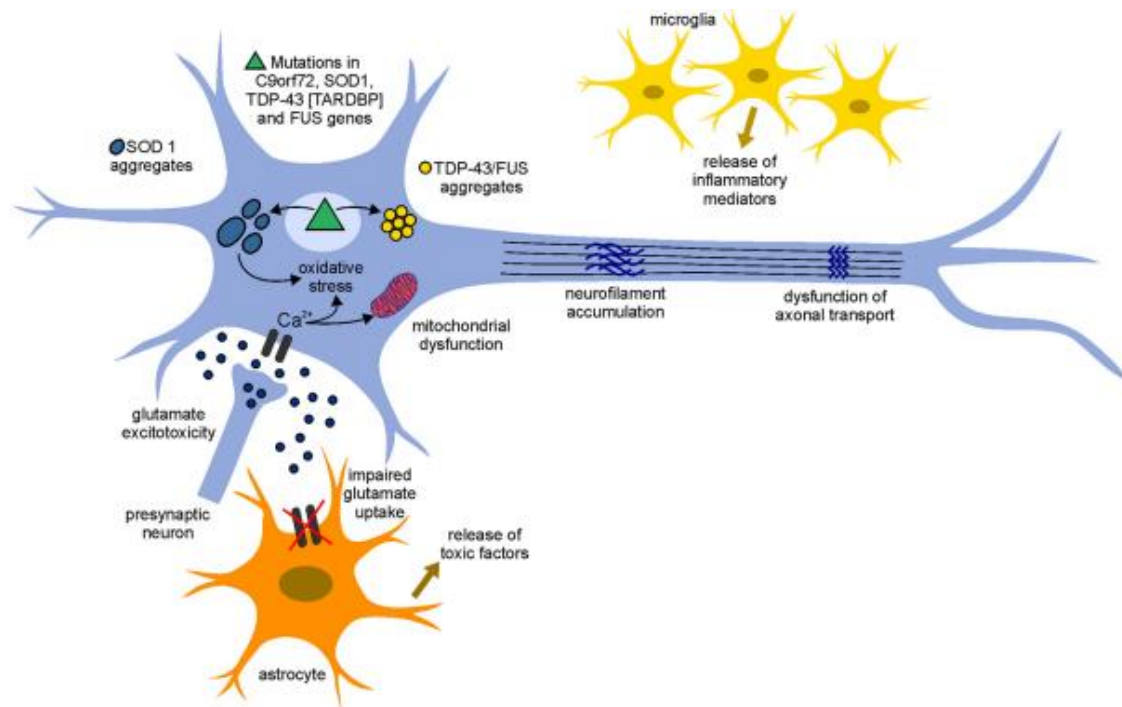
One of the main difficulties in deconstructing the early features of ALS come from the absence of a complete animal model of the disease. There are no naturally occurring animal forms of ALS. With the discovery that variants in SOD1 can lead to human ALS in 1991, and the generation of the first transgenic mouse with a form of ALS in 1994, there has been a large literature that has sought to understand the mechanism of disease in ALS in mouse models. Of the familial forms of ALS, the most studied is the SOD1 involving toxic gain-of-function mutations. Protein misfolding is often suggested as a common initial trigger in ALS, often thought to be linked to over 140 individual point mutations throughout the primary SOD1 structure⁶⁴. The expression of the mutant form of human SOD1 in transgenic mice models show selective motor neuron degeneration and death, which may resemble the pathology of sporadic and familial ALS ⁶⁵. Recent studies have shown that the expression of mSOD1 dictates the full disease phenotype not only on motor neurons but also in astrocytes and microglia cells ⁶⁶. These mSOD1 studies provide an opportunity to monitor the pre-symptomatic sequence of events at a molecular and cellular stage, which often lead to weakness, paralysis, and death, allowing us to understand the cellular and mechanisms involved in the pathogenesis of ALS.

While the SOD1 mouse is an incomplete model of human disease, work recently reported published by Lalancette-Hebert et al ⁶⁷ has demonstrated this “shared” feature of ALS in selective sparing of gamma motor neurons. Gamma motor neurons themselves innervate muscle spindles regulating the primary proprioceptive afferent feedback system to alpha motor neurons. The demonstration of the elimination of Ia inputs or even the partial elimination of gamma motor neurons was protective in SOD-1 mutant mice. The inference made was that the surviving gamma motor neurons contribute to the alpha motor neuron loss by increasing the muscle afferent mediated excitation ⁶⁷.

SOD1 gene mutations may cause a dominant gain of function, giving rise to an increase of SOD1 activity, allowing for an excessive production of Hydrogen peroxide (H₂O₂), or alternatively a dominant loss of function with a decrease in enzyme activity which results in insufficient degradation of reactive oxygen species (ROS) ⁶⁸. Studies^{69, 70} have suggested that the mutated SOD1 could return to its normal antioxidant actions thereby generating a toxic superoxide (where the mutated protein takes on electrons from other sources and donates them to molecular oxygen, giving rise to a superoxide and in the process SOD1 becomes the source of oxidative stress). Higher levels of free radicals were noted in the CSF and urine samples of ALS patients which could be attributed to the mutated site of the SOD1 gene ⁷¹.

The ability to identify the molecular mechanisms of neurodegeneration in ALS is vital for the understanding of disease progress and for the potential of developing new therapeutic approaches.

While SOD1 mutations have been linked to ALS for more than two decades, the pathways underlying the mode of action of a mutant SOD1 and the ensuing neurotoxicity are still uncertain ⁵⁸. Hypotheses have been proposed in this area, with the most likely being that the combination of mechanisms, rather than a single pathway, contributing to neurodegeneration in ALS, paving the way to a multifactorial pathogenesis ⁵⁸.



"The pathophysiological mechanism of the disease appears to be multifactorial and several mechanisms contribute to neurodegeneration. An increase of the neurotransmitter glutamate in the synaptic cleft (glutamate excitotoxicity), due to the impairment of its uptake by astrocytes, leads to an increased influx of Ca^{2+} ions in the motoneurons. The increased levels of Ca^{2+} ions, which in physiological conditions could be removed by mitochondria (calcium homeostasis), remain high in the cytoplasm due to mitochondrial dysfunction and can cause neurodegeneration through activation of Ca^{2+} -dependent enzymatic pathways contributing to oxidative stress. Mutant misfolding proteins (such as superoxide dismutase 1 gene (SOD1), chromosome 9 open reading frame 72 (C9orf72), TAR DNA-binding protein 43 (TDP-43) and fused in sarcoma (FUS) form intercellular aggregates, contribute to an increase of oxidative stress, contribute to mitochondrial dysfunction and could lead to the accumulation of neurofilaments (NFs) and dysfunction of axonal transport."

Figure 1-2 Pathogenetic mechanisms involved in amyotrophic lateral sclerosis (ALS). From Bonafede et al. (2017)⁵⁸.

One of the early hypotheses put forward was that of glutamate excitotoxicity as a possible mechanism of ALS⁷². Motor cortices and spinal cords of ALS patients and transgenic mutant SOD1 mouse models were found to have reduced levels of the isoform 2 of the astroglial glutamate transporter (EAAT2), leading to a noted increase of synaptic glutamate concentration and an over-stimulation of postsynaptic glutamate receptors, defining excitotoxic neuronal degeneration^{71, 73}. It was noted in other studies a similar loss of functional EAAT2 in affected brain regions of other neurodegenerative diseases, such as Alzheimer's and Huntington's diseases⁷⁴.

Recently the innate immune system has been implicated in the pathogenesis of ALS. The complement system has been a target for potential treatment in ALS. Studies have shown that an improper activation of the complement system can play a role in the pathogenesis of ALS⁷⁵.

One of the features of ALS and other neurodegenerative diseases is the neuroinflammatory response, often defined by microglial activity at sites of neuronal injury with infiltrating immune cells. While ALS involves the selective degeneration of motoneurons, there is evidence to show that neuronal injury is not cell dependent but rather depends on a finely tuned pathway between glial cells and motoneurons. Uncontrolled messaging between glial cells and neurons can impact the neuronal balance and its own survival. The effect of mutant glia in motoneuron dysfunction and degeneration is well documented ⁵⁸.

Microglial cells are the resident macrophages in the CNS and are the first line of defence against any injury or assault. Their role is to monitor the extracellular environment as well as interact with neurons and astrocytes. Microglial cells have immunological facets that are both neuroprotective and have neurotoxic potential. After injury, neuroinflammation is mainly driven by microglial activation. In ALS, the synergy between motoneurons and microglia is to initially protect neurons. As motoneuron damage continues deteriorate, motoneurons and astrocytes release misfolded proteins (such as the mutated SOD1) as well as other toxic molecules that stimulate the activation of microglial cells, which switch from an anti-inflammatory and neuroprotective to a pro-inflammatory and neurotoxic phenotype ^{76, 77}. Studies have shown correlation between microglial pathology and ALS progression ^{78, 79}. Notwithstanding, recent research points to neuroinflammation being a primary event resulting in a destructive interaction between motor neurons and surrounding cells ⁸⁰.

Whether the motor neurons themselves activate a neuroinflammatory response or the innate immune system kick starts a neurodegenerative process- one thing we know is that there is mounting evidence that ALS is a complex systemic disease, rather than a process of the CNS solely ^{81, 82}.

Reactive astrogliosis has been postulated to be involved in the neurodegeneration and progression of ALS. An important astrocyte function is the support it lends to neurons to maintain low concentrations of glutamate in the synaptic cleft through EAAT2 glutamate receptors⁵⁸. In sporadic and familial forms of ALS, as well as in SOD1 transgenic mice, astrocytes were shown to downregulate the EAAT2 transporter, giving rise to a less efficient uptake of glutamate and contributing to excitotoxicity ⁸³. It has also been shown in other studies that astrocyte activation with

inefficient release of neurotrophic factors along with the release of neurotoxic factors are involved in the neurodegeneration in ALS ⁸⁴. This was implied by analysing astrocytes in the post-mortem tissue of ALS patients, showing an upregulation of 22 genes encoding chemokines, proinflammatory cytokines and components of the complement cascade, which could exacerbate neural damage and loss of already compromised neurons ⁷⁷.

TDP-43, a protein present and characteristic in sporadic ALS, has been implicated in a “prion like” spread of disease ⁸⁵. These misfolded toxic form of protein inclusions in the cellular cytoplasm mirror the propagation process in prion disease, implying that these abnormal proteins spread to other nearby cells ⁸⁶. This infectious spread of a misfolded protein is thought to require a cell-to-cell mechanism of transfer. The current thought is that phagocytosis is the process by which these toxic proteins are ingested and transported from distal dendrite to the cell body ⁸⁷. These misfolded proteins when releases into the extracellular space during the apoptotic process is thought when they can “infect” their otherwise healthy cell neighbour again through a phagocytic ingestion ^{86, 87}.

By increasing our understanding of the complex nature of interacting pathways in ALS and other neurodegenerative conditions could lead to a generation of targeted therapies. However, without a means to quantifiably measure physiological changes these therapies could potentially be aimed at the wrong subgroups of patients, thereby yielding less than the desired for effects.

The efforts in the search for mapping disease progression as well as early diagnostic biomarkers has well and truly begun. Only when we improve our diagnostic power can we enhance care in ALS ⁸⁸.

1.1.2. Clinical Management of ALS

Current disease modifying Treatments

1.1.2.1. Riluzole

Up until very recently, riluzole was the only medication to shown efficacy in clinical trials in its ability to slow ALS progression. On foot of a double blind randomised controlled trial in 1994 ALS patients were found to have a higher survival rate and reduced rate of muscle strength progression the treated cohort ⁸⁹, a later study assessing drug efficacy at differing doses found 100mg total daily dose to have the best risk/benefit ratio⁹⁰. In 2012 a Cochrane review showed short survival benefit of 2 - 3 months for those taking riluzole ⁹¹. In view of the lack of effective alternative treatment options for patients with ALS, the licensing of riluzole to ALS patients was granted by several licensing bodies around the world ^{92, 93}.

The exact mechanism of action of riluzole remains unknown. It has been suggested that riluzole acts via several differing pathways including through modulation of the anti-glutamate of excitotoxic pathway ⁹⁴. Given the wide ranging possible modes of action, it is conceivable that riluzole may act on differing therapeutic pathways at different stages of the disease ⁹⁵.

1.1.2.2. Edaravone

Edaravone became the second medication licensed by the FDA in 2017 with a reported neuroprotective effect in ALS. A free radical scavenger, edaravone was initially licensed in Japan as a treatment of acute ischaemic stroke. A phase 3, randomised, double-blind trial of ALS patients was carried out targeting a sub-group which found a significantly smaller decline in the treatment arm in ALSFRS-R ⁹⁶.

Concerns have been raised about the possible clinical utility of this treatment. Debate around the trial follow up duration, outcome measures and lack of available biomarkers have been discussed ⁹⁷. In view of the high costs to the patients as well as time and effort spent to attend regular intravenous infusions, further studies assessing overall risk to benefit ratio for patients were recommended ⁹⁷.

1.1.3. Moving Towards Precision Medicine

Innovative models to the application of biomedical technologies have given rise to the concept of precision medicine, a way to individualize treatment by targeting molecular drivers of a specific disease. This allows for the classification of patients into subgroups that either differ in their vulnerability to disease, differ in disease

biology or differ in their response to a specific treatment ^{98, 99}. This approach is only starting to be considered in ALS, it has been used successfully in cancer treatment. With its complex, multifactorial aetiology, along with variations in phenotype Precision medicine is the only way forward to effectively offer a potential treatment that works.

1.1.4. A window into ALS complexity

1.1.4.1. ALS Going Forward or Going Back

In the quest to understand ALS pathogenesis the debate still rages on whether disease onset is primarily central or peripheral ¹⁰⁰.

The dying forward hypothesis is rooted in the view that ALS is a “brain disease”.

Often supported by the argument of increasingly recognised associations with frontotemporal dementia ¹⁰¹, as well as a consistent involvement of betz cell and motor cortex in pathology studies. The trademark TDP-43 pathology mainly restricted to corticofugal neuronal projections is seen in over 95% of ALS patients ¹⁰¹. The dying forward hypothesis speculates that there is a metabolic deficit at the anterior horn cell resulting from a glutamate excitotoxicity at the motor cortex ¹⁰².

Imaging and TMS studies have indicated and supported the idea of a cortical origin for ALS ^{103, 104}. While Charcot describes ALS starting in the motor cortex ¹ this later being detailed within the dying forward hypothesis ¹⁰⁵, arguments against the central role of cortical dysfunction in ALS has arisen.

The dying-back hypothesis suggested that ALS was a disorder of LMNs, with the pathogenesis centred around the retrograde transport from the NMJ to CNS where its effect is felt. Much of the support for the dying back model has been evidenced by transgenic mouse studies which have shown axonal transport abnormalities as an early feature preceding neuronal degeneration ¹⁰⁶. One of the limiting factors of this hypothesis relates to the widespread cortical dysfunction found in ALS patients ¹⁵ as well as its reliance on animal models, not addressing the complexity of human neuroanatomy.

As such evidence points to considering ALS as a distal axonopathy rather than a central disease of neurodegeneration ¹⁰⁷. It is theorised that motor neuron dysfunction is a result of pathogenic event within the NMJ giving rise to the loss of contact between nerve terminals and muscles fibres causing the degeneration of the motor neurons ⁸¹. NMJs are located within skeletal muscles, exposed to the

peripheral immune system. An immune system when triggered into dysregulation may be a mediator of ALS progression¹⁰⁸. There is an argument often made that ALS is primarily a neuromuscular disorder with brain dysfunction associated with it. The argument is often supported by the fact that ALS is defined as a disease of the upper (those neurons residing within the spinal cord prior to synapse) and lower motor neurons, with any cortical changes a consequence of a primary distal disease.

To better understand the arguments put forward I review in this section what we know of the pathophysiology of motor networks in the setting of ALS as well as evaluate what potential neurophysiologic biomarkers are in use clinically and in research that may have diagnostic and prognostic value.

There is an undeniable clinical importance attached to the full understanding of the role of descending motor pathways in movement, especially in the setting of motor neuron disorders. Clinical trials are already underway to assess varying therapeutic approaches to delaying or even stopping the progression of motor neuron diseases.

For progress to continue, it is increasingly more important to understand fully the functional contribution of the different descending systems that may have been injured and whether the surviving systems can play compensatory roles and how these various processes can be boosted by appropriate therapy in the future.

The focus of this review will be to reassess our understanding of the ALS pathology. In being able to devise and use diagnostic techniques one must be able to recognize and understand the underlying pathology in MND. For the purposes of this review I have focused on ALS as an example of a motor neuron disorder.

1.1.5. Anatomy of ALS

1.1.5.1. Cortical structure

One of the main difficulties in recognizing UMN signs in ALS is attributed to concurrent LMN weakness, obscuring the pattern of corticospinal weakness⁴⁰. As has been taught by Charcot, and reinforced over many generations, the definite diagnosis of ALS requires confirmation at autopsy. For definitive diagnosis, this includes the presence of UMN lesions together with LMN dysfunction, with the deposition of TDP-43 protein in affected regions. A lesion of the UMN includes the degeneration of the corticospinal tract (CST) and related descending pathways of the

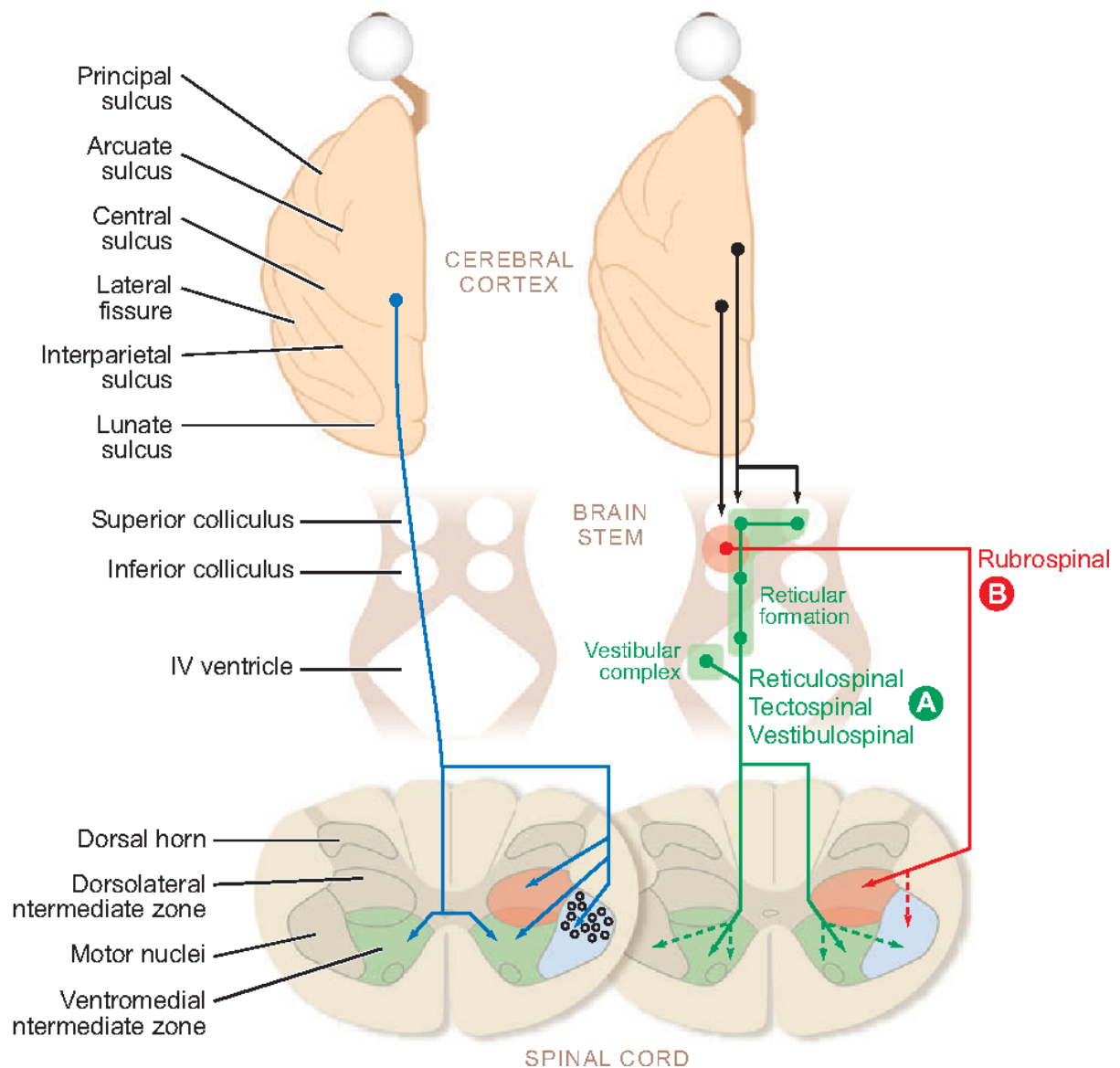
internal capsule, brainstem and spinal cord (Figure 1-3). Pathological changes have been noted in layer V of the motor cortex with the loss of Betz cells. More widespread pathology in the anterior brain involving the deep temporal and frontal white matter as well as the corpus callosum have also been described ¹⁰⁹.

Histological studies have demonstrated the presence of two general types of CST neuron. In the first type, axons contact spinal interneurons as they come to terminate in the intermediate zone of the spinal cord. Some of these interneurons mediate part of the descending commands for movement by synapsing with spinal motoneurons ¹¹⁰. In the second type - CST neuron axons monosynaptically synapse with spinal motoneurons in the ventral horn of the spinal cord. These corticomotoneuronal cells play a role in the generation and control of movement including fine finger movements¹¹¹.

In reality the involvement of UMN lesions is almost never confined to the fibres arising from the cortical Betz cells but also involves other pathways including those descending through the internal capsule and its caudal projections. (See Table 1-5)

Table 1-5 Pathways involved in UMN lesions in ALS

-
- Corticospinal Tract
 - Corticobulbar Tract
 - Reticulospinal Tract
 - Vestibulospinal Tract
-



"On the right, **Group A** fibers (reticulospinal, tectospinal, vestibulospinal) are shown in green, arising from the brainstem reticular formation, superior colliculus, and vestibular complex. These fibers terminate bilaterally in the ventromedial part of the intermediate zone (IZ) shown as a green area in the spinal sections, with some direct projections to motoneurons supplying trunk and girdle muscles (dashed green lines). **Group B** fibers (rubrospinal) are shown in red, arising from the red nucleus. These fibers terminate contralaterally in the dorsolateral region of the IZ (shown in red in the spinal sections) with some projections to the lateral group of motor nuclei innervating the arm and hand (dashed red lines). These brainstem pathways receive significant cortical projections (black). On the left, corticospinal projections are shown in blue: Some parallel the group A fibers and terminate bilaterally in the ventromedial IZ (green area), whereas the majority parallel the group B system and terminate contralaterally in the dorsolateral IZ (red area) and directly on motoneurons innervating the arm and hand (blue region with small black circles)."

Figure 1-3 Schematic representations corticospinal From Lemon et al (2008) ¹¹²

1.1.5.2. *Corticospinal Pathway*

A neuroanatomical pathway can mediate different functions ¹¹². The CST is a prime example. CST many functions include :

- (a) descending control of afferent inputs, including these nociceptive inputs ¹¹³
- (b) selection, gating, and gain control of spinal reflexes ¹¹⁴
- (c) excitation and inhibition of motoneurons ¹¹⁵
- (d) autonomic control ¹¹⁶
- (e) long-term plasticity of spinal cord circuits ¹¹⁷
- (f) trophic functions ¹¹⁸

The CST has been noted, from different animal studies, to originate from a wide variety of cortical areas, including, the primary motor cortex (M1), the dorsal and ventral premotor cortices, supplementary motor area (SMA), and cingulate motor areas ^{112, 119}. The origins of the CST from many different cortical areas make it less likely that it fulfils a single role. Notably, the CST terminates broadly within the spinal grey matter, possibly reflecting control of somatosensory, reflex, autonomic, and somatic motor functions.

CST projections to the dorsal horn are an important source of presynaptic inhibition of primary sensory afferent fibres ¹²⁰, and this mechanism could allow removal of predictable sources of afferent input associated with feedforward motor commands for voluntary movement. Lesions of the CST cause a breakdown in fine sensorimotor control, implying a deterioration not only in motor function, but also in the capacity to interrogate correctly the sensory feedback from the hand ¹¹².

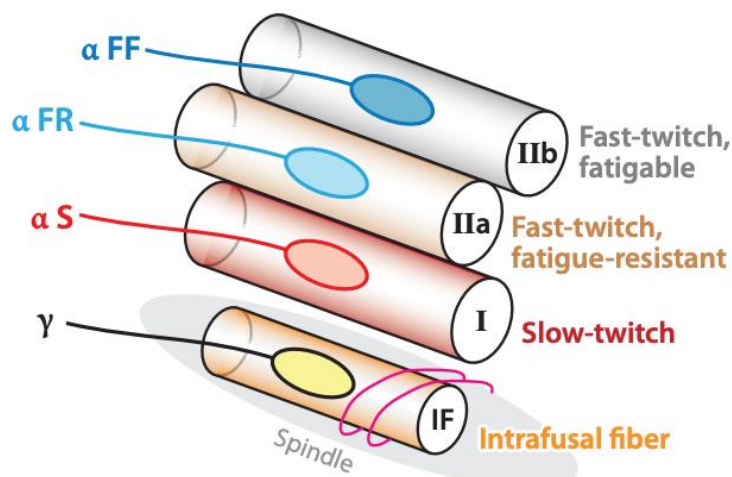
1.1.5.3. Motoneurons

The human body consists of more than 300 pairs of muscles, consisting of more than 100 million fibres, which are innervated by more than 120,000 motor neurons in the spinal cord ¹²¹. In humans, motor neurons are classified into functionally differing subtypes (Figure 1-4), namely into - alpha (α), beta (β), and gamma (γ) motor neurons. These motor neurons are divided according to the type of muscle fibre it innervates (Figure 1-4).

Extrafusal skeletal muscles are innervated by alpha motor neurons and are known to drive muscle contraction. Intrafusal muscle fibres are innervated by Gamma motor neurons and have a complex role in motor control. The third less defined subtype of motor neuron are called β -motor neurons, which innervate both intra- and extrafusal fibres ¹²¹.

Alpha motor neurons are the most abundant of these subtypes. Alpha motor neurons themselves can be grouped into subtypes based on the contractile effects of the motor units they form with muscle fibres:

- Fast-twitch fatigable (FF),
- Fast-twitch fatigue-resistant (FR),
- Slow-twitch fatigue resistant (S) ¹²².

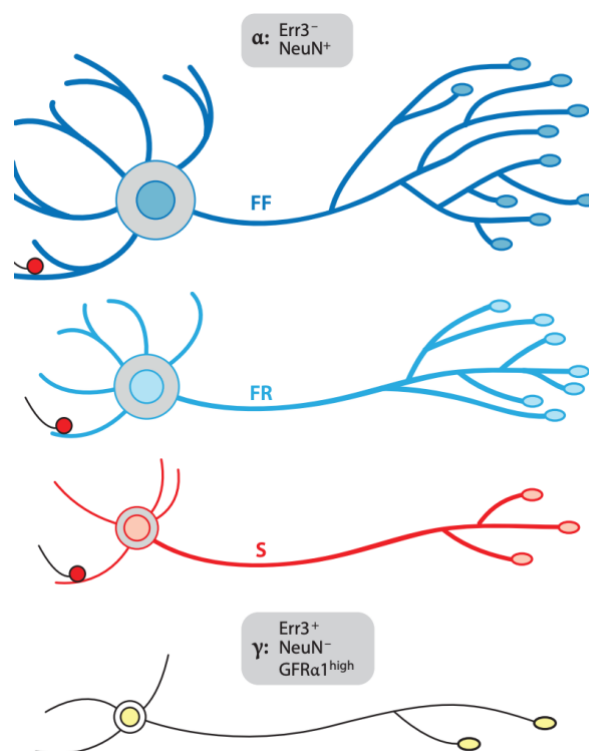


*“Distinct motor units within skeletal muscle. **Alpha** motor neurons innervate extrafusal muscle fibres to form three subtypes of motor unit: Slow-twitch (S) units control Type I fibres, fast-twitch fatigue-resistant (FR) units control Type IIa fibres, and fast-twitch fatigable (FF) units control Type IIb/x fibres. Intrafusal (IF) muscle fibres are innervated by γ -MNs and also by β -MNs (not shown).”*

Figure 1-4 Characteristics of motor neuron sub-classes from Kanning et al(2010) ¹²¹.

α -MNs innervating extrafusal muscle fibres allows for movement of the skeletal structure, whereas γ -MNs innervating intrafusal fibres regulate the sensitivity of muscle spindles to stretch ^{123, 124}.

Reflecting differing functions and targets, mature α - and γ -MNs show marked differences in size and form (Figure 1-5). Gamma motor neurons are smaller: with their average soma diameter being half that of the smallest α -MNs. Axonal conduction velocities are also slower, reflecting their smaller axon calibre ^{125, 126}. The dendritic extensions of α - and γ -MNs are of similar length, but those of γ motor neurons are significantly less branched and are simpler overall ¹²⁷. Distinctions also extend to connectivity within the spinal cord. All γ -MNs lack monosynaptic Ia input from proprioceptive sensory neurons, whereas most (but not all) α -MNs receive direct Ia input ^{128 129}. Few γ -MNs have intraspinal axon collaterals, so they are unlikely to contribute to recurrent inhibition within the spinal cord ¹²⁷. Thus α - and γ -MNs have largely distinct postsynaptic targets and presynaptic inputs. These marked differences between the two populations imply the existence of separate programs for the determination of α - and γ -MN identity.



“Alpha motor neurons diminish progressively from FF through FR to S motor units. Most α -MNs receive direct Ia innervation from VGLUT1⁺ (vesicular glutamate transporter 1) proprioceptive sensory neurons (red terminals). Gamma motor neurons are smaller still and do not receive Ia innervation. At postnatal stages, α - and γ -MN cell bodies can be distinguished by their size, connectivity, and the indicated molecular markers.”

Figure 1-5 Morphological features of Motor neurons from Kanning et al (2010) ¹²¹.

In most individuals, the numbers of approximate motor neurons remain largely constant for much of their lifetime, with the overall profile of motor neuron subtypes

remaining unchanged. However, in neuromuscular disorders such as spinal muscular atrophy (SMA) and amyotrophic lateral sclerosis (ALS), motor function is lost as the motor neurons degenerate and expire¹³⁰. In both conditions, specific pools and subtypes of motor neurons are differentially affected, providing insights into the pathogenic processes involved. ALS is characterized by axonal degeneration and cell death of both α - and γ -MNs in the spinal cord^{131, 132} and motor cortex, with progressive fatal decline.

Histological studies have shown that within the cord, interneurons degenerate alongside the loss of α motor neurons¹³³. γ and β innervation to muscle spindles is also lost in ALS, with the preservation of primary and secondary sensory innervation to the muscle spindles¹³². As such in ALS the α/γ co-activation during voluntary contraction is no longer achievable. However, although there is considerable evidence that motor neurons with larger cell bodies are more likely to degenerate, the pathological processes underlying this selective neuronal death is still poorly understood.

Despite clinical differences between ALS and SMA, there are also comparisons that can be drawn in terms of subtype and pools of motor neurons involved. Muscle biopsies taken from SMA patients, showed extensive atrophy of type II (fast) fibres, whereas type I (slow) fibres showed some compensatory hypertrophy¹³⁴. Similar to ALS, slow motor units are more resistant to disease progression. Clinically, patients with SMA show facial muscle sparing as well as sparing of the urethral and anal sphincters. a sparing of many facial muscles including those involved in eye movement¹³⁵⁻¹³⁷. The overlapping evidence between ALS and other neurodegenerative conditions shows the inherent resistance of some motor units (slow units) and pools (extraocular and onuf's nucleus) against multiple assaults.

ALS is a condition where there is neuronal loss in the primary and premotor cortex, frontal lobes and more widely in the basal ganglia and thalamic neurons⁸⁶. ALS therefore affects regions beyond the limits of the motor system, while the condition predominantly affects degeneration of the anterior brain, sparing primary sensory systems and the occipito-parietal cortex. The reasons for this vulnerability are not

well understood, but properties implying motor cell specific qualities determine them inherently susceptible. Processes that have remained independent of nuclear transcription have allowed motor cell to function. These included synaptic prion like proteins and mitochondria, this autonomy allows for a more effective neural communication system ¹³⁸. However, this system has a flaw, despite its independence these prion like proteins may replicate unchecked, allowing for the possibility of abnormal proteins to aggregate ¹³⁹. The vulnerability of the corticomotoneural synapse in ALS thus hinges on the fact that longer axons have larger synapses allowing for increasing probability of this autonomous system to malfunction.

1.1.5.4. *Renshaw cell*

One of the most abundant neurons present in the spinal cord are interneurons, which are mainly inhibitory. They are involved in the coordination of locomotor patterns, one such pattern being left-right alternating activity ¹⁴⁰. However, unlike our animal counterparts little is known about human spinal interneurons ¹³³.

Over the last 50 years the most researched interneuron in animal models has been the Renshaw cell, named after its discoverer Birdsey Renshaw ¹⁴¹. The first paper to explore at Renshaw cells in Humans was published in 1975 by Pierrot-Deseilligny et al ¹⁴².

The focus has largely remained on this special class of interneuron mainly because it is the only interneuron that receives afferents directly from the spinal α motoneurons and facilitates recurrent inhibition to the same motor neuron through the production and release of GABA and glycine¹⁴³.

Renshaw cells synapse directly on to Ia inhibitory interneurons, ventral spinocerebellar neurons as well as onto α motoneurons. They are also known to mediate recurrent inhibition of motoneurons, as they modulate recurrent inhibition produced by other Renshaw cells and Ia reciprocal inhibition between antagonist motor pools¹⁴⁴.

There has been no consensus as of yet on the functional meaning of recurrent inhibition in the adult spinal cord. The hypothesis is that alterations in force generation and maintenance as well as rigidity, spasticity or tremor are a consequence of Renshaw cell dysfunction¹⁴⁴.

Two hypotheses currently exist with regards to role of Renshaw cells in motor neuron degeneration.

The first hypothesis reasons that hyperexcitability is caused by the loss of recurrent inhibition by the Renshaw cells. Experimental models of familial ALS from rodents suggested that there is a progressive loss of glycine boutons occurring around the soma of motor neurons. This tends to start early in the symptomatic stage before degeneration occurs. In contrast GABAergic terminals tend to be affected only in the final stages, which have been noted in conjunction with Renshaw cell loss. These changes have been reported in early and late symptomatic stages ¹⁴⁵.

The second hypothesis suggests that Renshaw cell loss is not the index event in motor neuron hyperexcitability and neurodegeneration, but rather the recurrent inhibitory system is itself altered prior to motor neuron degeneration and is not the result of Renshaw cell loss.

Mazzocchio et al. has shown that activation of Renshaw cells has a weak effect on motor neuron soma activity. Experimental ALS models in symptomatic stages have shown the interneurons to be preserved, suggesting that progressive motor neuron degeneration is independent of the Renshaw cells¹⁴⁶.

Recently the involvement of cholinergic interneurons-V0c has been implicated in the control and modulation of motor neuron activity. These cells innervate the soma of motor neurons and inhibitory neurons¹⁴⁷.

1.1.6. “Experimental Models”

If each brain area was to be considered functionally independent, then pattern of atrophy in neurodegenerative diseases becomes a long list of unrelated and seemingly haphazard regions ¹⁴⁸. With the ongoing advances in technologies over the last decade, the focus of neuroscience and cognition altered gradually from the thought of an “area” or “region” to the concept of a “network”¹⁴⁸. This type “network” analysis has been applied to ALS patients within our own research group, showing increased resting-state connectivity in the sensorimotor network ¹⁴⁹⁻¹⁵¹.

The gradual change in perception and focus on neurodegenerative disorders has major implications for the way in which we interpret and study ALS.

If we consider the well-known Hebb’s principle ¹⁵²- “What fires together, wires together”, then this hypothesis can potentially show correlated activity leading to lasting synaptic changes. This has been confirmed in several neurophysiological studies ^{153, 154}. Strong synaptic connections not only provide for the smooth functioning of our neural systems they also provide the same pathways for the propagation of neurodegenerative disorders. “Cells that fire and wire together are also more prone to die together”¹⁴⁸.

It is this concept that can only best explain the commonality in neurodegenerative diseases through the notion of network-based disturbances and the heterogeneity seen in MND.

For this reason, we have elected to use other related neurodegenerative processes (SMA, PLS, Polio) as “experimental models” to ALS. While we acknowledge that

clinical presentation differs significantly there remains a common link based around network disturbances that can be explored and hypothesis/conclusions drawn.

1.2. PLS: A model for a pure UMN disorder

Primary Lateral Sclerosis (PLS) is an adult-onset disorder characterised by progressive degeneration of the upper motor neurons with the absence of clinical signs of lower motor neuron involvement ¹⁵⁵. Hereditary spastic paraparesis by contrast, presents in patients usually younger and are more likely to report a positive family history ¹⁵⁶. PLS is a rare disorder only accounting for approximately 1-3% of all new diagnoses of MND ¹⁵⁶. PLS is a diagnosis of exclusion. PLS is considered to exist on a spectrum of motor neuron disorders, these include progressive muscular atrophy (LMN involvement) and ALS (mixed upper and lower motor neuron involvement). While PLS is clinically distinct from ALS, controversy remains as to whether PLS is pathologically different from ALS.

1.2.1. Clinical Features

Unlike hereditary forms of spastic paraparesis, PLS symptoms usually present in the 5th-6th decade, with considerable heterogeneity between patients ¹⁵⁷. As with ALS, there is a slight male predominance. Due to its insidious onset patients are less likely to reach specialised neurological services after their earliest symptoms.

Erb's original 20th century description still stands true today, with the most common clinical presentation including spasticity, hyperreflexia and mild weakness ¹⁵⁸.

Patients often report poor coordination, stiffness and as the disease progresses increasing falls. Bulbar symptoms may present with dysarthria, dysphagia and emotional lability. Clinical examination typically only shows upper motor neuron signs, spread of reflexes, spasticity and the absence of lower motor neuron signs (muscle wasting and fasciculations). What the patient often reports as weakness is usually a mixture of increased tone, mild weakness and decreased coordination. A pattern of upper motor neuron weakness may present in the extensors of the upper extremity and flexors in the lower extremity. Stiffness as a presenting complaint is more likely to be seen in PLS (47%) than ALS (4%), with limb wasting being quite rare in PLS (2%) ¹⁵⁹.

There is increasing recognition of cognitive changes in PLS patients, with reports of frontal lobe dysfunction in 10-20% of patients ^{160, 161}.

Typically, PLS is a very slowly progressive disorder. Patterns of progression usually manifest by side to side spread and from region to region, with most patients

developing spastic quadriparesis with some degree of bulbar involvement. From various case series the average symptom duration ranged from 7.2-14.5 years^{157, 162, 163}.

1.2.1.1. Diagnosis

PLS is a diagnosis of exclusion. PLS is diagnosed in the presence of upper motor neuron dysfunction with the absence of other neurological findings or alternatives on diagnostic testing (Table 1-6).

Several diagnostic criteria have been proposed^{157, 164}. The Pringle criteria suggest symptoms be present ≥ 3 years, while the singer criteria proposed symptoms be present for ≥ 4 years. Consensus diagnostic criteria was described in 2019 as the result of a meeting of international PLS experts (Philadelphia, Pennsylvania, USA), in an effort to encourage therapeutic development and to push research into the direction of basic PLS histopathology (Table 1-7)¹⁶⁵. These revised criteria were seen to facilitate an earlier diagnosis of PLS (Table1-7). The use of an age cut-off of 25 years at point of diagnosis ('probable' or 'definite' PLS) reflects a decision to reduce the risk of atypical cases distorting outcome in future clinical trials.

Table 1-6 Differential Diagnosis for PLS. From Turner et al.(2020) ¹⁶⁵.

Structural
Tumour
Cervical spondylomyelopathy
Spinal arterio-venous fistula
Arnold Chiari Malformation
Demyelinating
Multiple Sclerosis/ Primary Progressive MS
Vitamin E deficiency
Hereditary
Hereditary spastic paraplegia
Leukodystrophy (metachromatic, adrenoleukodystrophy)
Polyglucosan body disease
Infectious / Inflammatory
Tropical Spastic Paraparesis (HTLV 1/2)
HIV
Syphilis
Sarcoidosis
Metabolic/Toxic
Subacute combined degeneration (B12 deficiency)
Vitamin E deficiency
Lathyrism
Neurodegenerative
Amyotrophic lateral sclerosis

Table 1-7 Consensus Diagnostic Criteria for PLS. From Turner et al.(2020) ¹⁶⁵.

1. Core principles
The diagnosis of PLS requires: A. the presence of: – age $\geq$ 25 years; – symptoms of progressive upper motor neuron (UMN) dysfunction for at least 2 years; – signs of UMN dysfunction* in at least two of three regions: lower extremity, upper extremity, bulbar. B. the absence of: – sensory symptoms (unexplained by comorbid condition); – active lower motor neuron (LMN) degeneration; alternative diagnosis: UMN pathology demonstrated on neuroimaging or identified through biofluid testing that provides a plausible alternative explanation for the clinical syndrome.
2. Diagnostic certainty
• Probable PLS is defined by the absence of significant active LMN degeneration 2–4 years from symptom onset. • Definite PLS is defined by the absence of significant active LMN degeneration 4 or more years from symptom onset.

1.2.1.2. Differential Diagnosis

HSP has the most common clinical overlap with PLS. However mimic disorders for PLS are rare. High resolution MRI of the brain and spinal cord often removes the majority of these (Table 1-6). The likelihood of many of the alternative diagnosis reduces dramatically, with the exception of neurodegenerative category, in relation to the duration of a progressive UMN syndrome.

1.2.1.3. Genomics

Genetic testing in suspected PLS cases is not considered routine. However, screening panels for pathogenic variants associated with spastic paraparesis is sometimes warranted especially in cases of progressive UMN syndromes confined symmetrically to the lower limbs.

At times in the presence of an UMN predominate phenotype, excluding a hereditary cause of ALS (e.g., C9orf72 expansion) may be warranted.

1.3. Polio: A model for a pure LMN disorder

Poliomyelitis is caused by a neurotropic enterovirus, with a predilection for the anterior horn cell. Polio epidemics occurred regularly until the advent of vaccination in the 1950s and 1960s, with almost complete eradication of the illness with some notable exceptions.

However, 15–20 million people across the world continue to experience sequelae from the infection, frequently presenting with a constellation of new neurological symptoms that has been described as Post-Polio Syndrome (PPS), characterized by new muscle weakness and fatigability occurring years after the initial acute phase. An estimated figure of 15-80 % of polio survivors go on to develop PPS, this variability exists in the literature largely due to population differences being studied and criteria used ¹⁶⁶.

1.3.1. Clinical features

Preparalytic poliomyelitis

Patients after a prodromal illness may develop pyrexia with pharyngitis, myalgia, nausea, vomiting and anorexia. These nonparalytic illness symptoms tended to subside with one to two weeks after initial onset ¹⁶⁷.

Paralytic phase

Following a meningitic phase, a spinal variation of polio would take hold. Most patients would experience severe muscular pain, associated with muscle spasms, followed by developing fasciculations and weakness. A notable asymmetry was associated with the weakness, with lower limbs being predominantly affected compared to upper limbs. Its peak is usually reached in 48 hours. However, a biphasic form was also noted, in which further weakness could occur after a short period of stability, with no further evolving symptoms after the fever settled.

On clinical examination the patient was often found to have flaccid muscle tone, with initially brisk followed by absent reflexes. Paraesthesia was often reported with no objective sensory disturbance.

Children were at a higher risk of developing a pure bulbar form of polio, especially with an association to adenoidectomies ¹⁶⁸. The most commonly affected cranial nerve nuclei lay around the medulla, causing dysphagia and respiratory failure ¹⁶⁹. Vasomotor changes and circulatory collapse lead to a high mortality in this form of polio ¹⁶⁷.

1.3.1.1. *Diagnosis*

Diagnosis of acute paralytic polio is a clinical diagnosis, especially in the presence of an asymmetric purely motor flaccid paralysis and an aseptic meningitis. Differential diagnosis mainly consists of other causes of neuromuscular acute paralysis (Table 1-8).

After the initial onset of symptoms the virus can be isolated for five days from the nasopharynx and for up to 5 weeks from the stool ¹⁷⁰.

CSF often shows raised protein content, pleocytosis in the presence of normal glucose levels. The earliest EMG findings is a noted reduction in recruitment pattern with diminished interference. After paralysis the muscle is electrically silent at rest.

After two to four weeks fibrillation potentials develop and persist indefinitely.

Fasciculations may be noted. Motor unit action potentials are initially reduced, but during recovery return, and eventually develop abnormally large amplitude with associated polyphasia and increased duration due to reinnervation. Motor conduction velocities remain normal ¹⁶⁷.

Table 1-8 Differential diagnosis for acute Polio

Guillain-Barre syndrome
HIV
Paralysis due to acute intermittent porphyria
Diphtheria
Lyme Disease
Botulism
Triorthocreslphosphate poisoning

The most frequent areas affected are the anterior horn cells of the spinal cord and the neurons in the intermediate and posterior horns, occasionally the dorsal root ganglia may be affected ¹⁶⁷. The vestibular nuclei and reticular formation in the medulla, pons and midbrain may all be affected ¹⁷¹.

1.3.2. *Management*

As is reminiscent of the most recent pandemic, patients with acute polio were to be isolated. Careful monitoring of bulbar function, vital capacity and cardiovascular system were put in place in anticipation of respiratory decline. Pain relief, splinting

and passive movement of joints were the main stay of treatment and prevention of contractures.

Acute respiratory failure was largely secondary to medullary involvement causing respiratory muscle weakness. Negative pressure ventilation in what was called “iron lungs” was used ¹⁶⁷.

1.3.2.1. *Late Polio Deterioration*

Many patients after a period of stability go through a period of functional deterioration which often manifests as impaired mobility, respiratory capacity and activities of daily living. Famously, there was a lot of speculation around the physical deterioration in later life of Franklin D Roosevelt, who contracted polio at the age of 39 ¹⁷². These late changes and deterioration are referred to as post-Polio syndrome. Definitions of this disorder varies, with an estimated figure of 15- 80 % of polio survivors going on to develop PPS, this variability exists in the literature largely due to population differences being studied and criteria used ¹⁶⁶. While some included all causes of progressive musculoskeletal deformities and nerve entrapment due to the original illness, ¹⁷³ yet others like Dalakas ¹⁷⁴ and Halstead ^{175, 176} included symptoms of fatigue, muscle joint pain and reduced exercise tolerance. Halstead’s definition evolved into what is currently known as the March of Dimes diagnostic criteria ¹⁷⁷ (Table 1-9).

The functional deterioration in late polio appears to be relatively mild ¹⁷⁵. In a series of 27 patients reported by Dalakas et al clinical assessment was carried out using an annual examination of 20 muscles groups using Medical Research Council grading ¹⁷⁸. The authors reported a 1 % per year decline over a follow-up period of just over 8 years. This series of post-polio syndrome was reported to be unrelated to any other neurological disorder. However, no patients were reported to have any progressive scoliosis although in our patients seen routinely in clinic this has been an extremely common finding especially in those who developed acute poliomyelitis before their growth spurt.

It is hypothesised that post-polio syndrome may be the clinical presentation of a continuous process of denervation and reinnervation occurring in all muscles clinically and sub-clinically affected by the original polio virus ¹⁷⁹. The electrophysiological evidence of active denervation include spontaneous increased activity and fibre density with jitter and blocking noted on single fibre studies. This

has been reported in both unaffected as well as affected muscle groups in patients with and without post-polio syndrome ¹⁷⁹. Muscle biopsy studies have reported evidence of ongoing denervation and group atrophy in both stable patients and those with late onset deterioration ¹⁸⁰.

Table 1-9 March of Dimes Diagnostic Criteria from Li Hi Shing et al 2019 ¹⁸¹

• Prior paralytic poliomyelitis with evidence of motor neuron loss, as confirmed by history of the acute paralytic illness, signs of residual weakness and muscle atrophy on examination, or signs of denervation on EMG.
• A period of partial or complete functional recovery after acute paralytic poliomyelitis, followed by an interval (usually 15 years or more) of stable neuromuscular function.
• Gradual onset (rarely abrupt) progressive and persistent new muscle weakness or abnormal muscle fatigability (decreased endurance), with or without generalized fatigue, muscle atrophy, or muscle and joint pain. Onset may at times follow trauma, surgery, or a period of inactivity. Less commonly, bulbar dysfunction or respiratory weakness occurs.
• Symptoms that persist for at least a year.
• Exclusion of alternative neuromuscular, medical, and orthopedic problems as causes of symptoms.

1.3.3. Aetiology

There are numerous theories have been put forward as to the mechanism of PPS ^{174, 182}.

Late post-polio deterioration may be reflective of the natural aging process of neuronal pools of the anterior horn cells, in which they were previously critically reduced from historical polio infection. However literature suggests that before the age of 60 years neuronal loss rarely occurs ¹⁸³, while the development of PPS is suggested to be linked to the severity of the acute phase rather than the patients age.

There were suggestions of various immunological mechanisms driving the syndrome, especially in the presence of mild inflammatory changes seen on muscle biopsy ^{180, 184} along with mild changes reported in the spinal cord ¹⁸⁵. Suggestions had been made of the presence of recurrent or persistent viral infection due to reports of intrathecal IgM antibodies in patients with PPS ¹⁸⁶. However further studies using PCR on CSF, have shown the enterovirus may continue in the central nervous system of those previously affected by polio, but no clinical or biological significance

are attributed to its presence ¹⁷⁰ and furthermore these findings have been contradicted in more recent studies ^{167, 187, 188}.

PPS may be a representation of an attritive process with premature neuronal exhaustion. During the acute phases large numbers of motor neurons are lost. During the recovery phase the surviving terminal axons sprout in an attempt to reinnervate the muscle, thus giving rise to enlarged motor units. These new motor units are considered unstable with ongoing denervation and reinnervation. This continuous process burdens the remaining neuronal cell bodies, which have a reduced capacity to maintain metabolic demands of all the new sprouts. Over time there may be losses of the terminal sprouts gradually, ultimately giving rise to atrophy of adjacent fibers.

1.4. SMA: An infant onset pure lower motor neuron disorder of genetic origin

Spinal muscular atrophy (SMA) encompasses a group of genetic conditions characterized by the degeneration of anterior horn cells and as a direct result muscle atrophy and weakness follows. The predominant form of SMA, accounting for 95% of cases, is the autosomal recessive variant resulting from a homozygous deletion or mutation in the 5q13 survival of motor neuron (SMN1) gene ¹⁸⁹. In a large cross ethnic genetic study for SMA, it was found the carrier frequency was 1 in 54 with an incidence rate of 1 in 11,000 ¹⁹⁰. Disease severity is variable in SMA, as such the condition is classified based on four phenotypes based on highest motor function achieved and age of onset ¹⁹¹.

Our knowledge of molecular genetics has led to successful changes in management and therapeutics of SMA ^{192, 193}. Along with active treatment comes the need for early diagnosis to allow for clinical intervention, leading to clinical standards of care being set out ^{194, 195}. For the purposes of this thesis, we will focus on SMA type 2 and 3 (Table 1-10).

1.4.1. Clinical Features

The main feature of SMA is atrophy and muscle weakness. Proximal muscles are usually more affected than distal groups in a symmetric pattern. In 1991 the international consortium on Spinal Muscular Atrophy formalized a classification

system to describe the many phenotypes of SMA ¹⁹¹. This classification system subdivided SMA into 3 types based on the highest level of motor function achieved and age of onset. Later versions of this system divided SMA type 3 by age of onset and adult onset was added. Type 0 was included to describe patients with prenatal onset and death within weeks of birth ^{196, 197}. Table 1-10

Table 1-10 SMA Classification from Farooq et al 2013. ¹⁹⁸

SMA Type	Other Names	Age of Onset	Life Span	Highest Motor Activity
Type 1 (Severe)	Werdnig-Hoffmann disease	0-6 months	2-5 years	Never sit
Type 2 (Intermediate)	SMA, Dubowitz type	7-18 months	>2 years	Sit, Never Stand
Type 3 (Mild)	Kugelberg-Welander disease	>18 months	Adulthood	Stand and Walk (may require assistance)
Type 4 (Adult)	----	Adulthood	Normal	Walk during adulthood-unassisted (some muscle weakness)

1.4.1.1. Genomics

Before determining the genetic cause of SMA, it often presented a conundrum with regards to severity, namely how one gene defect can code for a heterogeneity of clinical phenotype in disease severity. The thread yarn started to unravel itself when the Melki Lab in 1995 discovered a homozygous deletion in the SMN1 gene on chromosome 5q13, present in 95% of SMA patients ¹⁹⁹.

There are two forms of the SMN gene that are present on each allele. The telomeric (SMN1) and centromeric (SMN2) form. All SMA patients lack a working SMN1 gene, and as such are dependent on their SMN2 gene. However, the SMN2 gene is not as efficient to produce SMN protein as its counterpart the SMN1 gene ¹⁸⁹. Due to this inefficacy, SMA is in essence caused by a deficiency of the SMN protein, which for reasons still unknown causes selective motor neuron loss. However, the conundrum of disease severity is answered by the variability of the SMN2 copy gene in SMA patients ²⁰⁰

The riddle of severity was solved by the variability of SMN2 gene copy number that was found in SMA patients[26].

1.4.1.2. *Gene Therapy*

Antisense oligonucleotides (ASOs) are RNA therapeutic molecules arranged to bind to their correlating equivalent sequences within a targeted intron or exon that can either augment or disrupt the targeted splicing event ¹⁸⁹. ASOs do not cross the blood brain barrier and as such must be administered intrathecally, directly into the central nervous system.

ASOs are generally well tolerated, with remarkable improvements seen in motor function in all type of SMA patients and in prevention of disease onset or progression in pre-symptomatic patients. The importance of early intervention and treatment cannot be overemphasized, as delays may impact on recovery of function especially in type 1 infants who show rapid loss of function. Infants identified prenatally or neonatally, therefore, should be treated emergently, before symptoms develop, followed in hierarchy by newly diagnosed type1 patients and other affected children who are at risk of losing function (e.g. an SMA type 3 toddler losing the ability to walk) ²⁰¹.

For clinical trials to succeed, the development of reliable outcome measures hinges on the measures to be sensitive enough to pick up on neurophysiological changes as well as functional effects of any given intervention or trial treatment.

Diagnostic classifications provided by disruptions in anatomic pathways have enabled cohorting of patients on clinical grounds.

1.5. Conclusion

In this chapter, I have introduced a spectrum of neurodegenerative conditions. As the neurophysiological complexity of the nervous system is increasingly recognized, an argument can be made for integrating modern functional tools with clinical and anatomic based evaluation. In this context, there is a need for dynamic markers of functional impairment of motor and extra-motor control in ALS. Such markers could then be incorporated as diagnostic tools, reducing diagnostic uncertainty and avoiding delays in potential disease modifying medication²⁰². In the next chapter, I further explore potential diagnostic or prognostic tools in the setting of ALS.

2. Chapter 2: Biomarkers in ALS

There has been a renewed focus over the past 20 years on the subject of biomarkers in ALS. The main reason for such strong focus on biomarkers is that one that is shown to be sensitive and specific could help clinicians reach diagnoses faster, improve trial design as well as improve outcome measures in these trials. While a larger body of research has gone into the area, very few have had promising clinical significance. This is mainly due to the variation in analytical techniques as well as non-standardised methodology, small samples and not enough longitudinal studies being carried out ²⁰³. In this section, I focus on reviewing the modern assessment methods of the upper and lower motor neurons at network level, and how these might relate to the clinical presentation and pathological correlates of disease in humans.

2.1. Diagnostic Biomarkers

ALS patients have been known to have a delay in diagnosis initially due to subtle signs at symptom onset, this delay can average 12-18 months ²⁰⁴. The most current theory is that there is propagation of disease through means of axonal transmission of misfolded proteins such as pTDP-43, pathological RNA processing, and “prion-like” spread ²⁰⁵⁻²⁰⁷. By improving time to diagnosis, it is hoped that future treatments can be administered in a timely fashion to limit or halt progression. An effective diagnostic biomarker would aide in clinical diagnosis of ALS at an earlier stage when signs are localized and subtle, allowing for treatment and trial enrolment.

Trial enrolment criteria is often based around the El-Escorial criteria. Allowing for lab-supported ALS, which is based on evidence of active and chronic denervation on EMG. The development of new biomarkers can contribute to the current “laboratory supported” diagnosis for more accurate patient stratification and trial enrolment.

2.2. Prognostic Biomarkers

ALS is a heterogeneous condition with such variability in site of onset, extra-motor involvement and progression rate ⁶³.

In trial design taking into account the heterogenous nature of ALS would allow for grouping of patients to allow for maximum benefit from a novel treatment when

statistical analysis is not confounded by population variation. If variability is taken into account and reduced then sample-size can also be decreased, reducing time and cost of clinical trials ²⁰³.

Often clinical trial enrolment criteria favour a slower disease progression rate, as a result it can raise difficulties in evaluating the true effect of a drug on progression ²⁰⁸. In most ALS randomised controlled trials, disease progression is scored based on the rate of decline in the ALSFRS-R. Given that the ALSFRS-R is a scale based around evaluating activities of daily living, it can be considered a crude measure of disease progression in a heterogenous condition, where decline is in a curvilinear pattern rather than linear ²⁰⁸.

A prognostic biomarker would be help patient stratification, allowing for better trial design.

2.3. Pharmacodynamic Biomarkers

Typically, clinical trial endpoint measures include survival and ALSFRS-R. These outcomes can take several years of monitoring before a conclusion can be drawn. Pharmacodynamic biomarkers are defined by their ability to reliably change in response to treatment. Alternatively, disease progression markers can effectively show serial measures that alter as the condition worsens in the absence of treatment. This can allow for another objective measure in randomized control trial design.

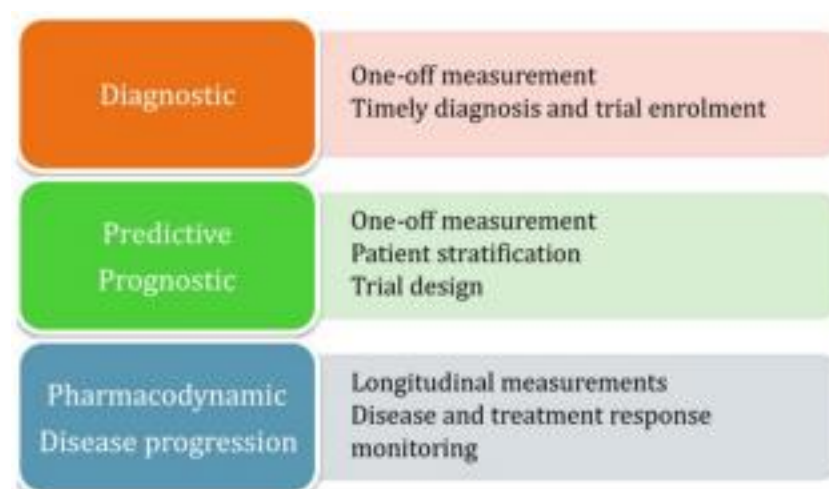


Figure 2-1 Outline of Biomarker categorization. From Verber et al. 2019 ²⁰³

2.4. Examples of Biomarkers

2.4.1. Neurofilament Proteins

The CSF biomarker showing the most promise to date are the neurofilament(Nfl) proteins, (currently in use in SOD1 and Nusinersen ASO clinical trials). A remnant of the neuronal cytoskeleton that has been shown to accumulate following degeneration and can be measured by sampling CSF ²⁰⁹. The two most relevant subunits are phosphorylated neurofilament heavy chain (pNfH) and neurofilament light chain (NfL). Research supports neurofilament titres as a potential diagnostic tool ^{210, 211}. In one multi-centre study validation of both subunits were confirmed as diagnostic biomarkers ²¹². Alternative marker use is still to be validated, although studies have provided some evidence to support additional marker utility. There is some variation in the literature regarding the prognostic value of Nfl. One longitudinal study showed that ALS patients with higher Nfl levels were associated with a worse prognosis ²¹³, while another on longitudinal analysis suggested that ALS groups that had higher baseline levels of Nfl were likely to plateau, possibly reflecting the peak neuronal death rate had been reached ²¹⁴. As such, further validation for disease progression and prognostics would be needed.

2.4.2. Blood Biomarkers

Altered proteins associated with genetic mutations have been studied in response to current clinical trials specific to SOD1 and C9ORF72 mutations. While most studies to date have primary outcome measures based in CSF protein changes ^{215, 216}. Proteins associated with genetic mutations have been studied in the context of sALS. One study showed overall SOD1 levels were reported to be increased in leukocytes ²¹⁷. Studies of TDP-43 is less straight forward. Total levels of TDP-43 did not distinguish between controls and ALS in the peripheral blood mononuclear cells ^{218, 219}.

2.4.3. Imaging Biomarkers

MRI high signalling has been well described in motor areas on T2-weighted or fluid-attenuated inversion recovery (FLAIR) imaging ^{220, 221}. However, T2 weighted signal changes is not specific and can relate to a number of different underlying pathologies, e.g., oedema, inflammation, demyelination, or gliosis (in the case of ALS).

Diffusion tensor imaging (DTI) has been shown to support the concept of ALS as a multi system disorder by highlighting widespread white matter tract damage. DTI has been reported to have a diagnostic sensitivity and specificity of 68 and 73%, respectively ²²².

Machine learning has been used to combine both volumetric gray matter and DTI measures, and has been shown to distinguish ALS patients from healthy controls with 86% sensitivity, 67% specificity, and 78% accuracy ²²³.

Assessing potential biomarkers for clinical robustness can only be done effectively if it can be reliably replicated. A multi-centre approach to biomarker validation is needed to provide sufficient statistical power and to judge whether it is useful or not. Once a potential biomarker is identified a round-robin method can be used to validate it ²²⁴. If deemed unsuccessful then a consensus body should be setup to move focus onto the next promising biomarker.

Most studies looking at diagnostic biomarkers use ALS patients against healthy controls. However, neurologists seeing a typical ALS are rarely in a diagnostic dilemma. Ideally, ALS should be compared to its disease mimics.

Electrophysiology biomarkers are described further in the section below (section 2.5)

2.5. Electrophysiology

The hallmark of ALS is the involvement of both upper and lower motor neurons of the corticospinal/bulbar tract. Understanding the complexity ALS symptoms, as well as how cortical circuitry can change in the face of neurodegeneration, can help us advance our understanding of the early stages of disease as well as enhance future therapeutic interventions. In this section, I review the literature regarding current tools (both in clinical and research based) used in the assessment of upper/ lower motor neuron involvement in ALS.

2.5.1. Current tools of U/LMN assessment

Clinical assessment is the only reliable way of diagnosing ALS, with the recognition of lower and upper motor neuron signs. The El Escorial criteria often used in clinical practice, allows for supportive evidence from neurophysiological studies. While electromyography can be used to detect subclinical involvement of LMNs, the

diagnosis can only be reached on clinical appraisal with the presence of UMN signs and a history of progression.

There have been many studies that have sought to ascertain contribution of upper and lower motor neuron dysfunction in impaired motor control and weakness in ALS. One study by Kent-Braun et al. studied 27 patients, measuring isometric maximum voluntary contraction in dorsiflexion. Their results suggested LMN loss was the primary cause of progressive weakness with UMN dysfunction leading to slow contraction speed and decreases in central activation ²²⁵.

There has been a recent increase in the number of studies that aim to develop neurophysiological biomarkers in ALS. The recognition of central and peripheral hyperexcitability may allow for the quantification of LMN and UMN loss in ALS. Various tools have been developed over the years to assess LMN involvement with fine tuning to allow for tracking of disease progression (see Table 2-1). Research mainly focused on the development of substituting markers of motor unit loss by using indirect techniques of motor unit estimation¹⁵. Various statistical approaches have been used to allow for a mean motor unit size calculation, which has given rise to reproducible estimates of motor unit numbers in patients with ALS. The main concern with these estimates is that they may be less accurate in patients with more advanced disease²²⁶.

There have been significant developments in the advancement of neurophysiological biomarkers of both U/LMN dysfunction in neurodegenerative disease. Evidence of UMN dysfunction in the setting of LMN degeneration is central to the diagnosis of ALS. There is a need for objective measures of UMN involvement to aid in assessing clinical phenotypes, monitoring of treatment response in clinical trials, as well as a measure of disease progression ²⁰².

In this section I will review the major advances in the development of neurophysiological markers in ALS in view of diagnosis, prognosis and utility in treatment monitoring.

2.5.2. H-reflex

The Hoffman reflex (H-reflex) can be considered as the neuro-electro-physiological counterpart of the deep tendon stretch reflex. Similar to the stretch reflex, it is a monosynaptic reflex triggered by stimulating the Ia sensory afferents in a mixed nerve. It is usually recorded from the soleus in adults ²²⁷. The H/M ratio (H reflex

relative to CMAP/M-wave) depends on the number of excitable motor neuron pools within the spinal cord activated by the Ia pathway ²²⁷.

Technique variations have been developed using varying facilitatory or inhibitory conditioning stimuli. This allows for the assessment of the state of excitability of the spinal motor neurons in pyramidal lesions ²²⁸.

In a paper of healthy patients by Bussel et al. recording the H reflex during vibratory stimulation of the soleus muscle at the same time as electrical stimulation was found to result in inhibition of impulses from spindle afferents with amplitude reduction of the H response ²²⁹. Abbruzzese et al. was able to demonstrate that this effect reflects presynaptic inhibition of Ia afferents and was elicited by other Ia fibres coming from the same muscle ²³⁰. Bringing these data together in ALS, a study by Drory et al. investigated the usefulness of F waves and H reflex functions in the assessment of UMN dysfunction in ALS patients ²²⁷. An interesting finding was those obtained by vibratory and recurrent inhibition of the H reflex. The method used in obtaining recurrent inhibition was based on that originally described by Bussel et al in 1977. This technique was based on the paired H reflex, where a monosynaptic reflex discharge activates Renshaw cells with the resulting recurrent inhibition evaluated by the subsequent H reflex- the H' test reflex²³¹. This study had shown that ALS patients, despite being predominately LMN presentations also had UMN dysfunction detected. The marked disinhibition seen in paired stimulation in the LMN predominant group cannot be fully explained by the mere drop-out of Renshaw cell activation as the patients studied had well preserved motor neuron pools as expressed by tibial CMAP values²²⁷, suggesting corticospinal involvement in patients with "pure" LMN syndrome presentations.

The overall vibratory effect on the reflex is a result of presynaptic inhibition, with recurrent inhibition being due to activation of inhibitory Renshaw cells (post synaptic) ²²⁷.

The implications of disruption of the spinal cord circuitry that cannot be simply attributed to a simple dichotomy of upper and lower motor neuron dysfunction. Whether this could be attributed to interneuron dysfunction, or a more complex disruption of motor circuitry remains to be determined.

2.5.2.1. The H_{max}/M_{max} Ratio

Two types electromyographic (EMG) responses are evoked on electrical stimulation of the posterior tibial nerve in the soleus muscle, namely the M and H wave. The M wave is a result of direct axonal activation of the α -motoneuron pool of the soleus muscle. The H wave, on the other hand, is the orthodromic afferent response via large diameter Ia fibres, arising to the reflex discharge from the same muscle pool²³².

The maximal H reflex (H_{max}) is produced by applying submaximal nerve stimulation and mainly arises due to the activation of slow twitch motor units²³³. The maximal M wave (M_{max}) is produced by supramaximal nerve stimulation, and is the electrical equivalent of all motor unit activation of a pool, including fast twitch units²³² (Figure 2-2).

The ratio of H_{max} and M_{max} is considered an appropriate index for demonstrating the level of reflex excitability of motor pool, which is dependent on the facilitation of the transmission between the Ia fibers and the α -motoneuron²³⁴.

The H_{max}/M_{max} ratio (maximal amplitudes of H-reflex relative to CMAP) has been used to assess a variety of neurological diseases. The H_{max}/M_{max} is usually increased in conditions characterised by spasticity and hyperreflexia²³⁵. It has been noted that despite the presence of UMN signs ALS patients do not have a higher H_{max}/M_{max} compared to normal controls²³⁶⁻²³⁸.

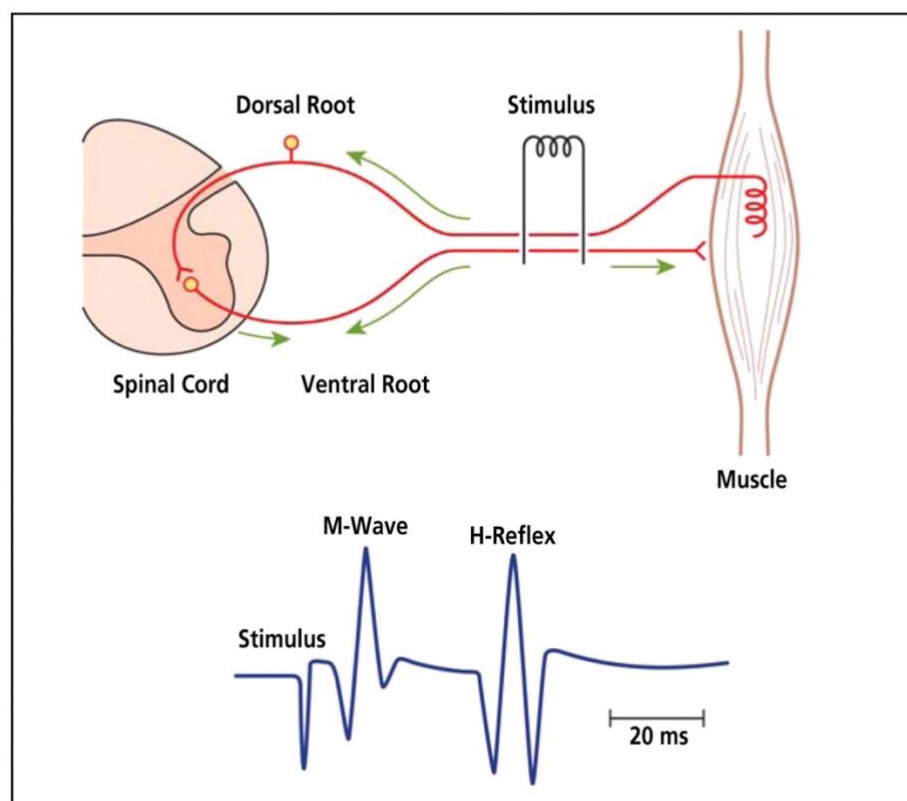
Simon et al. goes further to explain the possible reason behind the similarities in the H_{max}/M_{max} between patient and control groups. The paper proposes that the increased collision of reflex discharges with antidromically conducted motor impulses may be exacerbated in ALS secondary to the preferential loss of large calibre α -motoneurons. This could explain the similarities in H_{max}/M_{max} between the ALS and control groups and therefore its limited use in MND.

2.5.2.2. The H_{θ}/M_{θ} Ratio

The alternative measure which is independent H_{θ}/M_{θ} of the effects of collision antidromic and orthodromic impulses generated by direct nerve stimulation and the H reflex respectively. H_{θ} and M_{θ} is calculated using the slope angle of the earliest rising phase of the H and M wave recruitment curves⁴¹. The size principle holds for H reflex recruitment, where the initial phase of H reflex recruitment curve is formed by

small calibre motor neurons^{41, 239}. The opposite also holds, that the largest calibre motor neurons have the lowest threshold to electrical stimulation and so the earliest part of the M wave recruitment curve is made up of potentials produced by the largest calibre motor axons²⁴⁰. The paper by Simon et al. was able to show H_0/M_0 was increased in ALS patients when compared to healthy controls. Assessing the ratio of the recruitment slope of the H reflex seems to be a more suitable measure of segmental motoneuronal hyperexcitability in ALS than the more traditional H_{max}/M_{max} .

238.



“Low intensity stimuli delivered to the tibial nerve activates only the largest fibres within the nerve, namely the Ia sensory fibres, generating a reflex electromyogram (EMG) response in the muscle—the H-reflex. Higher intensity stimuli also activate motor fibres. Action potential conduction within these fibres in the orthodromic direction generates a short latency EMG response in the muscle—the M-wave. Action potentials traveling in the opposite direction, antidromically, travel toward the spinal cord and collide with action potentials generated reflex from sensory fibre stimulation. These collisions prevent the reflex generated signal from traveling to the muscle. With increasing intensity of stimuli, more motor fibres are stimulated, causing these collisions in a greater proportion of the motor fibres. The intensity of the H-reflex signal decreases as the M-wave becomes larger, disappearing with stimuli greater than 20 mA.”

Figure 2-2 Illustration of H reflex and M wave from Wynne et al. 2006 ²⁴¹.

2.5.3. F-Waves

The F wave is the result of motor neuron discharges that are stimulated by an antidromically travelling impulses ²⁴². F waves give a means of exploring

transmission between stimulation sites of the arm or the leg and the related motor neurons in the cervical and lumbosacral cord ²⁴³.

F wave excitability has been described as possible indicators of UMN dysfunction²⁴⁴. F waves were named owing to their origin in recording of small muscles of the foot²⁴⁵. Antidromic origins of the F wave have been shown in man by the existence of F waves in de-afferented dorsal roots²⁴⁶. F waves are passed orthodromically through the axon which has discharged the preceding antidromic impulse. It is due to this reason that the effect of change in the motor neuron pool excitability produces variable F waves²⁴⁵.

The role of Renshaw cells has been discussed in relation to F waves. Given that Renshaw cells inhibit large motoneurons, their activation gives a physiological model for selective discharge of large motoneurons in F waves²⁴⁵.

In the setting of ALS, F wave excitability is measured with respect to its persistence and the F wave- CMAP amplitude ratios (F/M ratio). The F/M ratio measures the proportion of motor neuron pool activated by antidromic stimulation²⁴⁵.

Eisen et al have reported on studies which showed inconsistent changes in measuring F waves in ALS. It has been noted that these changes include prolonged latencies with associated increased amplitude and normal frequency as well as prolonged latency associated with decreased F wave frequency²⁴⁷. De Carvalho et al have shown that F wave persistence decreases with disease progression²⁴⁸. Raised F/M ratios relative to controls has been reported by Drory et al ²²⁷, however this was not influenced by the degree of clinical UMN involvement. The use of the F/M ratio as a measure of UMN dysfunction is limited as increased ratios has also been demonstrated in other conditions e.g. polyneuropathy²⁴⁹ and as such makes it non-specific in detecting UMN dysfunction in conditions like ALS.

Table 2-1. Current neurophysiological tools available in either clinical or research based settings and their corresponding pathophysiological measures.

ASSESSMENT	PATHOPHYSIOLOGY
H REFLEX	Ia/Renshaw cells involvement in ALS
F WAVE	Renshaw cell involvement in ALS
TMS	Assessment of corticomotoneural pathway
COHERENCE	Assessment of integrity of corticomotoneural pathway (in classic form)
MUNIX/MUNE	Assessment of estimated Motor units in ALS

2.5.4. MUNE

In the attempt to track ALS progression, the development of a biomarker for the loss of motor units has been developed. Quantifying axonal loss essentially began by McComas et al. in the 1970s giving rise to what we know as MUNE (Motor Unit Number Estimation) ²⁵⁰. MUNE is based on the ratio of maximal CMAP (Compound Muscle Action Potential) and average single motor unit.

CMAP is recorded using the usual nerve conduction methods. There are a few different methods in recording single motor units. MUNE represents the estimate of the number of functioning axons and not its true total.

Various methods to obtain an average motor unit size have been developed these include the incremental stimulation technique which allows for the gradual increase of stimulus intensity to recruit additional motor units.

The biggest limitation to the incremental MUNE method is alternation, where stimuli of the same strength could activate different combinations of individual motor units ²⁵¹. It has been estimated that the likelihood of alteration is >65% when 10 units are activated by graded incremental stimulation ²⁵².

The Multiple point stimulation(MPS) technique is based on stimulating the nerve at different sites to sample different motor axons ²⁵¹. With the development of the MPS technique it took into account the phenomenon of alternation. The MPS method uses a series of low intensity stimuli given at different sites of the peripheral nerve. Both the incremental and the MPS methods have shown good test-retest reproducibility and seem sensitive enough to track disease progression ²⁵³.

Eliciting F-waves is another MUNE based technique that has been used to obtain average motor unit size ²⁵⁴.

In more recent times the combination of incremental and multipoint stimulation technique has given rise to multipoint incremental method, this method overcomes the bias presented by alternation. The multiple point incremental stimulation method was deemed both sensitive and reliable in a paper by Shefner et al ²⁵⁵. When investigated in longitudinal studies this method seemed to be reliable in even slowly progressing denervation ²⁵⁵. In another paper the rate of decline of the multipoint incremental (MPI) method was compared against the ALSFRS-R. The MPI method was found to be more sensitive when expressed as a percentage change from baseline ²⁵⁶.

Spike trigger technique uses a modification that allows for rapid collection by using voluntary muscle contractions that will activate motor units which can be captured by voltage trigger and averaged from background activity ^{257 258}. The need for specialized software and operator input have limited its uptake and use ²⁵⁹.

At present two dominant methods in the assessment of motor unit numbers in ALS is MUNIX (Motor Unit Number index) and the multipoint incremental technique. MUNIX is used largely in the European centres with the tendency of the multipoint increment method to be used in North America ²⁵⁹.

2.5.5. MUNIX

A new method in tracking LMN progression in ALS has been the development of MUNIX by Nandedkar et al. ²⁶⁰ A mathematical model is applied by using the area and power of the compound action potential (CMAP) after supramaximal stimulation of a mixed peripheral nerve. The area and power of surface electromyography at different levels of voluntary isometric contraction is also taken into account²⁶¹.

This is used to calculate the “ideal case motor unit count” to give an estimate of the functioning motor neurons²⁶².

In contrast to other MUNE techniques, MUNIX does not pinpoint individual motor unit potentials, which makes performing this technique faster- on average 5 mins per muscle compared to a conservative 20mins with MUNE ²⁶².

MUNIX calculates the number of motor units and then the average size, in contrast most MUNE methods compute the average size of motor units first to then allow for the calculation of the motor unit number.

It has been noted previously that neurophysiological abnormalities occur before it is seen in force measurements^{42, 263-265}. The study by Neuwirth et al. ²⁶⁶ confirms this precedence of neurophysiological changes for MUNIX. The study demonstrated a decline in MUNIX values 12 months ahead of clinical muscle weakness detected by manual muscle testing. Interestingly their results showed a MUNIX decline ahead of any clinical weakness which was more marked than the CMAP amplitude. They explain this by the compensatory nature of reinnervation, which will preserve motor function and hence CMAP amplitude ⁴². MUNIX seems to overcome what the authors called a “blind spot” in pre-symptomatic LMN loss by detecting that 50% of LMN loss to a muscle prior to clinical symptoms^{263, 267, 268}.

As cited by the authors, this aspect of MUNIX is relevant in early phase II clinical ALS trials as well as detecting potential effective medication (in the way they slow down motor neuron loss) thereby prolonging phase III trials.^{269, 270}.

One of the attractive advantages of using MUNIX is its reproducibility. The method allows relatively easy acquisition of the skill while maintaining both a good inter-rater and between rater reliability. MUNIX reproducibility has previously been studied in small samples- these studies showed high intra-class correlation coefficient(ICC) in healthy controls. The ALS patients in these studies had higher coefficient of variation(COV) and a high ICC^{271, 272}.

Along with being a reproducible quantitative tool for disorders that lead to denervation, MUNIX was sensitive enough to indicate loss of motor neurons in patients who meet at least “possible” according to the revised El Escorial criteria ¹².

Reliability and sensitivity have been reported while tracking motor unit loss for six muscles in upper and lower limbs during the progression of ALS. The longitudinal study by Neuwirth et al. showed MUNIX declined by 2.4-4.2% per month in the studied muscles²⁶².

Some prospective studies in small ALS groups indicated decreases in motor unit population as reflected in progressive decreases in MUNIX values^{260, 266}.

MUNIX has also been shown to demonstrate greater change than other matrices including ALSFRS and FVC ²⁷³, allowing MUNIX to be the favoured endpoint measure.

Benchmarking MUNIX against longer established methods have shown it in a favourable light. Comparison studies between IS-MUNE and MUNIX have been carried out. In one such study by Furtula et al, assessing the ADM muscle in 13 ALS patients, showed that a reduction of incremental stimulation MUNE (IS-MUNE) was comparable to the progressive reduction in MUNIX ²⁷⁴. MUNIX was also shown to have similar performance to high-density MUNE (HD MUNE)²⁷⁵. Taking into account its reliability, its efficiency and ease of which the skill can be acquired it is fast becoming the most widely accepted form of assessing motor units in ALS²⁵⁹.

2.5.5.1. Split hand Phenomenon

Split hand is an interesting phenomenon first described by Dr Asa Wilbourn. Split hand refers to the muscle wasting that predominantly affects the thenar and first dorsal interosseous(FDI) muscles, with relative sparing of the hypothenar muscles²⁷⁶.

APB and FDI muscles that make up the constituents of the split hand, are innervated through the C8/T1 spinal segment. However, both the FDI and ADM muscles share ulnar innervation, but interestingly are affected differentially. The underlying pathology of split hand is complex, and recent studies have shed light to the possible mechanisms involving split hand, that being both cortical and peripheral^{277, 278}.

Debate surrounds whether the split hand phenomenon reflects peripheral or cortical processes. There has been the hypothesis that the human use of APB is greater than other small muscles of the hand and so lends itself to greater oxidative stress compared to that of ADM²⁷⁹. This argument is reinforced by the tendency of normal aging processes with cumulative oxidative stress is also associated with a bigger reduction in CMAPs of APB and FDI compared to ADM²⁸⁰. Other arguments for a peripheral mechanism take into account the different excitability properties in the respective peripheral nerves²⁷⁷.

A cortical mechanism argument gets support from evolutionary concepts where thumb movements (pincer grip specifically) is specific to humans where cortico-

motor neuron studies show upper motor neuron integrity is needed for precision of movement^{279, 281, 282}.

The spilt hand phenomenon has been used in electro-diagnostics in ALS. A study carried out by Menon et al. used CMAPs in calculating the split hand index, they showed a high specificity (80%) and sensitivity (74%) with an index of 5.2 or less, which reliably differentiates ALS from other neurological disorders²⁸³.

$$SI_{\text{cmap}} = APB_{\text{cmap}} \times FDI_{\text{cmap}} / ADM_{\text{cmap}}$$

A recent study used MUNIX in the calculation of the split hand index²⁸⁴. Kim et al were able to show a better diagnostic accuracy with SI_{munix} compared to SI_{cmap} ²⁸⁴. The caveat being that patients with ALS mimic disorders were not included in his study. However, these studies throw up some interesting adaptations of what we know in electro-diagnostics.

$$SI_{\text{munix}} = APB_{\text{munix}} \times FDI_{\text{munix}} / ADM_{\text{munix}}$$

The split hand index in the literature is yet to be validated across the ALS spectrum and in multiple centres.

2.6. Research Based Electrophysiological Methods

With the increasing recognition that ALS is a network-based disorder, there has been increasing neurophysiology-based studies exploring the possible network changes in ALS. Studies such as EEG based measures, TMS and corticomuscular coherence have a role to play in evaluating cortical network involvement, as well as disease progression.

2.6.1. EEG

EEG is often misguidedly labelled as an inaccurate or variable source of data when assessing functional neuronal activity in research settings. However, EEG holds a unique position in its combined ability to represent neuronal activity both directly (i.e without relying on an intermediate response system) and in real-time by non-invasive means. EEG based measures are also comparatively inexpensive when compared to other modalities such as MRI or MEG systems²⁸⁵. If we consider EEG as a time varying measure of voltage in electrical fields, then we are constrained by the limit imposed on us by measuring potentials that require points of reference. This

limitation can be mitigated by the use of surface Laplacian techniques, which is a mathematical transformation used on EEG surface recordings ²⁸⁵. EEG signals reflect the summation of cortical neurons activating in synchrony, that have similar spatial orientation. Neuronal firing in synchrony is thought to be a determinant of the processing capabilities in brain networks ²⁸⁶. As such, the study of synchrony in EEG recordings has been used in an attempt to better understand the basic mechanisms of brain function ²⁸⁷. While time-frequency analysis relates to the use of EEG signals to extract frequency rhythms (such as alpha, beta and gamma waves). The frequency domain illustrates how much of an EEG signal lies within a given frequency band, while time domain graphs signal changes over time. Our wider research group at the academic unit of neurology, has previously shown the utility of EEG as a tool to map complex network disruptions and connectivity patterns in motor and nonmotor networks in ALS ^{150, 151, 288}.

Due to the complex nature of neuronal signalling and networks in neurodegenerative conditions, it was decided to assess ALS simultaneously from both an UMN/LMN perspective with the use of EEG and EMG measures. This was achieved by employing corticomuscular coherence.

2.6.2. Corticomotoneural Pathway Assessment

2.6.2.1. Coherence

Historically we can trace our knowledge of neurophysiology back to Grimaldi in 1665 ²⁸⁹ where he first described the rumbling sounds of contracting skeletal muscles. It wasn't until 1810 when William Wollaston first formally described the rhythmic muscle discharges- these sounds were compared to London carriages driven over cobbles- he estimated the frequency to be 20-30 cycles/s. Fast forwarding to the early 1990's when the first evidence of a central origin to motor unit synchrony emerged ²⁹⁰ to the present day- where focus has turned to certain patterns of oscillatory drive to muscle may be of clinical significance.

With a growing interest in the neural signal analysis and coherence measures to assess motor circuits, we look at the literature to assess if the patterns of oscillatory drives to muscles are of diagnostic potential in neurodegenerative disease.

A useful way of examining neuronal synchrony is frequency analysis and is based on the cross correlation between two different signals in the time and frequency

domains²⁹¹. The main measure of the correlation between two separate signals in the frequency domain is coherence.

EMG signals are often analysed to extract information about synaptic input received by motor neurons- and conclude an indirect measure of excitation of signal received by motor neurons.

The linear correlation to Quantify the strength of corticospinal input to motor neurons can be based on the frequency domain between EMG and concurrently recorded EEG. It is the general consensus that motor unit synchronization is in the beta band (15-30Hz) and low gamma(30-60Hz) band is mainly driven from the primary motor cortex²⁹².

Coupling between the periphery and motor cortex seems to be demonstrable even in muscles with the smallest cortical representation that is the para-spinals and abdominal wall muscles²⁹³.

Beta band coherence appears during weak tonic contraction, and especially so when directed towards a motor task²⁹⁴ and disappears on movement (Table 2-1).

The gamma band is more apparent on strong contractions of muscle and can persist during slow movement²⁹⁵.

Oscillations at 20Hz were found to be coherent with EMG activity during sustained contractions²⁹⁶. This interaction is labelled as corticomuscular coherence. This corticomuscular coherence indicates there is an effective relay of cortical oscillations along the corticospinal tract down to the eventual trains of motor unit action potentials (and back to the sensory cortex via afferent pathways).

There is an assumption that the degree of significant coherence between cortical and muscular activity relates to the strength of supra-spinal contributions to the output generated by spinal motor neurons²⁹⁶. Significant coherence is explained by an adequate number of motor neurons receiving linear transmission of a synaptic input²⁹⁶.

The distribution of the cortico-muscular coherence patterns roughly follows that of the somatotopic organisation²⁹⁷. The areas corresponding to small muscles of the hand as well as arm muscles overlap. This convergence is suggested to reflect the overlapping representations of muscles that may mediate cognitive and motor control required in neural processing of finger and wrist actions²⁹⁸. TMS and fMRI

studies have supported this theory^{299, 300}. The coherence of EMGs in maintained precision grip tasks is likely to be mediated by this mechanism²⁹⁵.

These techniques have been applied in a range of pathological conditions²⁹¹.

Table 2-2 CMC frequency ranges and its associated physiological correlates

Frequency	Physiological correlate
1-2 Hz	“Common drive” ³⁰¹
8-12 Hz	Physiological tremor ³⁰²
15-30 Hz	Beta band activity-short term motor unit synchronisation ³⁰³
30-60 Hz	Gamma band activity ³⁰⁴

Frequency analysis is being applied increasingly to different fields within neurology. Various studies have looked at cortical myoclonus³⁰⁵, Parkinson’s disease^{306, 307} and stroke patients³⁰⁸. However very few papers have focused on coherence in the setting of MND.

One such paper is by Fisher et al.²⁹² which focused on intermuscular coherence as a novel biomarker in ALS in assessing upper motor neuron dysfunction. They looked at eight patients with PLS, six with PMA and 16 age matched controls. Beta band coherence was noted in all controls and PMA patients with absent coherence in PLS patients. The conclusion was drawn that intermuscular coherence in the 15-30Hz band is dependent on an intact corticospinal tract, however this persists in selective anterior horn cell degeneration²⁹². This gives rise to the tantalising possibility of using intermuscular coherence as a quantitative test of subclinical UMN degeneration in ALS and other neurodegenerative diseases.

2.6.2.2. Transcranial Magnetic Stimulation

The quantification of UMN dysfunction through cortical hyper-excitability has been an important development in neurophysiological biomarkers in ALS⁴¹. TMS techniques have been the focus of assessing the corticomotoneuronal system in ALS. Single, Paired and triple-pulse techniques have all been used in ALS research.

Reviewing the literature, the outcome parameters commonly used in TMS in assessing the corticospinal tract integrity are⁴¹:

- Motor threshold(MT)
- Motor evoked potential(MEP) amplitude

- Central motor conduction(CMC) time
- Cortical silent period(CSP) duration
- Short interval intra-cortical inhibition(SICI)
- Intra-cortical facilitation(ICF)

Cortical hyperexcitability in ALS has been shown during transcranial magnetic stimulation by decreases in short-interval intracortical inhibition, which is the ability of a subthreshold stimulus to quell the response to a later suprathreshold stimulus by an interval of up to 10ms¹⁵. It has been noted that abnormalities in the trans-callosal inhibition is reflected by reduced SICI identified early in the disease process of ALS, as well as possibly underlying mirror movements in the condition. Early stage as defined by the paper was based on the El Escorial Criteria of clinically “suspect” or “possible” ALS⁴¹.

The mechanism of SICI appears to be mediated by GABA-secreting inhibitory cortical interneurons by means of GABA_A receptors³⁰⁹.

The length of the cortical silent period (CSP) seems to be consistently reduced across all ALS phenotypes³¹⁰. The duration of CSP appears to occur in the early phase of cortical inhibition of anterior horn cells³¹¹ and by GABA_A receptors in later phases in cortical processes³¹². Measurements of the CSP can therefore be exacted to pinpoint disinhibition of the anterior horn cells or to identify the progressive dysfunction of cortical inhibitory interneurons that act through GABA_A receptors³¹³.

Supporting the notion that cortical hyper-excitability is an early process in ALS is a significant reduction in MT³¹⁴. Prominent increases of MEP amplitudes, as an early feature in ALS, were noted in both familial and sporadic forms. This MEP amplitude increase is not seen in mimic disorders thereby giving credence to the excitotoxicity pathogenesis in ALS³¹⁵. Another feature not seen in mimic disorders and seems to be specific to ALS is the decrease in CSP duration, which is also seen in ALS patients with no upper motor neuron signs³¹⁶.

It has been found that prolonged CMCT in ALS reflects the degeneration of the corticomotoneuronal tracts along with increased desynchronization of the descending volleys^{314, 317}. The literature has noted that CMCT abnormalities can deteriorate over the course of ALS are correlated with UMN signs³¹⁸.

Cortical hyper-excitability has been identified as an early feature in sporadic ALS³¹⁴,³¹⁹. Vucic et al. paper supports the central origin theory of ALS is the presence of cortical hyper- excitability as a pre-symptomatic feature in familial ALS³¹⁴.

2.7. Discussion

While the search continues for the definitive biological marker for the clinical diagnosis of ALS, clinical neurophysiology continues to be the main supportive diagnostic tool in the diagnosis of suspected ALS.

While the role of clinical conduction studies remains to be that of excluding disease mimics in early ALS, many promising methods are emerging in the assessment of LMNs.

With the increasing understanding and insight into the dysfunction of anterior horn cells and their central connections, one hopes that in time specific biomarkers may be developed.

There has been strong evidence to support the loss of inhibitory neuronal function as the main culprit in ALS pathogenesis. Interneurons are in a unique position with the ability to inhibit both cortical and spinal motor neurons. Our understanding of interneurons has allowed us to unravel the mystery of the clinical heterogeneity of ALS, with their presence explaining the process of UMN and LMN degeneration resulting in the typical ALS end phenotype.

Confirmation of interneuron involvement would open the gates to new targets for disease modifying drugs aiming to increase inhibitory influence, reinstating a balance with excitatory drive.

With the complexity of the neurodegenerative processes especially in ALS, it is hard to confine any level of change to one particular area of the brain, whether in cellular changes, atrophy, hypoperfusion or hypometabolism. Studies carried out in Alzheimer's disease in the early 90s had shown that even in an event of an initially relatively well circumscribed area of degeneration, the pattern of spread was usually in systematic manner to other, not necessarily adjacent regions of the brain³²⁰.

Over the last two decades significant advances have been made in the field clinical neurophysiology of the upper motor neuron network in ALS. The importance of

recognising the need to assess upper motor neuron involvement in patients lies in the fact that even in patients with clinically predominant LMN syndromes have been shown to have UMN involvement³²¹. One study went so far to show that up to 70% of patients with a clinically “pure” LMN syndrome had corticospinal tract involvement at autopsy ³²².

The methods with the most potential in the assessment of cortical abnormalities seem to be cortical excitability studies and coherence studies. It remains to be seen if these methods can be transferred to widespread clinical use. However, we are tantalisingly close to being able to accurately stratify patients, this will allow for targeted therapy both in clinical trials and beyond.

Neural signal analysis can provide direct estimates of network activity with high spatiotemporal resolution. Subjective measures of symptoms manifest from neural degeneration may now be objectively assessed by directly quantifying network activity, allowing for potentially earlier intervention, with greater probability of success in clinical trials. From my review, it is clear that the complexity of neurodegenerative conditions makes any one modality difficult to interpret or carry forward into a clinical setting. As such, CMC offers the potential to assess dysfunction systems on a whole, both from a cortical as well as from a motor unit perspective. CMC allows us the potential to interrogate for the first time neurodegenerative systems at a network based level, allowing for the complexity of the diseases.

A consequence of this review process is that ALS is increasingly being considered as a disease primarily of neural networks that is defined by the involvement of upper and lower motor neurons but that can also affect other cell populations and extend to other neuronal networks. One of the main reasons research is ongoing into mechanisms of motor neuron degeneration is the hope that this work will lead to rational strategies for preventing disease progression. In ALS and SMA, gene therapy, antisense oligonucleotides and novel chemical compounds are all currently being explored ³²³⁻³²⁵. However, increasing our understanding of the neurodegenerative process also means we have an obligation to improve our ability to monitor disease progression objectively and ideally from a neurophysiological standpoint.

3. Chapter 3: Aims and Objectives

In this chapter, the aims and objectives of this project are described along with the summary of the rationale for the proposed approach in completing these objectives. In section 3.1, what I aimed to achieve is described. In section 3.2 the specific objectives planned in order to meet these aims, and their rationale, are outlined.

3.1 Aims

The overarching aim of this thesis is to characterize the dysfunction in motor networks spanning LMN, UMN, and cortical brain networks using non-invasive methods based on neurophysiological and signal analysis. This is facilitated by including 4 groupings (ALS, PLS, PPS and SMA patients compared to healthy controls) that span the spectrum of LMN/UMN involvement in the motor system in a selective way. This approach aims to improve our understanding of the neurophysiological markers associated with the various patterns of motor system dysfunction that underpin ALS, SMA and PLS.

The aim is to provide quantitative data that can support sub-phenotyping, stratification, as well as network-specific prognostic biomarkers of the disease, in clinical settings. This will be achieved by neuro-electrophysiological recordings, i.e., HD-EMG and sEMG, with additional neural signal analysis to study the spectral characteristics and the synchrony of the neuro-electric signals, the most important measure of which is cortico-muscular coherence (CMC).

The primary working hypothesis of this study is that CMC between EEG-EMG can inform of the specific alterations in the brain's motor networks within and beyond the primary motor cortex. Specifically, I aimed to determine the following:

1. To develop CMC-based biomarker candidates as potential tools for assessing UMN and cortical network disruption in selected motor subsystems in ALS patient subgroups during functional isometric motor tasks.
2. To determine the nature of the pathologically decreased/increased neurophysiological measures of CMC in 4 patient groups including ALS, PLS and Polio and SMA.
3. To define reliable quantitative neurophysiological biomarkers of cortical and spinal network integrity in ALS, validated against clinical UMN/LMN motor scores.

3.1. Objectives

3.1.1. Objective A

To determine the nature of neurophysiological measures in CMC in the setting of LMN conditions (Polio/SMA).

Hypothesis: The motor subsystems are disrupted in patient subgroups during functional isometric motor tasks, and measures which interrogate specific alterations in motor networks can also uncover potential compensatory drives in LMN and mixed conditions, namely in Polio/SMA and ALS respectively.

Rationale: Prior to the onset of this project few coherence studies were carried out on ALS patients. Due to the heterogeneity of ALS, its characterisation required a network based approach that recognises the complex relationships between transcortical and corticofugal pathways and the spinal cord circuitry ³²⁶.

Characterisation of disease heterogeneity in terms of underlying network disruptions require newer techniques. Most of the clinical neurophysiological methods used today have a primary focus on quantifying structural degeneration of either upper or lower motor neurons (UMN/LMN), and imaging studies focus on the characterisation of the general features of disruption in the brain as a whole ^{149, 151} or in the cognitive domain³²⁷. Consequently, the motor decline, as the main feature of ALS, has never been quantified at a network level.

Motor neuronal loss is known to follow a distinct pattern ³²⁶, with the loss of the largest α -motoneurons ahead of the smallest, with sparing of the γ -motoneurons. These circuitry changes in ALS disrupt the “normal homeostatic” mechanisms with the potential to accelerate the disease process, namely the loss of motor function ³²⁶. Emerging preliminary evidence suggests that interruption of one component in the motor circuit can lead to compensatory changes both up and down stream, as has been demonstrated by recent MRI observations of cortical reorganisation in SMA ³²⁸. I hypothesise that LMN changes may have a wider networked disruption than originally thought, and by employing CMC one can assess for changes on a larger scale (cortical and muscular).

3.1.2. Objective B

To determine the nature of neurophysiological measures in CMC in the setting of UMN conditions (PLS).

Hypothesis: Cortico-muscular coherence (CMC) between EEG-EMG can inform to the specific alterations in motor networks within and beyond the primary motor cortex in PLS.

Rationale: PLS and Polio/SMA are at the 2 ends of the UMN-LMN pathology spectrum. I postulate that Cortico-muscular Coherence (CMC) between EEG and EMG is a measure that has the potential to provide key insights into the composition of the descending motor drive ³⁰³ and involve non-invasive recording techniques that make them suitable for clinical application. In fact, CMC measures assess neuronal synchrony by frequency analysis based on the cross correlation between two different signals (i.e. EEG/EMG) in the frequency domains ²⁹⁷ using spectral coherence ³²⁹.

Simultaneous recordings of multi-channel EEG and EMG for time-series analysis can be used to quantify the level of effective communication between all cortical brain regions while quantifying oscillatory motor drives to muscles during motor tasks ³²⁹. EMG signals are often analysed to extract information about synaptic input received by motor neurons, and conclude an indirect measure of excitation of signal received by motor neurons²⁹⁶. The linear correlation to quantify the strength of corticospinal input to motor neurons can be based on the frequency domain between EMG and concurrently recorded EEG ³³⁰. It is my hypothesis that such network disruptions are likely to form a spectrum, based on the foci of degeneration in lower (Spinal Muscular Atrophy, (SMA) or similarly in Polio patients ³³¹), upper (PLS) or both motor neurons (ALS). These network disruptions include and extend beyond UMN/LMN regions and may not directly manifest as clinical symptoms ^{41, 42}.

3.1.3. Objective C

To define reliable quantitative neurophysiological biomarkers of cortical and spinal network integrity in ALS, validated against clinical UMN/LMN motor scores.

Hypothesis: Cortico-muscular coherence between EEG-EMG can quantify the network specific impairments which are clinically valid.

Rationale: The correlation of neurophysiological findings with clinical profiles makes these network-based neurophysiological measures a potentially suitable biomarker of network failure in neurodegeneration/neural disorders. I postulate that in the era of

gene therapy, neurodegenerative conditions need to be assessed on a networked basis, as such the potential to use CMC as a biomarker to gauge outcome measures in future clinical trials warrants further investigation.

4. Chapter 4: General Materials and Methods

This chapter details the general methodologies and analyses used in this thesis. Ethical approval and participant written consent are described in section 4.1. The recruitment of patient and control cohorts and their evaluation by clinical, and cognitive tests are described in 4.2 and 4.3 respectively. Those materials and methods employed for corticomuscular coherence studies are described in 4.4 and 4.5. Data analysis and statistical tests used across this project are described in 4.6 and 4.7. Study-specific methods, participant recruitment and demographics and inclusion and exclusion criteria are described for each study in the respective results chapters 5 to 8.

The data processing and analysis for this project as described in this chapter was carried out by a team of engineers at the academic Unit of Neurology at Trinity College Dublin.

4.1. Ethical Approval and Informed Consent

Ethical approval was granted by the *Tallaght University Hospital / St. James's Hospital Joint Research Ethics Committee - Dublin* [REC Reference: 2019-05 List 17 (01)] (appendix 4.1) and performed in accordance with the Declaration of Helsinki. All participants provided informed written consent to the procedures before undergoing assessment (appendix 4.2).

4.2. Recruitment of Patients and Healthy Controls

Patient Recruitment

Five population groups were recruited into this study: ALS, PLS, Polio, SMA patients and healthy controls. All Participants took part in a voluntary basis. All participants were capable of informed consent.

4.2.1.1. Inclusion and Exclusion Criteria

Inclusion criteria varied between patients' cohorts and as such are defined across the relevant results chapters 5-8.

Participants with the following were excluded from the study:

- History of major head trauma,
- Neurological conditions that could affect cognition,
- Alcohol dependence syndrome,

- Current use of neuroleptic medications,
- High dose psychoactive medication.

4.2.1.2. Clinical and Functional Assessment

Clinical evaluation consisted of detailed phenotyping- including clinical staging, disease burden (based on specific functional rating scales for ALS, PLS, Polio, SMA) and specific motor measures recorded. These measures include MRC muscle strengths, hypo/hyper-reflexivity profiles, Ashworth scores, and pathological signs namely: Wasting, fasciculations, clonus, and Hoffmans/ Babinski sign (appendix 4.3). These measures were subsequently used to calculate an adapted upper or lower motor neuron score relevant to each patient group.

4.2.2. ALS and PLS patient recruitment

Throughout the research study, individuals diagnosed with possible, probable or definite ALS according to the El Escorial Criteria Revised ¹² were recruited from the Irish National ALS Clinic, at Beaumont Hospital Dublin. In the PLS group, patients whose diagnosis was confirmed as PLS after 4 years of first symptom onset were approached to be included in the study.

Patients were approached, if appropriate, by a member of the Academic Unit of Neurology, to ask if they were interested in hearing about ongoing research of ALS at a future time. If the patient consented, they were contacted by the experimenter after at least one week by phone or email to discuss specific information about the study/studies of interest and any questions regarding participation from the patient were answered. If the participant was then willing to take part and deemed suitable according to the study's inclusion and exclusion criteria, they were scheduled for a research session appointment at their earliest convenience.

4.2.3. SMA patient recruitment

Throughout the study, patients clinically diagnosed with SMA type 2 or 3 were recruited from the SMA Clinic, at Beaumont Hospital Dublin. Patients were approached, if appropriate, by a member of the Academic Unit of Neurology, to ask if they were interested in hearing about ongoing research on SMA at a future time. If the patient consented, they were contacted by the experimenter after at least one week by phone or email to discuss specific information about the study/studies of

interest and any questions regarding participation from the patient were answered. If the participant was then willing to take part and deemed suitable according to the study's inclusion and exclusion criteria, they were scheduled for a research session appointment at their earliest convenience.

4.2.4. Polio patient recruitment

Throughout the research, adult Polio survivors who were clinically diagnosed with Polio in infancy or childhood were recruited from the Polio Clinic, at Beaumont Hospital Dublin. Patients were approached, if appropriate, by a member of the Academic Unit of Neurology, to ask if they were interested in hearing about ongoing research on polio at a future time. If the patient consented, they were contacted by the experimenter after at least one week by phone or email to discuss specific information about the study/studies of interest and any questions regarding participation from the patient were answered. If the participant was then willing to take part and deemed suitable according to the study's inclusion and exclusion criteria, they were scheduled for a research session appointment at their earliest convenience.

4.2.5. Healthy Control recruitment

Throughout the project, individuals without any neurological, psychiatric or muscular disease diagnoses were recruited to the CMC study. Those with a first degree relative with ALS were also not eligible, to avoid potential inclusion of individuals with premorbid familial ALS pathology or ALS-related. Control participants were recruited via advertisement of the study and call for volunteers to spouses and friends of ALS patients and through public advertising of the Academic Unit of Neurology's research studies. In order to maintain age-matched patient and control cohorts, recruitment of control volunteers between the ages of eighteen and thirty years old was limited to match the low frequency of ALS, PLS and Polio patient participants of this age bracket within each study.

4.3. Clinical, cognitive and behavioural measures

Cognitive scores were collected by the team at the Academic Unit of neurology and the National ALS Clinic, were obtained for correlation analysis with ALSFRS-R and some of the cognitive data collected as part of this project.

4.3.1. ALS functional rating scale revised (ALSFRS-R)

The ALSFRS-R is a 48 point semi-quantitative scoring scale for the measurement of functional motor severity in ALS. The ALSFRS-R is a revised version of the original, 40 point scale, which consists of 10 sub-scores ranging from 0 to 4 (4 being normal function)³³². These sub-scores address impairments of daily living due to motor symptoms of ALS. The ALSFRS-R replaces one of these sub-scores, regarding breathing, with three respiratory sub-scores regarding dyspnoea, orthopnoea and use of mechanical respiratory aids, such that the total score range is 0 to 48 (48 being normal function)³³³.

The ALSFRS-R was recorded for all ALS patients who took part in this project on the day of EEG/EMG recording.

4.3.2. Date of Onset

As part of study enrolment, ALS/PLS patients were asked to recall date of symptom onset as well as date of diagnosis. Polio and SMA patients were asked similarly age of symptom onset.

4.3.3. Cognitive testing

ECAS (Edinburgh cognitive and behavioural assessment scale) score were collected jointly by me and trained members of the academic unit of neurology as part of concurrent PLS/ALS imaging studies.

The ECAS is a screening tool designed to detect the cognitive profile and behavioural changes in ALS as well as to differentiate this profile from other disorders. The ECAS takes approximately 20 minutes to complete, enabling screening of ALS patients for cognitive and behavioural impairment during clinic visits. The Screen includes an ALS- specific score, incorporating executive functions, social cognition, verbal fluency and language tasks, an ALS non-specific score, incorporating memory and visuospatial tasks^{334, 335}. Higher scores indicate better cognitive performance.

4.4. Experimental Procedure

4.4.1. General Procedure Set-Up of surface EEG Acquisition

After discussion of the study protocols and informed written consent, participants were brought to the recording room and seated in a chair. Participants were seated comfortably in a chair that supported their posture, in front of a 23" computer monitor (distance from eyes: ~1m), with right upper arm elevated at approximately 40° from shoulder and elbow at 90° resting on a pillow over a desk. Participant's feet were adjusted to rest on the floor. If wheelchair-bound patients were unable to easily move to the provided chair, tasks were performed while seated in their own wheelchair. Participants were seated in front of a desk upon which a screen and

force sensor block were positioned for delivery of visual stimuli and detection of force responses. Participants' head measurements were taken to select a correctly- sized, electrode-positioning cap. Eight external reference/electro-oculography electrodes were positioned above and below the left eye and bilaterally on the earlobes, temples and mastoids. The cap was subsequently positioned with electrode A1 over the vertex (the intersection of the horizontal axis between the tragi of the ears and the vertical axis between the nasion and inion). The chin strap of the cap was closed beneath the chin of the participant to ensure secure positioning of the cap while avoiding discomfort to the patient. Correct positioning of the cap was subsequently ensured by alignment of electrode locations horizontally relative to the inter-tragus axis and rotationally and antero-posteriorly relative to the nasion-inion axis.

Conductive gel was then syringed into each electrode-holding hole in the cap, forming a bridge between the scalp and electrode. Hair was moved and the scalp was lightly abraded with the syringe tip to minimise electrode impedance. Electrodes were inserted into their assigned location in the cap, with cables attached to the back of the chair with slack to facilitate limited participant movement. Following hardware setup, direct current offset of each recording electrode relative to the common sense (CMS) electrode was checked via Actiview software, and additional abrasion/gelling was performed if necessary to ensure sufficient quality of electrode-scalp contact (i.e., all offset values were $<25\text{mV}$ and $>-25\text{mV}$).

4.4.2. Surface EMG Acquisition

Surface EMG was recorded from 8 muscles in the right hand and forearm:

- FDI (first dorsal interosseous);
- EDC (Extensor Digitorum Communis);
- FDS (Flexor Digitorum Superficialis);
- APL (Abductor Pollicis Longus) and EPB (Extensor Pollicis Brevis);
- FPB (Flexor Pollicis Brevis);
- APB (Abductor Pollicis Brevis);
- ADM (Abductor Digiti Minimi);
- FDMB (Flexor Digiti Minimi Brevis).

Muscles are located based on surface anatomy and voluntary muscle contraction. Surface EMG recordings are conducted using BioSemi® ActiveTwo system with flat active sintered Ag-AgCl electrodes (BioSemi B.V., Amsterdam, The Netherlands), which provide a circular recording area of ($d=3\text{mm}$) in a $17\times 10\text{mm}$ support surface area.

Subsequently, online signals were briefly monitored to check for artefacts in single electrodes which indicate insufficient recording quality. Following improvement of recording from any noisy channels to the required standard, motor tasks were undertaken by the participant.

The recording session then started, lasting for about 2 hour and 10 minutes (from beginning of equipment setup to end of the paradigm). This included 45 minutes for head measurement, external electrode placement, cap fitting, application of gel and placement of electrodes, and EMG electrode placement, 10 minutes for checking and maximisation of recording quality based on online recording and electrode impedance values, 6 minutes for resting state recordings, 5 minutes for grip strength paradigm setup and explanation of the task to the participant, 45 minutes for the force paradigm, 15-20 minutes for full history and clinical examination.

4.4.3. Resting state Recording

All participants were asked to minimise their eye movements and to relax during the experiment. Resting-state EEG recordings were obtained with the eyes open. This included 3 blocks of 2 minutes. This recording allows for comparisons of the findings in subsequent experiments to the recently found ALS-related changes in resting-state EEG ¹⁵¹.

4.5. Experiments for Corticomuscular Coherence

Participants were instructed to attempt maximum voluntary contraction (MVC) of the pincer grip between the thumb and the index finger of their right hand (Fig. 1) guided by visual cues. Each MVC exertion was requested for 5s, with 30s delays between trials. MVC was taken as the average peak force of the three trials which were within 10% of each other. Note MVC trials (Task 1) were used for the purpose of calibration of force and visual interface and not for physiological signal recordings.

Subsequently participants were guided through a series of motor tasks that included the following:

- Task 2: 30 attempts of voluntary isometric pincer grip tasks between the thumb and the index finger, according to visual cues. The onset and offset of the exertion was signalled to the subject via visual cues. Each exertion was requested at 10%MVC for 5s, with 10s delays in-between for rest. Subjects were told to use their *preferred pace* for increasing and decreasing the grip force while

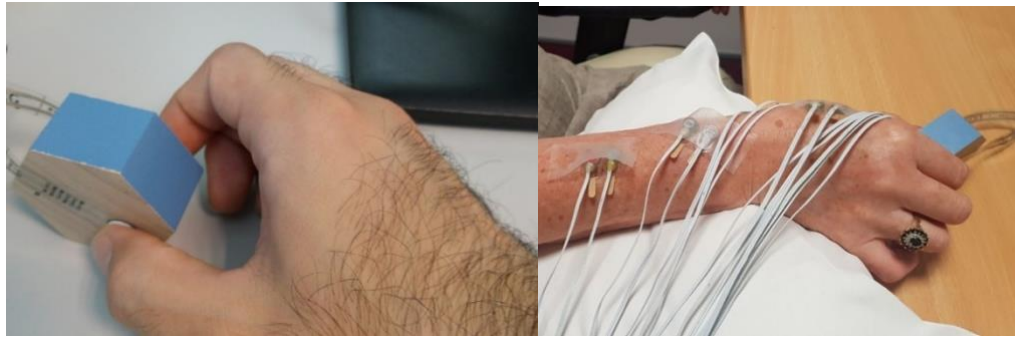
avoiding abrupt changes. The exerted force level by the subject was deemed as correct, if the error was less than 10% of the range. This experiment aimed to capture the cortico-muscular coherence during low-force levels, as well as during slow force onset and offset.

- Task 3: 30 attempted voluntary isometric pincer grips between the thumb and the index finger, according to visual cues. The onset and offset of the exertion was signalled to the subject by visual cues. Each exertion was requested at 10% MVC for 5s, with 10s delays in-between for rest. Subjects were told to increase and decrease the grip force to the requested levels, *as fast as possible*. This experiment aimed to capture the cortico-muscular coherence during fast force onset and offset.
- Task 4: Tracked isometric pincer grips between the thumb and the index finger to generate sinusoidal force profiles according to visual cues. Subjects generated a 2Hz sinusoidal force profile between 2% and 10% MVC (centred at 6%), for 2x60s. This experiment aimed to quantify corticomuscular coherence during rhythmic force tracking and resembles a clinical diagnostic task that includes rhythmic pinching/tapping of the finger against thumb.
- Task 5: 30 attempted voluntary pincer grips between the thumb and the index finger, according to visual cues. They were required to hold and lift the wooden flat object over 2 soft springs, giving the behaviour of an elasticated object, according to visual cues. Each exertion was requested for 5s, with 10s delays in-between for rest. This experiment aimed to quantify the (potentially increased) corticomuscular coherence during a combined position-force control.

If patient was unable to complete all outlined tasks due to fatigue the number of attempted recordings were halved. In patients with significant motor impairment affecting grip strength recordings were taken to measure “flicker of movement” equivalent to MRC power score 1 (these patients were largely in the SMA group and for the purpose of statistical analysis the patients were analysed individually as opposed to on group level).



(a)



(b)

*Assessing cortico-muscular coherence (a) and the recording of sEMG during the experiment (b).
Figure 4-1 Pincer grip in isometric task*

4.6. Data Acquisition

4.6.1. Hardware

EEG: EEG data were recorded in a special purpose laboratory, electromagnetically shielded as a Faraday cage, using 128-channel scalp electrode cap, filtered over the range 0–400 Hz and digitized at 2048 Hz using the BioSemi® ActiveTwo system (BioSemi B.V, Amsterdam, Netherlands). Each participant was fitted with an appropriately sized EEG cap. Data were recorded in a Faraday cage enclosed room, using a battery-powered amplifier to prevent introduction of electrical mains noise to the signal. Data were transmitted to computers in a neighbouring room by a fibre optic cable, where signals were monitored and recorded on a computer (Dell Inc., TX, USA) equipped with the Windows 7 operating system (Microsoft Corporation, WA, USA). A similar, second computer was used to deliver task stimuli to the participant within the electrically isolated room and to record responses. This task computer was plugged in via sockets in the neighbouring room to avoid introduction of electrical noise.

EMG: Surface EMG was recorded (Fig. 1) from 8 muscles in the right upper arm: FDI (first dorsal interosseous); EDC (Extensor Digitorum Communis); FDS (Flexor

Digitorum Superficialis); APL (Abductor Pollicis Longus) and EPB (Extensor Pollicis Brevis); FPB (Flexor Pollicis Brevis); APB (Abductor Pollicis Brevis); ADM (Abductor Digiti Minimi); FDMB (Flexor Digiti Minimi Brevis). These 8 muscles were chosen for recording surface EMG and were not the same as the 9 muscle pairs chosen for strength-based clinical assessment. Bipolar channels were used according to the provided recommendation by SENIAM (Hermens et al., 2000; Merletti & Hermens, 2000). Surface EMG recordings were conducted using flat active sintered Ag-AgCl electrodes (BioSemi B.V., Amsterdam, The Netherlands), which provided a circular recording area ($d=3\text{mm}$) in a $17\times 10\text{mm}$ support surface area. An interelectrode distance of 1cm (up to a maximum of 2 cm) was used for bipolar recording. The placement of EMG electrodes with respect to the muscle locations followed previously reported recommendations³³⁶⁻³³⁸. The sampling frequency and the filter settings for the EMG channels were the same as the EEG channels.

Force: Grip force was recorded using 2 flat resistive force sensors (FlexiForce A201 Sensor, Tekscan, Inc., Boston, MA, USA) with their circular sensing area ($d=9.7\text{mm}$) attached to the 2 bases of a wooden hexagon (Fig. 1). The resistance was converted to analogue voltage using a small circuit board (Tekscan, Inc., Boston, MA, USA) and was recorded and digitised using a Data Acquisition Card (PCIe-6321, National Instruments, Austin, TX, USA) at 2000Hz in real time, and subsequently visualised and presented to the participant.

4.6.2. Software

Visual Cues: The visual stimuli and the visual feedback of the grip force was programmed in MATLAB® (Mathworks, Inc., Natick, MA, USA) using Psychophysics Toolbox³³⁹ at a screen refresh rate of 60Hz. The typical delay between the visualisation loop and the recording loop was 1-3ms.

4.7. Data Analysis

4.7.1. Pre-processing

During all motor task experiments, 152 (128 EEG + 16 unipolar EMG + 8 External) channels were recorded in total. Signal pre-processing of all EEG/EMG data was preformed using MATLAB with the help of FieldTrip toolbox³⁴⁰. The following steps were performed during pre-processing of EEG/EMG signals.

4.7.2. CMC pre-processing

4.7.2.1. Signal Extraction

The EEG/EMG signals were extracted from Biosemi (.bdf) file into MATLAB (.mat) file. Instead of extracting whole data, the segments of data which were being analysed were extracted to save memory and increase processing speed. The extraction was based on the events/triggers stored in .bdf file. For example, during Task 2, the start of a trial was marked as event 20; after 5 seconds, visual cues were presented to perform the motor task which was marked as event 21; and 5 seconds after that, visual cues were presented to end the motor task which was marked as event 22 followed by 5 second resting period before another trial begun. Thus, a trial of Task 2 was 15 seconds long and the motor task data was between event 21 and event 22. Four seconds of motor task data, 1 second after event 21 until event 22, were extracted for the analysis. So, for each participant 120 seconds (30 trials x 4 seconds) motor task data were extracted. During the signal extraction, the 16 unipolar EMG channels were converted into 8 bipolar EMG signals by simple subtraction.

4.7.2.2. Force Processing

The force exerted by the participant on the force sensors during each motor task were stored separately. During pre-processing of a motor task data, the corresponding force profiles were processed to identify the trials where the applied force was deemed incorrect. The trials with the force value within $\pm 10\%$ of target force (10% of MVC) were marked as correct trials, rest were marked incorrect. The incorrect trials were removed from the data.

4.7.3. Channel Selection and Re-referencing

4.7.3.1. Sensor Space

Five EEG channels A1, A19, B22, C21, D19 (International 10-20 System equivalent Cz, Pz, C4, Fz, C3 respectively) and 3 EMG channels (APB, FDI, FPB), that were deemed most relevant for assessing cortico-muscular coherence (CMC), were chosen for analysis. The EEG channels were re-referenced using surface Laplacian spatial filter to reduce the effect of volume conduction^{341, 342}. The Laplacian filters for 5 selected EEG channels were created by using corresponding 4 neighbouring channels. The neighbouring channels chosen for 5 EEG channels were A19, B22, C21, D19 for A1(Cz); A1, A23, B22, D19 for A19(Pz); A1, B26, A19, C21 for B22(C4); A1, B22, C17, D19 for C21(Pz); and A1, A19, C21, D23 for D19(C3). Also, the EMG data (signal amplitude) were normalized by MVC.

4.7.3.2. Source Space

For source space analysis, during this pre-processing step, all 128 EEG channels and 3 EMG channels (APB, FDI, FPB) were chosen. Also, the EEG channels were re-referenced

using common-average referencing. Just like in sensor space pre-processing, the EMG signal amplitudes were normalized by MVC.

4.7.3.3. Filtering

EEG/EMG data was filtered between 49-51 Hz using a dual-pass 4th order Butterworth band stop filter to remove 50Hz noise (power line noise). This was followed by a 1-100Hz bandpass filtering of EEG signal using dual-pass 4th order Butterworth filter and 10-100Hz bandpass filtering of EMG signal using dual-pass 4th order Butterworth filter.

4.7.3.4. Artefacts Detection and Rejection

For the detection of the EEG artefacts such as eyeblinks, muscle artefacts, jump artefacts, and ECG artefacts, a threshold based automatic artefact detection method implemented in FieldTrip toolbox was used. In this method, the original data is copied and filtered according to the nature of the artefact. For example, to detect eyeblinks artefacts, the copy of original data is bandpass filtered between 2-15Hz. After that, the filtered data was converted into z-scores using mean and standard deviation calculated over all trials for each channel individually. Next, a single time series data was generated by averaging the z-scores over all channels. This single time series data contained the accumulated artefacts. Finally, the artefactual trials were detected using threshold z-values i.e., if average z-score was greater than threshold z-value for any timepoint in a trial, the trial was considered artefactual and removed from original data. The threshold z-values were selected using visual inspection such that no more than 20% of trials were rejected as artefactual trials.

4.7.4. Resting-state Pre-processing

The whole EEG signals were extracted from Biosemi (.bdf) files to Matlab (.mat) files. Bad epochs were rejected using a custom artefact rejection method based on a statistical threshold ³⁴³, before being made continuous. After resampling at 256 Hz, the EEG signals were band-passed ([1-97Hz]) and notch filtered (around 50Hz) to remove power line noise. A baseline correction was then performed to remove potential temporal drifts. Next, noisy channels were removed using a custom algorithm based on the PREPpipeline and the work of Kohe ^{344, 345}. It detected abnormal correlations between EEG channels, standard deviations and ratios of high to low frequencies. Channels removed were regenerated by interpolating from the remaining electrodes using FieldTrip toolbox spline interpolation ³⁴⁰. Last, channels were referenced to the common average.

4.8. Signal processing

4.8.1. Sensor Space- CMC

The auto-spectrum of each EEG/EMG signal, and cross-spectrum between all combinations of EEG-EMG signals (frequency resolution 1Hz, bandwidth 2-100Hz) was calculated using FieldTrip toolbox (Hanning taper and frequency smoothing at 1Hz). The auto/cross spectrum at each frequency (2-100Hz) was converted into 8 band values- delta (2-4Hz), theta (5-7Hz), lower alpha (8-10Hz), higher alpha (11-13Hz), lower beta (14-20Hz), higher beta (21-30Hz), lower gamma (31-47Hz), and higher gamma (53-97Hz)- by taking Spatial Median^{346, 347} of spectrum at corresponding frequencies. For example, the auto/cross spectrum for delta band was the spatial median value of auto/cross spectra at 2, 3, and 4 Hz. The spectral coherence (cortico-muscular coherence in this study) was obtained by normalizing cross-spectrum by respective auto-spectra³⁴⁷.

4.8.2. Source Space- CMC

The pre-processed sensor space EEG signals were converted into source space EEG using Linear Constrained Minimum Variance (LCMV) beamformer³⁴⁸ with the help of FieldTrip toolbox. For reconstruction of source signals, a template structural MRI data (<https://identifiers.org/neurovault.image:29404>) was used to compute forward model (lead-field matrix). Ten anatomical brain regions, 5 on each side of the brain, were chosen as regions of interests (ROIs) using automated anatomical labelling atlas³⁴⁹. The 10 ROIs chosen were Precentral_L/Precentral_R, Postcentral_L/Postcentral_R, Supp_Motor_Area_L/Supp_Motor_Area_R, Frontal_Mid_L/Frontal_Mid_R and Parietal_Sup_L/Parietal_Sup_R. The lead-field corresponding to the centre of anatomical ROI was chosen as current dipole to estimate the time series EEG data for each ROI. The centre of these anatomical brain regions corresponds to left/right Primary Motor, left/right Primary Sensory, left/right Supplementary Motor, left/right Prefrontal and left/right Superior Parietal cortical sources respectively. After the estimation of source EEG signals, the source space CMC were calculated using the same method as sensor space CMC.

4.8.3. Source Space -Resting State

After pre-processing, the resting-state EEG signals were converted into source space EEG following the protocol described by Dukic et al. ¹⁵⁰. More precisely, the linear constrained minimum variance beamformer from the FieldTrip toolbox ³⁴⁰ was used on an ICBM152 structural MRI template ³⁵⁰ to compute the forward model. 90 anatomical brain regions from the automated anatomical labelling atlas ³⁵¹ were used as a reference to estimate localised source-space signals. A fast Fourier transform was then applied on 2s epochs of the signals to estimate the normalised spectral power in six frequency bands of interest i.e., α (7-13 Hz), β (13-30Hz), γ_l (30-47 Hz), γ_h (53-97 Hz), θ (4-7 Hz), δ (2-4 Hz). The functional connectivity between brain region was also estimated using amplitude envelope correlation (*AEC*) to measure the co-modulation between two signals.

4.9. Statistics

Subject-level statistics was performed using one-sample non-parametric rank statistics for spectral coherence ³⁴⁷. This method gave individual p-values for spectral cortico-muscular coherence at each frequency bands for both patients and control groups. Stouffer's method ^{352, 353} was used to combine individual p values to get group average p value. This procedure is equivalent to the pooled coherence analysis ³⁵⁴.

Correction for multiple comparison was performed using the adaptive False Discovery Rate at $q = 0.05$ ³⁵⁵, which was applied by correcting the p-values in the coherence spectra. Negative logarithm of p-values, i.e. $-\log_{10}(p)$, was used to visualize cortico-muscular coherence.

Resting State Statistics was handled by the Empirical Bayesian Inference framework ³⁵⁶ through an available Toolbox implemented in MATLAB ³⁵⁷.

5. Chapter 5: ALS Results

5.1. Corticomuscular Coherence in ALS

5.1.1. Introduction

ALS is a disorder that not only affects the motor system but also extra-motor areas affecting cognition, behavioural impairments with overlaps to frontotemporal dementia (FTD) ^{202, 358, 359}.

With at least 30 genes identified to be associated with ALS ³⁶⁰, with no clear correlation to clinical or neuropathology phenotypes, ALS can no longer be thought of as a homogenous disease but rather one that may have multiple factors that trigger varying pathophysiological and clinical courses of disease.

Imaging and neurophysiology studies previously published in the literature suggest a differential disruption of neural networks and can possibly reflect the clinically heterogeneous picture we see in ALS presentations ²⁰⁷.

Functional MR images have been used to identify increased patterns of connectivity within the sensorimotor networks of ALS ³⁶¹. Network impairments, however, can also be interrogated using EEG to assess the neuroelectric signals. These signals show in various frequency bands and can substantially differ across neural networks ³⁶².

Previous EEG studies have shown changes in network patterns, such as increased frontoparietal connectivity in ALS ^{149, 151}. These spectral signatures require a high temporal resolution and cannot be captured by MRI ³⁶³.

The characterisation of the disease heterogeneity in terms of the underlying network disruptions requires alternative tools to explore these network changes and how they apply to clinical presentations. Most of the neurophysiological methods to date have a primary focus on quantifying the structural degeneration of either upper or lower motor neurons (UMN/LMN), and imaging studies focus on the characterisation of the general features of disruption in the brain as a whole ^{149, 151} or in the cognitive domain³²⁷. Consequently, the motor decline, as the main feature of ALS, has never not been quantified at a network level.

Motor neuronal loss is known to follow a distinct pattern ³²⁶, with the loss of the largest α -motoneurons ahead of the smallest, with sparing of the γ -motoneurons. These circuitry changes in ALS disrupt the “normal homeostatic” mechanisms with

the potential to accelerate the disease process, namely the loss of motor function ³²⁶. This α - γ reweighting varies across patients, which contributes to clinically heterogeneous picture seen in ALS. Such network disruptions are likely to form a spectrum, based on the foci of degeneration in lower (Progressive Muscular Atrophy, (PMA) or similarly in Post-Polio Syndrome ³³¹, PPS), upper (PLS) or both motor neurons (ALS). These network disruptions include and extend beyond UMN/LMN regions and may not directly manifest as clinical symptoms ^{41, 42}.

While neurophysiological measurement tools already have the capability to measure progressive loss of motor units and are reliable predictors of late disease progression, very little has been directed toward investigating reliable markers of disruption in UMN and importantly other cortical networks. Given the promise of the methods based on neural (EEG/EMG) signal analysis ²⁹² for interrogating UMN dysfunction, measures based on Cortico-muscular Coherence (CMC) patterns are the most promising measures for quantifying the neural dysfunction in UMN and more broadly other cortical motor networks.

The simultaneous recordings of multi-channel electroencephalogram (EEG) and electromyogram (EMG) for time-series analysis can be used to quantify the level of effective communication between all cortical brain regions while quantifying the oscillatory motor drives to muscles during motor tasks ³²⁹.

EMG signals are often analysed to extract information about synaptic input received by motor neurons and conclude an indirect measure of excitation of signal received by motor neurons²⁹⁶. The linear correlation to quantify the strength of corticospinal input to motor neurons can be based on the frequency domain between EMG and concurrently recorded EEG ³³⁰. Cortico-muscular Coherence (CMC) between EEG and EMG, has the potential to provide key insights into the composition of the descending motor drive ³⁰³ and involve non-invasive recording techniques that make them suitable for clinical application. In fact, CMC measures assess neuronal synchrony by frequency analysis based on the cross correlation between two different signals (i.e. EEG/EMG) in the frequency domains ²⁹⁷ using spectral coherence ³²⁹.

Here I have hypothesized that cortico-muscular coherence (CMC) between EEG-EMG can interrogate disease-specific alterations in the brain's motor networks within and beyond the primary motor cortex in MND

5.1.2. Methods

5.1.2.1. Ethics

The study was approved by the *Tallaght University Hospital / St. James's Hospital Joint Research Ethics Committee - Dublin* [REC Reference: 2019-05 List 17 (01)] as described in chapter 4 section 4.1.

5.1.2.2. Inclusion Criteria

All patients were over the age of 18 and diagnosed with ALS. Patients were prospectively recruited from the multidisciplinary ALS clinic based in Beaumont Hospital, Dublin. All patients were diagnosed as having definite/probable/possible ALS in accordance with the El-Escorial Revised Criteria ¹².

5.1.2.3. Exclusion Criteria

Patients diagnosed with primary lateral sclerosis (PLS), progressive muscular atrophy (PMA), ALS restricted phenotypes ³⁶⁴ or suffering from any other neurological condition were excluded. Patients with a history of major head trauma or other neurological conditions that could affect cognition, alcohol dependence syndrome, current use of neuroleptic medications or high-dose psychoactive medication were excluded. Those with uncontrolled diabetes mellitus, a history of cerebrovascular disease was also excluded.

5.1.2.4. Clinical Assessment

22 ALS Patients were prospectively recruited in this cross sectional study between June 2017- Nov 2019 through the national clinic for ALS at Beaumont Hospital.

On the day of EEG recording all patients underwent an extensive clinical assessment. Disease duration from symptom onset, site of disease onset and phenotype were recorded. Disability was assessed using the Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised ³³³ (ALSFRS-R, range 0-48, the lower the score reflects greater disability) encompassing evaluation of gross and fine motor tasks, bulbar functions and respiratory functions. Additionally, the fine motor function sub-score was derived from ALSFRS-R: this sub-score is based on the four items of the scale (handwriting, cutting food and handling utensils, dressing and hygiene, turning in bed and adjusting bed clothes) and it reflects coordinated actions related to cortical motor control³³³.

ALSFRS-R progression rate was calculated with the following formula ³⁶⁵:

$$(48 - \text{ALSFRS-R}) / \text{disease duration in months}$$

This reflects the pace of linear decrease based on the change of ALSFRS-R score in a given time interval (that is, between the approximate date of symptom onset and the date of recording).

The fine motor function (FMF) – progression rate was also calculated with the following formula:

$$(16 - \text{FMF sub-score}) / \text{disease duration in months}.$$

Clinical assessment was performed on the same day of EEG/EMG recording. Muscle strength was assessed with the Medical Research Council (MRC) score in the following upper limb muscles: deltoid, triceps, biceps, wrist flexors and extensors, fingers flexors and extensors, FDI, APB. The degree of clinical lower motor neuron (LMN) involvement in the upper limbs was graded by a LMN score³⁶⁶ which ranged from 90 (absent LMN signs) to 0 (severe LMN signs). Clinical examination also included deep tendon reflexes' testing in bicipital, tricipital and brachioradial districts as well as Hoffmann sign evaluation. The degree of clinical upper motor neuron (UMN) involvement in the upper limbs was graded by an adapted UMN score⁷⁹ which ranged from 0 (absent UMN signs) to 8 (severe UMN signs).

5.1.2.5. *Experimental Paradigm and Data acquisition*

Assessment was conducted in the same manner for the patients and control groups. EEG/EMG acquisition and the employed experimental paradigm are described in detail in the methods section 4.4.

5.1.2.6. *Data Analysis*

EEG/EMG data analysis was blinded to clinical assessments. Five EEG channels (Cz, Pz, C4, Fz, C3) and 3 EMG channels (APB, FDI, FPB) were chosen prior to the analysis of cortico-muscular coherence (CMC). This selection was based on the biomechanical involvement of the muscles in the pincer grip task³⁶⁷, and the suitability of the EEG and EMG channels for assessing CMC³⁶⁸. A time window/epoch duration of 4s (starting 1s after the visual cue) was chosen for analysis; data epochs where the target force was not correctly achieved were excluded. The raw EEG data was (re-)referenced using (small) surface Laplacian spatial filter^{369, 370} which is a spatial filter for removing spurious signal components in

EEG channels, and EMG data (signal amplitude) were normalized with respect to the EMG amplitude during 100 % MVC. EEG/EMG data were filtered between 1-100Hz using a dual-pass 4th order Butterworth bandpass filter. This was followed by a 50Hz Discrete Fourier Transform (DFT) filter in the FieldTrip toolbox to remove power line noise. An automatic artefact detection and rejection was performed using FieldTrip toolbox ³⁴⁰ to remove eyeblinks, muscle, and electrode jump artefacts from EEG signals. The auto-spectrum of each EEG/EMG signal, and cross-spectrum between all combinations of EEG-EMG signals (frequency resolution 1Hz, bandwidth 2-100Hz) was calculated using FieldTrip toolbox (Hanning taper and frequency smoothing at 1Hz). The auto- and cross-spectra at each frequency (2-100Hz) was converted into 8 band values- delta (2-4Hz), theta (5-7Hz), lower alpha (8-10Hz), higher alpha (11-13Hz), lower beta (14-20Hz), higher beta (21-30Hz), lower gamma (31-47Hz), and higher gamma (53-97Hz), excluding the 48-52Hz range to avoid mains power noise. The formation of band-specific values was carried out by taking Spatial Median (a variation of the median operator for complex-valued spectra, chosen and preferred over the algebraic averaging to provide robustness against outlier values) ^{346, 347} of the spectra at corresponding frequencies. The spectral coherence (cortico-muscular coherence in this study) was obtained by normalizing the cross-spectrum by the respective auto-spectra ³⁴⁷.

The selection of the parameters and methods for signal processing (e.g. band-specific analysis and the use of non-parametric methods) was based on our previous EEG studies ^{150, 151} that provided robust estimations not sensitive to outliers or observations in individual subjects ³⁷¹.

5.1.2.7. Statistics

Participant-level statistics were calculated using one-sample non-parametric rank statistics for spectral coherence ³⁴⁷. This method gave individual p-values for spectral cortico-muscular coherence in each frequency band for both patients and control groups. Stouffer's method ^{352, 353} was used to combine individual p values to derive group average p value. This procedure is equivalent to the pooled coherence analysis ³⁵⁴.

Correction for multiple comparisons was performed using the adaptive false discovery rate at $q = 0.05$ ³⁵⁵, which was applied by correcting the p-values in the

coherence spectra. Negative logarithm of p-values, i.e. $-\log_{10}(p)$, was used to visualize cortico-muscular coherence. The band-specific values of coherence, expressed in $-\log_{10}(p)$, was used to represent the values for all of the frequencies in that frequency band.

5.1.3. Results

5.1.3.1. Patients clinical profile

A total of 22 patients affected by ALS were successfully recruited from a cohort ALS clinic based in Beaumont hospital, Dublin (see Tables 5-1). The analysed patient group included 16 male and 6 female patients (mean age of 67.9 ± 9.6 (Standard deviation), 21 were right hand dominant). A total of 11 healthy controls were successfully recruited (mean age of 61.09 ± 14.8 standard deviation).

The mean disease duration from symptom onset was 30.7 ± 26.5 months. The mean degree of functional impairment, as defined by the ALSFRS-R, was 37.0 ± 4.7 points, while the degree of muscle weakness, as graded by the LMN score, was 75.9 ± 11.3 points. The mean UMN score was 4.1 ± 4.1 points. At the time of recording, only one patient was not on *Riluzole*.

Table 5-1 Clinical and demographic data of analysed patients.

Patient Demographics	
Age (years)	67.9 ± 9.6
Sex (female / male)	6 / 16
Site of onset (spinal/bulbar/respiratory)	17 / 3 / 2
ALSFRS-R	37.0 ± 4.7
Fine motor functions sub-score	12.1 ± 2.1
Disease duration (months)	30.7 ± 26.5
ALSFRS-R progression rate	0.53 ± 0.38
Fine motor function - progression rate	0.18 ± 0.11
Lower motoneuron score (LMN-s)	75.9 ± 11.3
Upper motoneuron score (UMN-s)	4.1 ± 4.1

5.1.3.2. Abnormal cortico-muscular coherence in the ALS

Cortico-muscular coherence (CMC), during steady low force isometric pincer grip, was calculated between intrinsic hand muscles (using bipolar EMG) and surface EEG electrodes over scalp. In this context, patterns of CMC were identified in the ALS group that differed from those of the controls. Specifically, the CMC in the patient group did not show the typical beta-band CMC over contralateral motor area (as intended by the task selection), and the CMC across the 5 tested EEG electrodes, 3 muscles, and the frequency bands were scattered and inconsistent (Fig.5.1). These null findings were in accordance with expectations from our pilot study in controls, as well as the anticipation of typical beta-band cortico-muscular coherence primarily over primary motor cortex, C3 electrode (seen in low force sustained muscle contraction ^{297, 298, 303}).

In the ALS group the CMC was statistically significant ($p < 0.05$, $q = 0.05$) at group level (Fig. 5.1) and appeared at frequencies different from the commonly observed and expected beta (14- 30Hz) frequency bands. Instead, the significant CMC appeared consistently in the alpha (8-12Hz) frequency band across several EEG-EMG channels (see Fig. 5.1). Importantly, this abnormal alpha -band coherence was observed at the ipsilateral motor area as well as (C4) area, but also over parietal and frontal areas (Fz and Pz). The abnormal CMC appeared in other (e.g. beta) frequency bands but was less consistently across EEG-EMG channels. These CMC

patterns differed from the control cohort in location (contralateral motor C3 vs Frontal Fz and Parietal Pz) ^{303, 372}, (Fig. 5.2).

The pathological alpha-band CMC was a consistent finding and was observed at both frontal and parietal electrodes and in both FPB and APB muscles (Fig. 5.1)

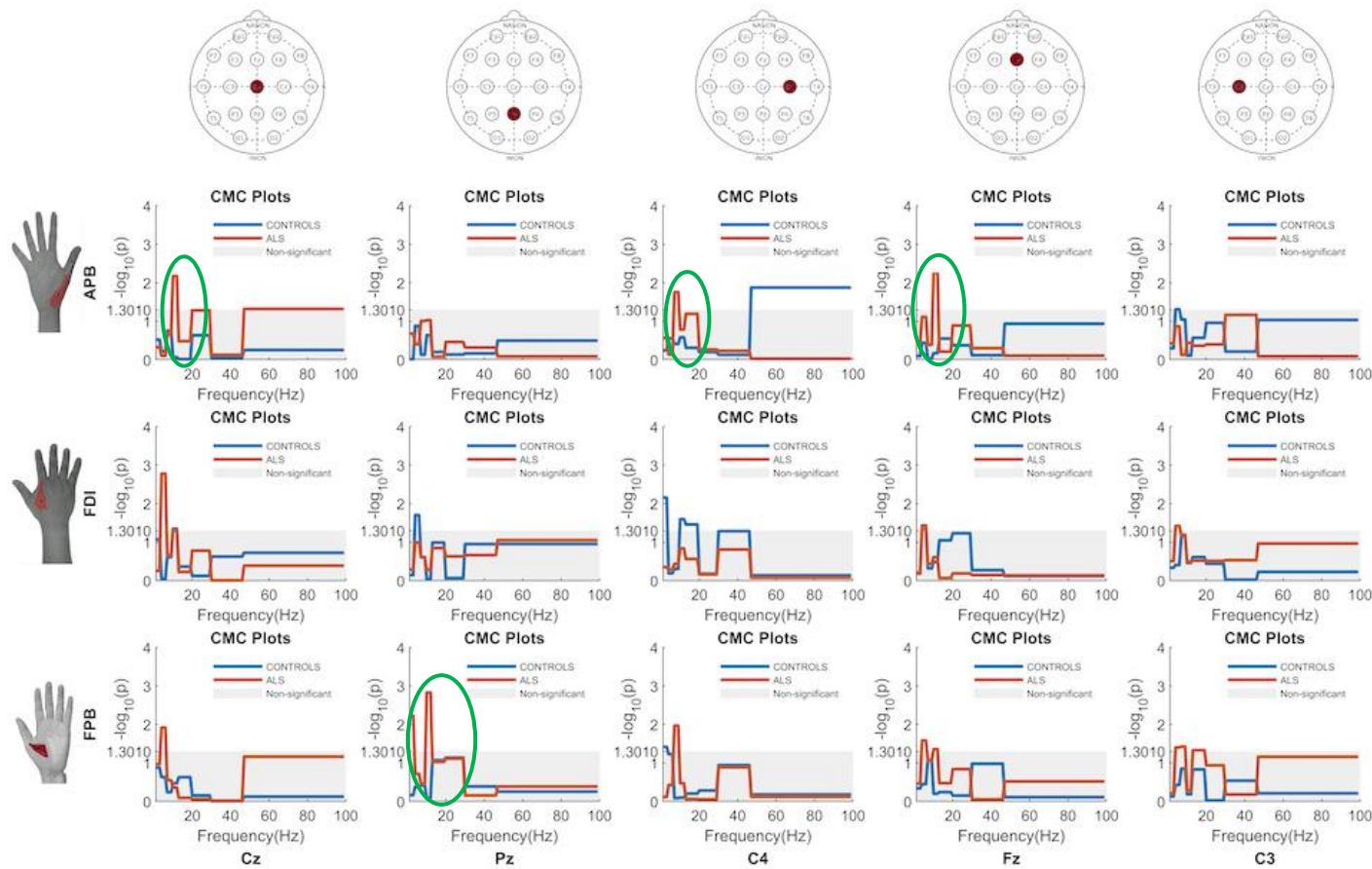


Figure 5-1 ALS Patients show abnormal group level CMC in non-primary motor areas.

The cortico-muscular coherence spectra, expressed in $-\log_{10}(p)$, show the synchrony between the EEG electrodes (over the frontal area, Fz, Central, Cz, and parietal areas, Pz) and EMG (APB, FDI, and FPB muscle) in different frequency bands. The lower values show less synchrony, whereas the higher value show higher EEG-EMG synchrony. The shaded area corresponds to the non-significant values at $\alpha=0.05$ threshold for p -values (corrected for multiple comparison using FDR at $q=0.05$). Notice the dominant abnormal coherence in ALS in alpha (8-12 Hz)/ low beta-band (14 - 30Hz) which is present in APB muscle and in both C4 and Fz electrodes, as well as alpha- band noted between Pz and Cz electrodes and APB and FPB muscles.

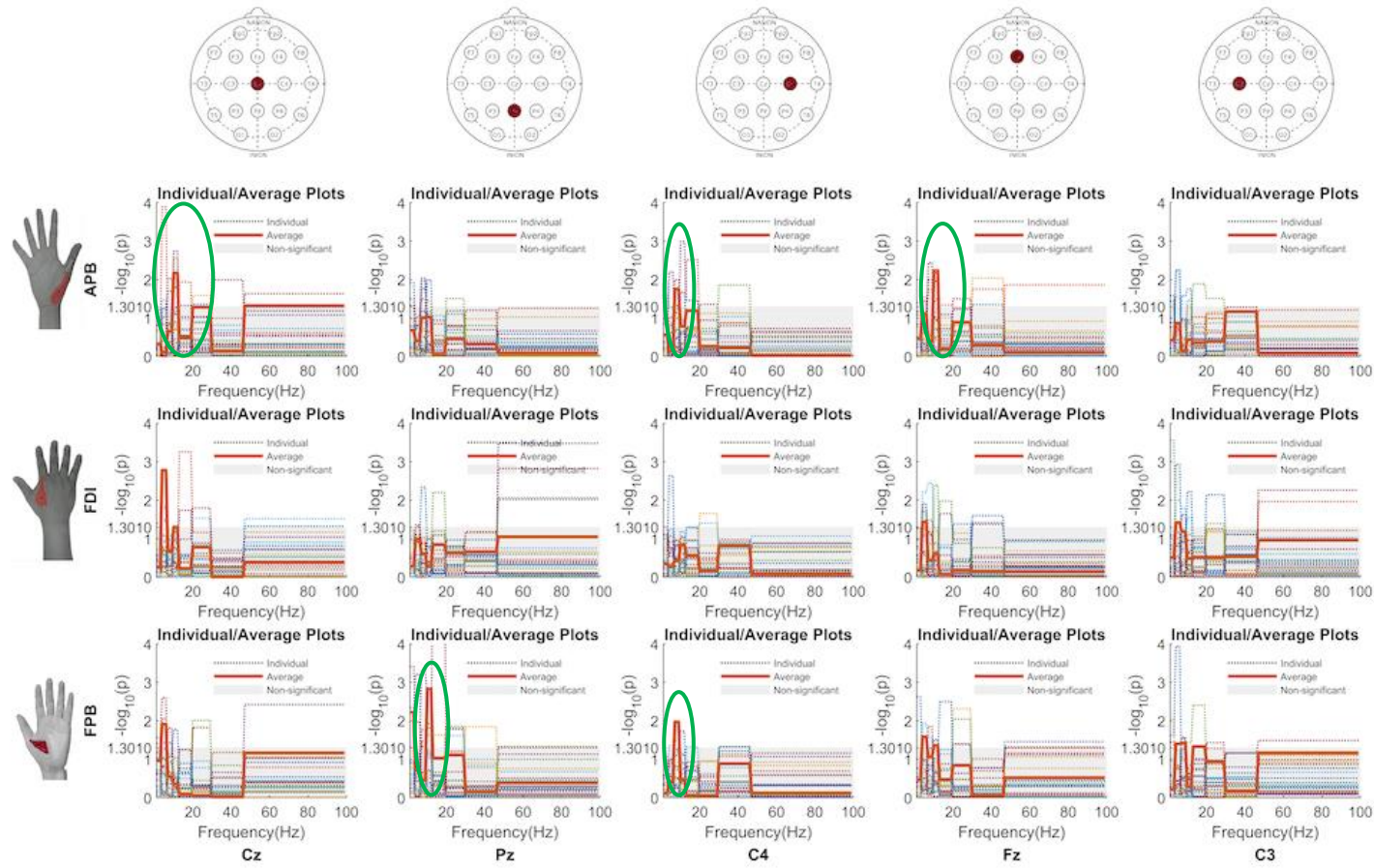


Figure 5-2 Significant pathological increases in CMC seen at individual as well as group level in ALS.

Analysis of ALS patients showed abnormal frequency band changes -namely alpha band increases over central (Cz) region. Abnormal coherence patterns noted between APB muscles and the frontal (Fz), ipsilateral (C4) and central (Cz) regions. Abnormal coherence patterns also noted between FPB and APB muscles and ipsilateral (C4) regions.

5.1.4. Discussion

Evidence of pathological alterations in cortical connectivity in ALS potentially show compensatory changes that retain the possibility to become markers of both disease and a target for potential novel therapeutics. Evidence of modulation of cortical circuitry in mixed (U/LMN) disease would provide compelling evidence for precision medicine.

Changes in CMC in ALS patients suggests changes in the supra-spinal networks. ALS, can present with muscular weakness that is linked to the dysfunction of the remaining surviving motor neurons that causes slow disintegration of terminals of the individual nerve axons³⁷³. Parallels can be drawn in the motor unit loss and associated muscle function that anticipates the death of motor neurons and can mirror the "die-back" phenomena often described in ALS³⁷⁴. These preliminary results suggest that interruption of one component in the motor circuit can also lead to compensatory changes both up and down stream. When considering the "Dying back vs the Dying forward" models of neurodegeneration, one has to reconcile what we know already in models already described in literature. We can speculate that the reason for ALS complexity goes as far as to combine, the "dying forward" and "dying back" theories, which I reason as being not entirely mutually exclusive but rather likely to be a combination of both. Whether the point of disease origin starts in the cortex or distally we can argue that there is a cyclical pattern that affects the cortex (if point of initial assault is at the NMJ) or affects the LMN and its associated motor neuron pools (if nidus of origin is at the motor cortex with a synaptic spread of disease). Regardless of origin, we must acknowledge that both scenarios describe a network based system in which disequilibrium in one affects the other.

The accentuated abnormal frequency band increases seen in the patient groupings may be reflective of compensatory activities from other regions that are not seen in healthy conditions. It may be inferred that this is a mechanism to compensate for primary motor (M1) corticospinal projections, considering the M1 degeneration in ALS¹⁵¹. The increased connectivity patterns seen in the frontal region supports previous MRI findings of cortical atrophy patterns and inter-connected cortical-subcortical grey matter regions³⁷⁵.

Further analysis is required to fully characterize the pathologic CMC patterns in the ALS patient group.

The distinct signatures exhibited by patients suggest that this methodology may provide a means of unmasking altered neural communication and can help to provide new patient stratification perspectives in ALS in the future.

While this study is ongoing the preliminary evidence present here indicates that cortico-muscular communication can potentially map the altered sensorimotor functions in the motor subsystems affected in ALS. By combining EMG/EEG I have aimed to closely reflect these pathological systems. These electrophysiological signatures will help to better understand the underlying disease mechanisms, providing new patient stratification models, and facilitate the development of diagnostic and perhaps even prognostic biomarkers.

The description of the heterogeneous nature of MND is near on ubiquitous in literature. This heterogeneity is believed to have contributed to, in part, to the failure of over 40 large clinical trials in a disorder where significant variability of survival is the norm, independent of treatment. To optimise trial design and allow fair evaluation of potential therapeutic agents, it is clear that more effective methods are required to sub-categorise ALS cohorts.

The application of this novel quantitative network-based biomarker in the clinical setting, has the potential to sub-classify, and assess measures of disease progression.

5.2. Resting State in ALS

EEG β -band measures in the motor network as potential biomarkers for disease severity and progression of motor symptoms in ALS.

The work described in this section is being prepared for submission to a peer-reviewed journal. This work is based on equal contribution from Antonio Fasano, Stefan Dukic and myself. It is intended that this work be published to include 3 first co-authors.

5.2.1. Introduction

Amyotrophic lateral sclerosis (ALS) is the most frequent adult onset motor neuron disease (MND) and it is characterized by degeneration of motor neurons in the brain and spinal cord leading to motor and extra-motor symptoms ³⁷⁶.

Different degrees of impairment within intracortical and cortical networks ³⁷⁷, corpus callosum ³¹⁶ and spinal γ -motor system ^{67, 378} affect the upper motor neurons in the motor networks and in the corticospinal projections, possibly contributing to the heterogeneous phenotypic presentation. Consequently, assessing cortical and spinal networks functions allows us to better explore and understand the complexity of ALS presentations. This type of approach has been shown as a potential promising biomarker candidate for upper motor neuron dysfunction, as well as other aspects of ALS onset, staging and progression ^{20, 310, 379}.

Spectral EEG measures have proven to be useful biomarker-candidates in other neurodegenerative disorders such as Parkinson's and Alzheimer's diseases ³⁸⁰. This technology has good temporal resolution since it is strictly related to neuro-electric signals generated along the nervous system's structures; additionally, the use of high-density EEG coupled with source-space localization techniques can dramatically improve its spatial resolution ²⁸⁸.

Previous resting-state EEG studies in ALS showed the capacity of this technique as a sensitive tool in different ALS aspects. Differences between ALS patients and healthy controls have been described in different brain regions: a significant decrease in θ frequency spectral power over bilateral motor areas (spreading to δ - and α -bands and to adjacent scalp locations) was shown in ALS patients and it correlated with MRI measures of gray and white matter degeneration in motor cortical regions and cortico-spinal tracts ³⁸¹. Another study within our group expanded these findings further by showing a decrease in the β -band power source-localized in the sensorimotor network ¹⁵⁰. From a clinical point of view, patients' ALSFRS-R scores correlate with a decline in average β -band connectivity change in the motor network, while connectivity changes within the frontal-parietal and frontal-temporal network are sensitive to patients' neuropsychological impairment ¹⁵⁰.

Conversely, the role of EEG-based measures in assessing and monitoring motor impairment in ALS patients has not yet been fully established to date.

The aim of this study is to investigate the utility of source-reconstructed resting-state EEG measures, reflecting cortical motor networks function, in quantifying the severity

and progression of ALS motor symptoms. Considering the study by Dukic et al. (2019)¹⁵⁰, our first candidate-biomarker was the β -band power in the motor network as a measure of the overall activity of the cortico-spinal projections from the motor system to the muscles³⁸². This frequency-band is mostly expressed in the somatosensory and motor cortices³⁸³ and it is mainly the result of electrical activity in layer V^o of cortical networks³⁸⁴. Additionally, previous studies indicated that the EEG β -band power over the sensorimotor network differs in ALS patients when compared with healthy controls³⁷¹. Our alternative candidate-biomarkers were the average β -band imaginary coherence and the average δ -band amplitude envelope correlation in the motor network, which measure the level of synchrony and co-modulation, respectively, of the motor cortex with other brain regions¹⁵⁰. The present findings provide evidence that the source-localized β -band power and the β -band synchrony in the motor network correlate with several important clinical and functional motor scores; thus, proving suitable as neurophysiological biomarkers candidates for disease severity, progression and response to treatment for ALS patients.

5.2.2. Materials and Methods

Ethics, clinical assessment and patient cohort has been described above in section 5.1.2.

5.2.2.1. EEG acquisition

Three blocks of 2-minute resting-state EEG data were acquired per participant using the BioSemi® Active Two system (BioSemi B.V., Amsterdam, The Netherlands) at the Clinical Research Facility in St. James's Hospital, Dublin. The participant was seated comfortably in a chair and instructed to remain still and awake without performing any specific task. The recording was made in the resting-state "eyes open" state with participants' visual fixation directed to the letter **X** (6 cm × 8 cm) printed on a sheet of paper approximately 1 m away. Blinks and saccades were monitored continuously using surface electrooculography.

EEG data analysis

EEG data were source-analyzed using the LCMV (Linearly Constrained Minimum Variance) beamformer³⁸⁵ to obtain time-varying signals originating from the brain.

Ninety brain regions used in the analysis were based on the AAL (Automated Anatomical Labelling) atlas ³⁵¹, excluding the cerebellum.

Left/Right pre-central gyri were used to define the motor network and they were averaged using algebraic mean. For detailed information regarding EEG data processing and how spectral power, synchrony and co-modulation were estimated, see Dukic et al. (2019) ¹⁵⁰.

5.2.2.2. Statistical analysis

The Shapiro-Wilk test was used to assess data distributions. The β -power and β -synchrony in motor network as well as the LMN score, the fine motor function sub-score and the fine motor function-progression rate had a normal distribution. Partial correlation was used to assess relationships between parameters while controlling for age. Values of $p < 0.05$ were regarded as statistically significant.

5.2.3. Results

5.2.3.1. Patients

18 ALS patients were included for the statistical analysis in this section. Patients were prospectively recruited from the *National ALS Clinic* in Beaumont Hospital, Dublin. The mean age at the time of recording was 65.3 ± 10.8 years (mean $\pm$ standard deviation), while the mean disease duration from symptom onset was 30.7 ± 26.5 months. The mean degree of functional impairment, as defined by the ALSFRS-R, was 37.0 ± 4.7 points, while the degree of muscle weakness, as graded by the LMN score, was 75.9 ± 11.3 points. The mean UMN score was 4.1 ± 4.1 points. At the time of testing, only one patient was not on *Riluzole*. For an overview, see Table 5-1.

5.2.3.2. Correlation analysis

Table 5-2 summarizes the results from the correlation analysis between the EEG-based measures and the clinical scores. The details of each result were further reported in subsections that follow.

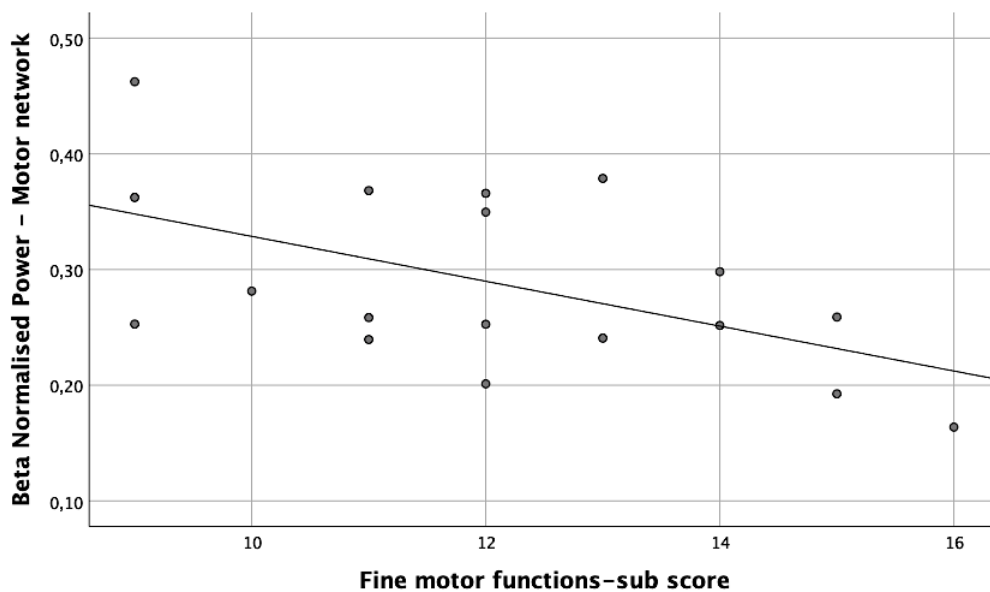
Table 5-2 Summary of correlation analysis results between EEG and clinical scores.

*FMF: Fine motor function; LMN: Lower motor neuron; UMN: Upper motor neuron; * statistically significant result (p value < 0.05).*

	ALSFRS-R score	ALSFRS-R Progression rate	FMF sub-score	FMF Progression rate	LMN score	UMN score
β -band power	-	-	-	0.576*	-	-
β -band synchrony	-	-	0.525*	-	0.555*	-
					0.747*	

Motor β -band power and ALSFRS-R scale

A strong, negative partial correlation was found between β -power values in the motor network and the fine motor functions sub-score of the ALSFRS-R ($r = -0.525$, $p = 0.03$), with high levels of β -power being associated with higher clinical impairment (Figure 5-3). No significant correlation has been described between β -power values and total ALSFRS-R score.



*(4 items of the ALSFRS-R scale): $r = -0.525$, $p = 0.03$.

Figure 5-3 The normalized β -band power in motor network shows a significant correlation with the fine motor functions sub-score

Motor β -band power and progression rate

We found a strong, positive partial correlation between β -band power in the motor and the fine motor functions - progression rate (Figure 5-4) ($r = 0.576$, $p = 0.016$). No

significant correlation was shown between β -power values and total ALSFRS-R progression rate.

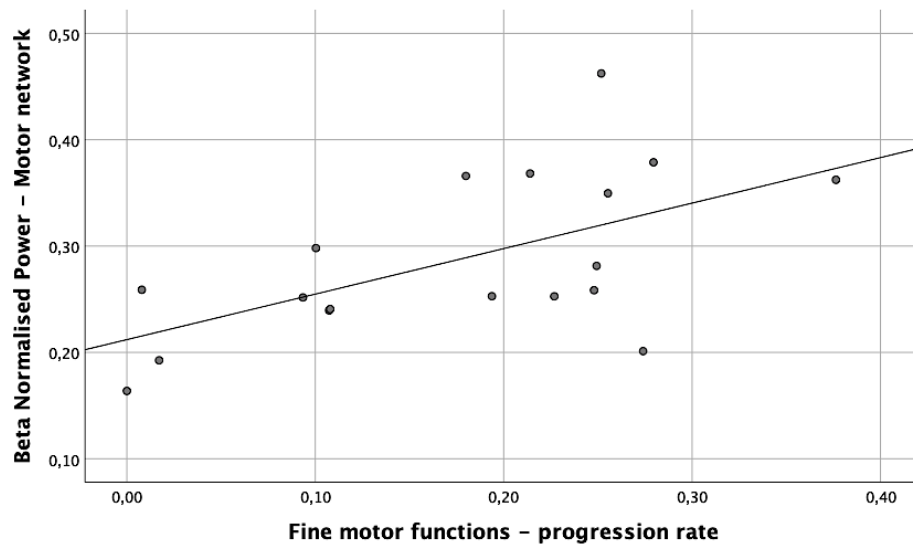


Figure 5-4 The normalized β -band power in motor network shows a significant correlation with the fine motor functions

Motor β -band power and LMN score

In addition, β -band power in the motor network strongly correlated with the burden of LMN impairment (Figure 5-5) ($r = -0.555$, $p = 0.021$). No interaction has been shown between the β -band power and the UMN-score.

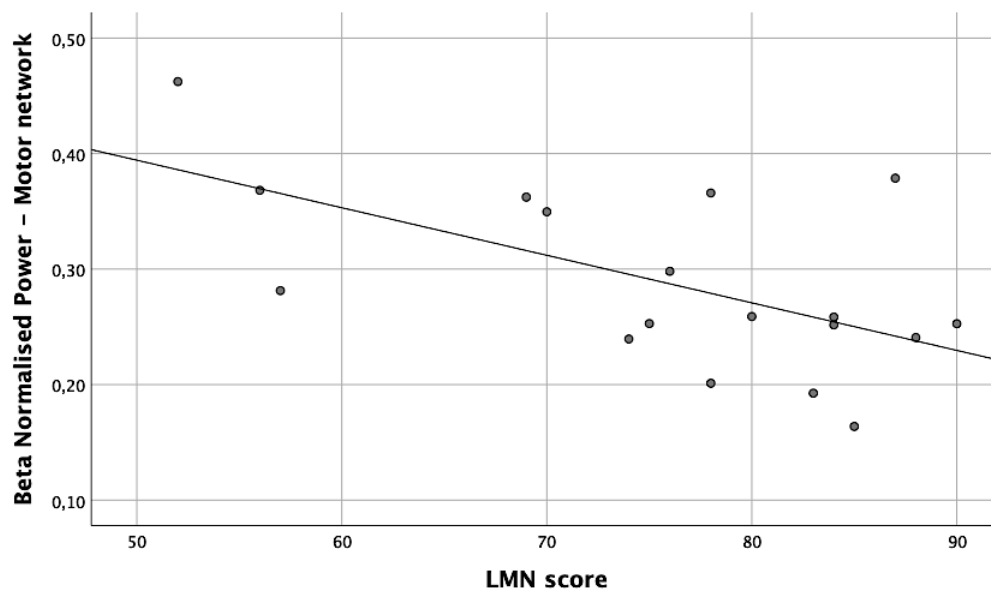


Figure 5-5 The normalized β -band power in motor network shows a significant correlation with LMN score

Motor β -band synchrony and LMN scores

Finally, we described a strong negative partial correlation between β -synchrony and the LMN score (Figure 5-6) ($r = -0.747$, $p = 0.001$). No other significant correlations were found between clinical measures and the candidate EEG measures of the motor network.

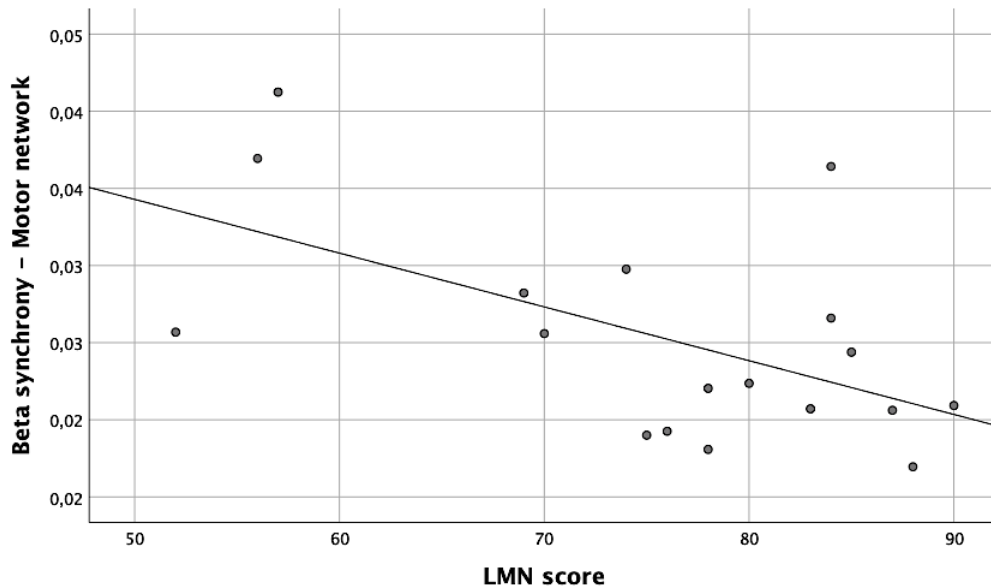


Figure 5-6 The β -synchrony shows a significant correlation with the LMN score

5.2.4. Discussion

In this cross-sectional study involving ALS patients in different stages of disease severity and progression, we show that source-localized EEG measures in the β -band reflect ALS motor impairment as assessed by clinical scales (ALSFRS-R, LMN/UMN scores and progression rate). These findings expand the previously described correlations between connectivity changes in cortical networks and patients' neuropsychological impairment, highlighting the use of EEG as a multi-dimensional approach that can capture different aspects of the disease¹⁵⁰. Our findings show that EEG-based measures have the potential to be developed as accessible and quantifiable biomarkers for severity and progression of motor disability, which remains a key unmet need of ALS therapeutic trials.

5.2.4.1. ALS and the neurophysiology of β -band oscillations

In the motor system, β -band oscillations are mostly generated by pyramidal neurons and GABA-A inhibitory interneurons in the V° cortical layer³⁸⁶. Within this layer, they reflect the functioning of GABA-A receptors, cellular gap junctions, K⁺ channels as well as that of the spiking somatostatin-positive inhibitory interneurons^{383, 384}.

Degeneration of layer V^o pyramidal cells and GABA-A parvalbumin positive interneurons has been extensively documented in ALS ³⁸⁷ and may underlay β -band EEG changes in ALS. In addition to this, a dysfunction of somatostatin-positive intracortical interneurons has been recently shown in a TDP-43 mouse model ³⁷⁷.

Evidences also suggest that the ascending sensory transmission coming from group Ia nerve fibers could modulate the cortical β -band allowing information to come back to the central nervous system from muscle spindles ³⁸⁸. In ALS, some patients exhibit subclinical sensory dysfunction ³⁸⁹ and a recent study showed an increased amplitude of cortical somatosensory-evoked potentials in ALS patients, suggesting that the sensory cortex is pathologically involved in the disease ³⁹⁰. Although it may be the case that sensory transmission could have contributed to our results (e.g., in the motor-related episodes or microstates in resting-state EEG), it is important to note that our participants were asked to sit still and relaxed without performing a specific task.

It is also noteworthy that EEG β -oscillations between prefrontal and motor cortex have been related to participant's attention ³⁸³: a study by Saleh et al³⁹¹ described an increased β -activity in the motor cortex during tasks demanding attention but not during motor execution. Computation modeling, in fact, suggested that β -oscillations are well suited for communication between distal areas ³⁹². This aspect is of particular interest in a disease where cognitive deficit ²¹ mapping and involvement of prefrontal cortices ³⁹³ have extensively been recognized as part of the clinical phenotype ^{8, 21, 358}.

5.2.4.2. *Correlating EEG changes with clinical scores in ALS patients*

Previous investigations of the motor network in ALS patients using source-localizing EEG techniques showed a reduced β -band power and a reduced β -band connectivity compared to healthy controls ^{150, 381}. It is, therefore, somewhat surprising that we have observed the opposite trend in our results from the correlation analyses. Namely, our findings suggest that patients with higher functional motor impairment will have higher EEG β -band power or connectivity. This result may be attributed to the complex mechanisms influencing β -band oscillations that could lead to its non-linear dysfunction in ALS ^{377, 384, 386}. Cortical excitability in ALS patients has been further characterized by transcranial magnetic stimulation (TMS) techniques showing a dysfunction of both inhibitory and

facilitatory intracortical circuits²⁰. These results suggest a non-linear trend of cortical excitability in ALS patients: while earlier disease stages are characterized by a hyper-excitable state of the motor cortex³¹⁰, later stages are defined by a progressively reduced excitability of the cortex, some reaching an unexcitable state³¹⁰. Thus, it may be argued that the progressive nonlinear impairment in cortical facilitatory and inhibitory networks captured using TMS may well explain our EEG correlations.

Regardless, the strong negative correlation between the β -band synchrony and the LMN-score coupled with the previous discovered relation with the ALSFRS-R score suggests that this parameter is sensitive not only to cortical impairment but that it might reflect the state of the entire motor system including the midbrain, LMNs, peripheral nerves and muscles. Additional analysis of longitudinal data is warranted to further shed light in this direction and to evaluate the changes of these measures over the course of disease.

Finally, we did not observe an interaction between EEG β -band power and the total ALSFRS-r score. This finding can be expected considering that the clinical scale encompasses evaluation of different functions (bulbar, respiratory, fine and gross motor actions) with different sensitivities and representations in the nervous system³³³. In addition to this, we did not observe any significant correlations with the UMN score: in this case, it should be considered that clinical signs of UMN impairment are not easily appreciated in patients affected by a high burden of LMN degeneration⁴¹.

5.2.4.3. *Neuro-electric biomarkers for measuring disease severity*

Our findings coupled with the link between the β -band oscillations and cortical dysfunction pave the way to a potential use of these measures as a prognostic, pharmacodynamic and disease progression biomarkers useful in ALS irrespective of its etiology. Despite the relentless course of disease progression, a proper quantification of differences among patients is still lacking, which then leads to a significant reduction of success in ALS therapy. ALS clinical trials, in fact, mostly rely on macroscopic outcome measures, such as survival or the progression rate of the ALSFRS-R score - measurements requiring a long period to determine the success or failure of a tested drug. A specific test sensitive to motor system activity or to the downstream loss of neuromuscular integrity, might shorten this period and might

help deciding whether to continue with a particular drug or switch to a new candidate.

EEG measures capturing the complex heterogeneous profiles of ALS patients would also allow more efficient stratification of patients in clinical trials. Although longitudinal data are still missing, in a clinical trial setting the EEG β -band would be an ideal outcome measure as it seems to modify with advanced motor impairment (as assessed by the fine motor function sub-score and LMN score). Consequently, an experimental therapeutic that stabilizes β -band power in the motor network over time would provide evidence of an underlying biological effect of the treatment. Moreover, the important finding regarding the correlation between EEG β -power and patients' motor symptoms-progression rate suggest that this measure might also have a prognostic value. Finally, considering the previous TMS findings of an early cortical hyperexcitability in ALS patients ³¹⁰ and taking into account the increased cerebral functional connectivity in asymptomatic genetic mutation carriers described with MEG³⁹⁴, further studies could unravel if the present EEG β -band alterations may also be observed early in pre-clinical disease stages and, therefore, if they can aid in reducing the diagnostic delay of patients with rapid deterioration. In conclusion, this study presents a quantifiable and easily accessible neurophysiological EEG measure, that is a promising biomarker-candidate useful in clinical trials, considering its ability to quantify motor disability and disease progression of ALS patients.

5.2.5. Limitations

Limitations in our study include the heterogeneity of ALS. Given the relatively small numbers studied, it was not possible to subclassify patients either by progression rate or stage of disease. I acknowledge that both aspects could have an impact on the present findings. This issue needs to be further studied in greater detail, using other supplementary measurements of the motor cortex and its connectivity in order to elucidate disease heterogeneity and relationships to survival. However, it is important to note that the findings were obtained despite such heterogeneity.

Secondly, we only considered clinical data of the upper limbs. Although it seems to be an important limitation, it is worth to note that representation of the leg in the

cortical homunculus is mainly located in the mesial surface of the frontal cortex and it is exceedingly small compared to that of the face, hand and upper limb ³⁹⁵.

Thirdly, the number of patients examined was small; notwithstanding, the results seem to be robust enough to draw a trend and represent strong enough evidence for a larger study. A larger and longitudinal study may also help determine the natural history of cortical dysfunction in ALS that may include a progression towards an unexcitable motor cortex in advanced stages of disease with consequent hardly predictable β -band abnormalities. Our cohort, in fact, did not include many patients in the advanced stage of the disease, which may also have affected the results.

Lastly, in the power analysis we did not consider the absolute value of EEG β -band in the motor network but its relative weight when compared to the total power. Therefore, variations in other frequencies' characteristics might also have given a contribution to our results and these aspects need to be addressed separately.

5.2.6. Conclusions

The biological properties of β -band oscillations coupled with new techniques of source-reconstructing EEG signals make the EEG β -power in the motor network a promising and non-invasive tool to monitor ALS progression. The intrinsic relation with the disease pathophysiology makes it a promising biomarker with diagnostic and therapeutic monitoring objectives. This measure can objectively quantify the overall (motor) dysfunction, which can be subsequently complemented with other (neurophysiological) biomarkers of dysfunction in specific networks for phenotyping and stratification aims.

6. Chapter 6: Poliomyelitis Results

The work described in this section has been published in the peer-reviewed journal *Clinical Neurophysiology* ³⁹⁶ see (Appendix 11.1):

Coffey A, Bista S, et al. Altered supraspinal motor networks in survivors of poliomyelitis: A cortico-muscular coherence study. *Clin Neurophysiol.* 2021 Jan;132(1):106-113.

This section contains all figures and tables as well as the results and discussion section text in full form from this publication. Introduction and methods section text from this publication have been abbreviated in this chapter to avoid repetition of the contents of previous chapters.

6.1. Corticomuscular Coherence in Polio

6.1.1. Introduction

The establishment of vaccination programmes against the polio virus in the 1950s, has rapidly reduced the occurrences of epidemics in the western world.

While acute polio no longer poses a risk in large parts of the world, there continues to be a paucity of information regarding our understanding of the mechanisms that allow remaining lower motor neurons to expand their innervation territories. Several studies give clues to the peripheral and interestingly central contributions to motor networks in Polio patients.

Poliomyelitis is characterized by anterior horn cell loss. Post-Polio Syndrome (PPS) itself was a term conceived by the very patients it affected to detail an array of unexplained symptoms they experienced in later years ^{178, 397}. PPS is reported to present as new and progressive asymmetrical muscle weakness, fatigue, reduced endurance, myalgia and muscular atrophy ¹⁷⁴.

While Post-polio syndrome is a phenomenon that is characterised by new muscle weakness and fatigability occurring years after the initial acute phase. An estimated figure of 15- 80 % of polio survivors goes on to develop PPS, this variability exists in the literature largely due to population differences being studied and criteria used ¹⁶⁶. The most accepted theory around the pathogenesis of PPS relates to an observation made by Wiechers and Hubell in 1981 ¹⁷⁹. They had noted that degeneration of the

distally enlarged motor units that had formed after the acute polio exposure reinnervated through collateral axonal sprouting after the acute infection, potentially occurring in response to denervation giving rise to enlarged motor units ^{166, 179}. PPS is thought to arise from an equilibrium imbalance – through which a dynamic balance is struck by the addition and loss of muscle fibres over time. The decompensation of this equilibrium is thought to give rise to PPS. Motor neurons are no longer able to maintain the axonal sprouts resulting in dysfunction/degeneration and giving rise to the characteristic new weakness that defines PPS^{166, 398}. It is thought that the overuse of functional motor units induces structural alterations in patients with PPS ^{399, 400}. Cellular contributors to muscle fatigue and myalgia are most likely due to adaptations seen in the muscle tissue, such as alteration from type II (fast) to type I (slow) muscle fibre ³⁹⁹, changes in contractile properties ^{401, 402}, and muscle hypertrophy ⁴⁰³.

Interestingly, in a study carried out in assessing fatigue in PPS patients, their findings suggested that motor fatigue did not correlate with subjective fatigue and was found not to be associated with mobility ⁴⁰⁴. There was no significant difference identified in lower limb fatigue ⁴⁰⁴. The study showed little muscle strength reduction over time that could not be accounted for by the physiological aging process ^{404, 405}.

Although the neurological manifestations of poliomyelitis are generally considered to be confined to the anterior horn cell, extra-motor features have been described in PPS. Studies have shown evidence that there is peripheral as well as central contributions to fatigue in PPS⁴⁰⁶⁻⁴⁰⁸. These studies suggest that the effects of polio extend beyond the lower motor neurons to affect central pathways for movement, either directly or as a compensatory process following the loss of motor neurons^{406, 408}. There are suggestive findings that describe encephalitic involvement in the initial disease process ^{171, 409} with gliosis of white matter tracts ⁴¹⁰, punctate lesions of grey matter within motor midbrain areas (particularly the reticular formation) ^{171, 409, 410} and lesions of the motor cortex ^{171, 409} have all been documented. It is believed that the disease phenotype of paralytic poliomyelitis (PPM) is a result of viral-induced motor neuron cell death ^{411, 412}. Studies have shown that the polio virus can trigger apoptosis through the mitochondrion-dependent intrinsic pathway ⁴¹³. The polio virus has several mechanisms of blocking cell death, which can defer host cell apoptosis,

and potentially act as a pro-viral tool to allow the virus more time to replicate and assemble new infectious particles ⁴¹¹.

Onset of cortical function in humans seems to occur at differing stages for different cortical regions ⁴¹⁴. For example, functional development of the prefrontal cortex is described as a more gradual process than the visual cortex ⁴¹⁴. Polio often occurs in infancy in those affected, therefore, the developing cortex has the ability to reorganise and can result in compensatory neuronal network changes compared to those affected in late childhood⁴¹⁵. The striking CMC pattern differences between those affected in infancy compared with those affected in childhood is likely to reflect the differences in compensatory patterns during the development of the motor system. These synaptic connections from the motor cortex to spinal α -motor neurons and interneurons are generally established early in humans compared to primates. Early corticospinal innervation was demonstrated by a study published in 2000 ⁴¹⁶, showing that in humans the cortex is intimately involved in the development of the spinal motor centre reflecting the uniquely dominant role of the corticomotoneuronal system in human control of movement ⁴¹⁶.

To assess the transcortical and the corticofugal pathways in PPS, cortico-muscular coherence was used in this study. Coherence in PPS was used for the assessment and quantification of pathway degeneration. Coherence is a mathematical approach to assessing the physiological phenomenon of synchrony between two neural signals³⁰³. Cortico-muscular coherence indicates the effective relay of cortical oscillations along the corticospinal tract to the eventual trains of motor unit action potentials^{297, 329}. Significant coherence is explained by an adequate number of motor neurons receiving linear transmission of synaptic input³⁰³. (Table 6-1)

CMC represents a track between the primary motor cortex and its corresponding muscles ^{297, 303, 354}. CMC at beta frequency occurs during weak to moderate isometric contractions of the forearm and reduces with the onset of movement ²⁹⁵. It is thought that the ability to detect these type of CMC patterns develops during childhood and is related to the maturation of intra-cortical as well as cortico-muscular connectivity ⁴¹⁷. CMC most likely mirrors the conduction patterns of the fast pyramidal pathways representing a marker of the integrity of the pyramidal system

⁴¹⁸. Accordingly, CMC plays a crucial role for sensorimotor integration representing a key mechanism for appropriate motor control ²⁹⁵. Changes of task-related brain processes are well documented during the healthy aging process ⁴¹⁸. Semmler et al. have found that in older participants motor-unit coherence during isometric contraction of the hand changed towards lower frequencies (i.e., 5–9 and 12– 13 Hz) ⁴¹⁹. The literature implies that the oscillatory input to motor neurons changes during healthy aging even during apparently simple motor tasks. These alterations have been related to a reduction in steadiness during sustained contraction seen in older participants ⁴¹⁹.

It has been shown that age is a factor that affects CMC. This is evident in studies carried out in children which showed larger distributions of cortical networks compared to adults ⁴²⁰. Studies comparing CMC patterns in infants and adults showed contrasting CMC patterns during motor development compared to adults ⁴¹⁷. Based on these findings , it is possible that survivors of poliomyelitis may have experienced changes in developing motor networks both peripherally and also centrally.

We hypothesize there is an adaptive cortical network change that may be present in patients with a history of poliomyelitis.

The aim of this work was to investigate by Cortico-muscular Coherence(CMC) the motor pathway disruption and cortical reorganisation in adult patients with a history of paralytic poliomyelitis, to determine the presence and neurophysiological characteristics of long-term reorganisation of cortical networks.

Table 6-1 Frequency bands and their physiological correlates described in literature in healthy controls

Band	Frequency	Physiological Correlates
Alpha Band	8-12Hz	Physiological tremor ³⁰²
Beta Band	15-30Hz	Sustained muscle contraction ³⁰³
Gamma Band	30-60Hz	Dynamic force output ³⁰⁴

6.1.2. Methods

6.1.2.1. Ethics

The study was approved by the *Tallaght University Hospital / St. James's Hospital Joint Research Ethics Committee - Dublin* [REC Reference: 2019-05 List 17 (01)] as described in section 4.1.

Inclusion Criteria

All patients over the age of 18 with a verified diagnosis of poliomyelitis in childhood, and all had supportive clinical and electromyographic findings.

Exclusion Criteria

Patients with a history of major head trauma or other neurological conditions that could affect cognition, alcohol dependence syndrome, current use of neuroleptic medications or high-dose psychoactive medication were excluded. Those with diabetes mellitus, a history of cerebrovascular disease, and those with neuropathy from other causes were also excluded.

6.1.2.2. Clinical assessment

25 Patients were prospectively recruited in this cross-sectional study between June 2017-November 2019 through the national clinic for polio survivors at Beaumont Hospital.

On the day of EEG recording all patients underwent an extensive clinical assessment. Disease duration from symptom onset and site of disease onset were recorded. Muscle strength was assessed using the Medical Research Council (*MRC*) score in 9 bilateral (i.e., 18) upper limb muscles, including deltoid, triceps, biceps, wrist flexors and extensors, fingers flexors and extensors, and abductors of the index fingers and thumbs. The degree of clinical lower motor neuron (LMN) involvement in the upper limbs was graded by a LMN score³⁶⁶ from these MRC scores, which ranged from 90 (absent LMN signs) to 0 (severe LMN signs).

Handedness was assessed in all participants with the Edinburgh Handedness Inventory⁴²¹.

6.1.2.3. *Experimental Paradigm and Data acquisition*

Assessment was conducted in the same manner for the patients and control groups. EEG/EMG acquisition and the employed experimental paradigm are described in detail in the methods section 4.4.

6.1.2.4. *Data Analysis*

EEG/EMG data analysis was blinded to clinical assessments. Five EEG channels (Cz, Pz, C4, Fz, C3) and 3 EMG channels (APB, FDI, FPB) were chosen prior to the analysis of cortico-muscular coherence (CMC). This selection was based on the biomechanical involvement of the muscles in the pincer grip task ³⁶⁷, and the suitability of the EEG and EMG channels for assessing CMC ³⁶⁸. A time window/epoch duration of 4s (starting 1s after the visual cue) was chosen for analysis; data epochs where the target force was not correctly achieved were excluded. The raw EEG data was (re-)referenced using (small) surface Laplacian spatial filter ^{369, 370} which is a spatial filter for removing spurious signal components in EEG channels, and EMG data (signal amplitude) were normalized with respect to the EMG amplitude during 100 % MVC. EEG/EMG data were filtered between 1-100Hz using a dual-pass 4th order Butterworth bandpass filter. This was followed by a 50Hz Discrete Fourier Transform (DFT) filter in the FieldTrip toolbox to remove power line noise. An automatic artefact detection and rejection was performed using FieldTrip toolbox ³⁴⁰ to remove eyeblinks, muscle, and electrode jump artefacts from EEG signals. The auto-spectrum of each EEG/EMG signal, and cross-spectrum between all combinations of EEG-EMG signals (frequency resolution 1Hz, bandwidth 2-100Hz) was calculated using FieldTrip toolbox (Hanning taper and frequency smoothing at 1Hz). The auto- and cross-spectra at each frequency (2-100Hz) was converted into 8 band values- delta (2-4Hz), theta (5-7Hz), lower alpha (8-10Hz), higher alpha (11-13Hz), lower beta (14-20Hz), higher beta (21-30Hz), lower gamma (31-47Hz), and higher gamma (53-97Hz), excluding the 48-52Hz range to avoid mains power noise. The formation of band-specific values was carried out by taking Spatial Median (a variation of the median operator for complex-valued spectra, chosen and preferred over the algebraic averaging to provide robustness against outlier values) ^{346, 347} of the spectra at corresponding frequencies. The spectral coherence (cortico-muscular coherence in this study) was obtained by normalizing the cross-spectrum by the respective auto-spectra ³⁴⁷.

The selection of the parameters and methods for signal processing (e.g. band-specific analysis and the use of non-parametric methods) was based on our previous EEG studies ^{150, 151} that provided robust estimations not sensitive to outliers or observations in individual subjects ³⁷¹.

6.1.2.5. *Statistics*

Participant-level statistics were calculated using one-sample non-parametric rank statistics for spectral coherence ³⁴⁷. This method gave individual p-values for spectral cortico-muscular coherence in each frequency band for both patients and control groups (Fig.6-1). Stouffer's method ^{352, 353} was used to combine individual p values to derive group average p value. This procedure is equivalent to the pooled coherence analysis ³⁵⁴.

Correction for multiple comparisons was performed using the adaptive false discovery rate at $q = 0.05$ ³⁵⁵, which was applied by correcting the p-values in the coherence spectra. Negative logarithm of p-values, i.e. $-\log_{10}(p)$, was used to visualize cortico-muscular coherence. The band-specific values of coherence, expressed in $-\log_{10}(p)$, was used to represent the values for all of the frequencies in that frequency band.

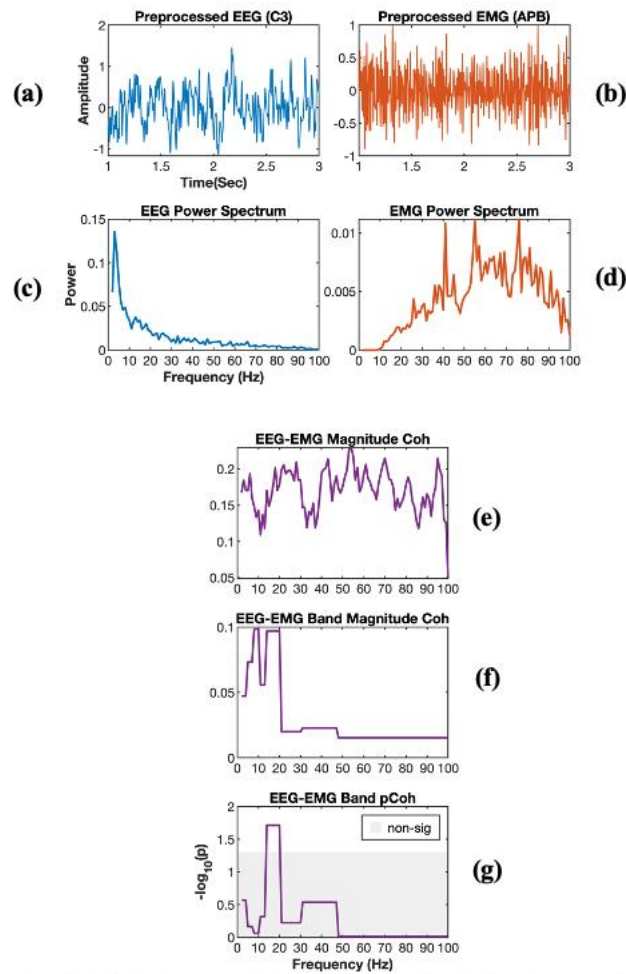


Figure 6-1 Illustration of methodological steps carried out to calculate banded cortico-muscular coherence (pCoh), and its classical counterpart

*data from a healthy control, **(a)** segment of a trial of pre-processed EEG data, obtained after surface Laplacian referencing, bandpass filtering, Discrete Fourier Transform (DFT) filtering and automatic artefact rejection of raw EEG data, **(b)** segment of a trial of pre-processed sEMG data, obtained after bandpass filtering, DFT filtering, and Maximum voluntary contraction (MVC) normalization of raw sEMG data, **(c, d)** EEG/EMG power spectrum obtained by Fourier transform of pre-processed data, 1 Hz frequency smoothing using Hanning taper, and 1Hz frequency resolution, from all of the trials **(e)** Classical cortico-muscular magnitude coherence (Coh) showing coherence value at each frequency, **(f)** Banded cortico-muscular magnitude coherence (Coh) showing coherence value for 8 frequency bands obtained by using spatial median operation, **(g)** The p-value of banded cortico-muscular coherence in $-\log_{10}(p)$ scale (pCoh) obtained from banded spectral coherence, which is used for assessing the significance and visualising the results.

6.1.3. Results- CMC in Poliomyelitis

Patients' Clinical Profile

A total of 25 patients affected by Poliomyelitis were successfully recruited from a cohort Polio clinic based in Beaumont hospital, Dublin (see Tables 6-2 and 6-3/4).

One patient was subsequently excluded from analysis as recording of the motor task was carried out using his left hand, due to inadequate strength in right hand. The analysed patient group included 17 female and 7 male patients (mean age of $67.04 \pm$

6.8 (Standard deviation), 22 right hand dominant). From this group, 8 patients suffered poliomyelitis in first 24 months of life with 16 contracting polio after 24 months of age. Muscle weakness, graded by the LMN score, was 85.2 ± 6.6 points. A total of 11 healthy controls were successfully recruited (mean age of 61.09 ± 14.8 standard deviation).

Table 6-2 Clinical and demographic data of analysed patients.

	Patient Group	Controls
Gender (F/M)	17/7	4/7
Average age at recording (F/M)	68.5 ± 3.6 / 63.5 ± 10.8	64.7 ± 4.2 / 59 ± 18.5
Onset < 24 months	8/16	-
LMN score (max 90)	85.2 ± 6.6	90

Table 6-3 Clinical and demographic data of patient sub-groups

	Infant-onset (<24months)	Childhood-onset (>24months)
Number of patients	8	16
Average age of onset (in yrs)	0.97 ± 0.5	4.12 ± 2.4
LMN score	86.6 ± 4.1	84.5 ± 7.6

Table 6-4 Demographic Characteristics of Patients

Case No	Age of Onset (yrs)	Sex	Affected limb	LMN Score
1	0.75	F	R lower limb	90
2	1.5	F	R lower limb	85
3	4	M	L lower limb	90
4	2	M	R lower limb	90
5	3	F	L lower limb	89
6	0.75	M	L lower limb	90
7	2	F	L lower limb	86
8	1.8	F	R lower limb	89
9	9	F	L lower limb	90
10	2	F	R lower limb	72
11	3	M	R lower limb	88
12	2	F	L lower limb	90
13	1.25	F	L lower limb	90
14	0.5	M	R lower limb	81
15	9	F	L upper limb	72
16	6	F	R upper limb	67
17	4	F	L lower limb	90
18	2.5	F	R lower limb	90
19	0.75	M	R lower limb	88
20	2	F	R lower limb	90
21	3	F	L lower limb	82
22	0.5	M	R lower limb	80
23	4	F	R lower limb	83
24	7	F	R lower limb	84

6.1.3.1. Abnormal cortico-muscular coherence in the PPS patient group

Cortico-muscular coherence (CMC), during steady low force isometric pincer grip (Fig. 6-3), was calculated between intrinsic hand muscles (using bipolar EMG) and surface EEG electrodes over scalp. In this context, we identified patterns of CMC in the PPS group that differed from those of the controls (see Table 6-1). Specifically,

the CMC in the patient group did not show the typical beta-band CMC over contralateral motor area (as intended by the task selection), and the CMC across the 5 tested EEG electrodes, 3 muscles, and the frequency bands were scattered and inconsistent (Fig. 6-2). These null findings were in accordance with expectations from our pilot study in controls, as well as the anticipation of typical beta-band cortico-muscular coherence primarily over primary motor cortex, C3 electrode (seen in low force sustained muscle contraction ^{297, 298, 303}).

In the PPS group the CMC was statistically significant ($p < 0.05$, $q = 0.05$) at the group level (Fig. 6-3) and appeared at frequencies different from the commonly observed and expected beta (14- 30Hz) frequency bands. Instead, the significant CMC appeared consistently in the low gamma (31-47Hz) frequency band across several EEG-EMG channels (see Fig.6-4). Importantly, this abnormal low gamma-band coherence was observed not only over primary motor (C3 and Cz, and C4) areas, but also over parietal and frontal areas (Fz and Pz). The abnormal CMC appeared in other (e.g. alpha) frequency bands but was less consistently across EEG-EMG channels. These CMC patterns differed from the control cohort in frequency (beta vs. low gamma) and location (contralateral motor C3 vs Frontal Fz and Parietal Pz) ^{303, 372}, (Fig. 6-3).

The pathological gamma-band CMC was a consistent finding and was observed at both frontal and parietal electrodes and in both FDI and FPB muscles (Fig. 6-4 and 6-3).

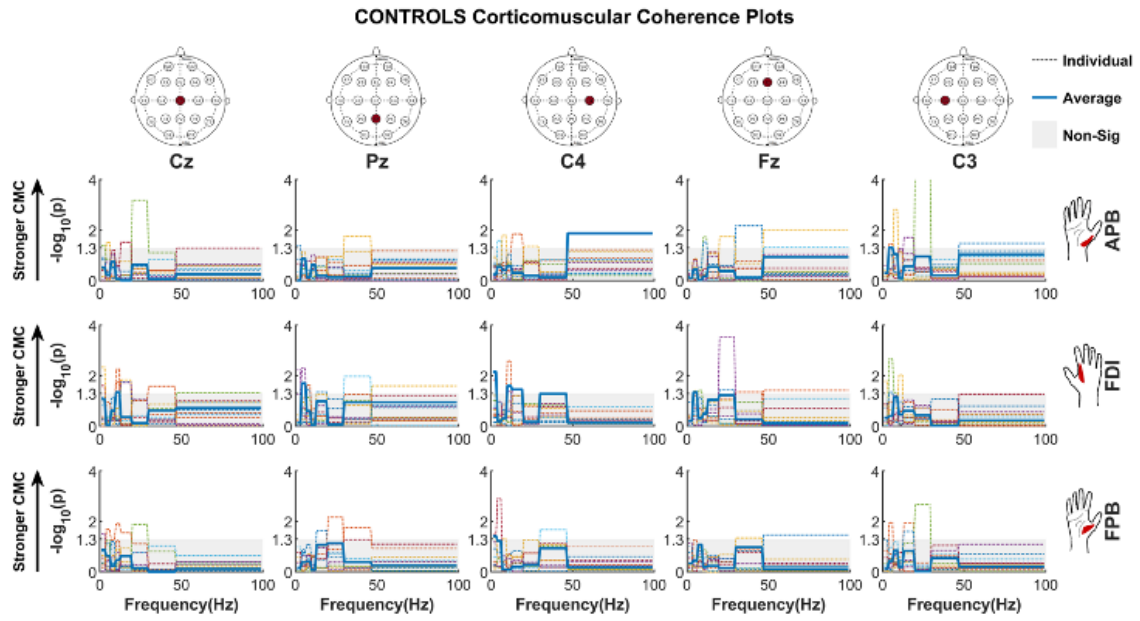


Figure 6-2 The cortico-muscular coherence spectra in the healthy control group, showing the significant values at individual and group level.

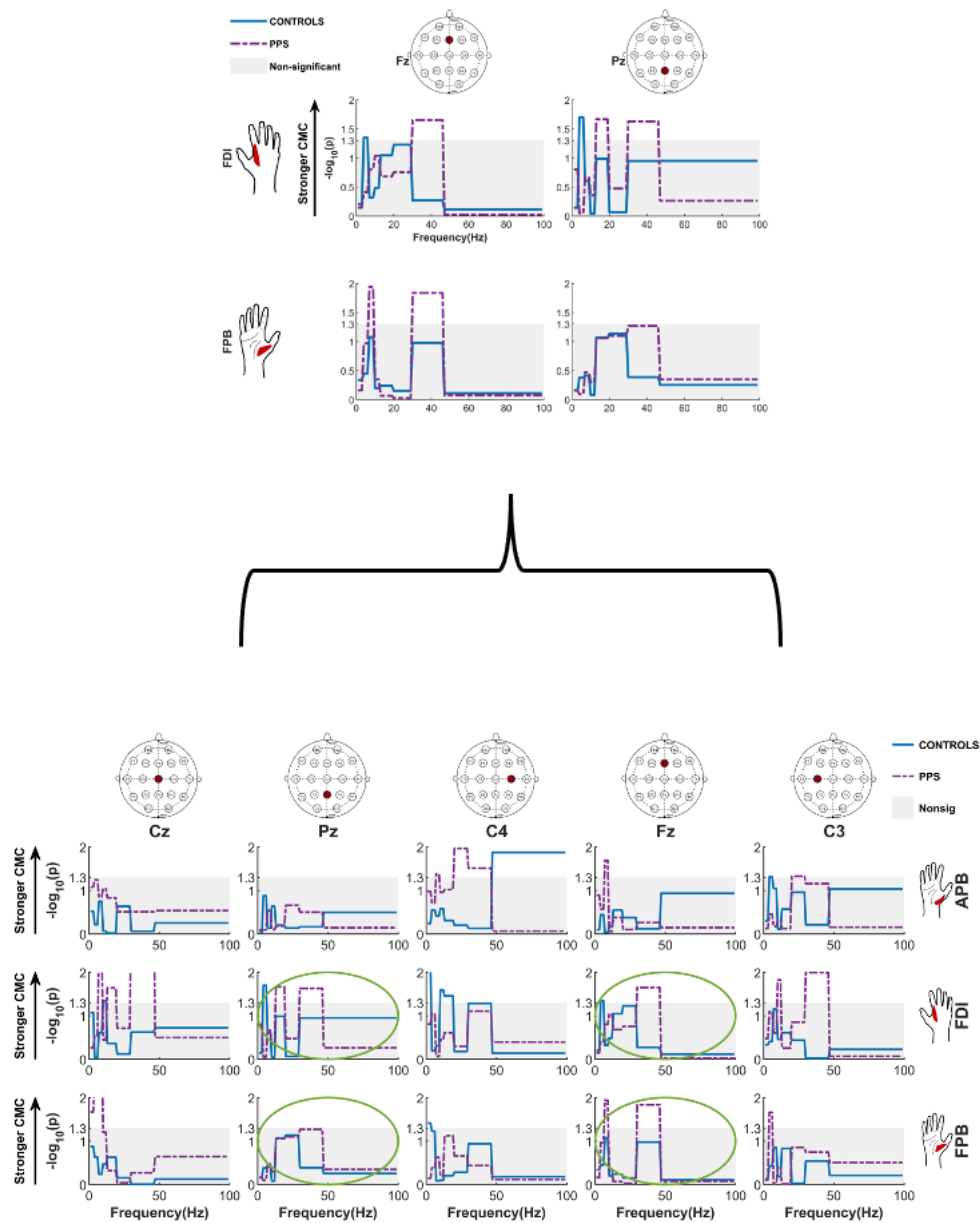


Figure 6-3 Patients with Post-Polio Syndrome (PPS) show abnormal group-level Cortico-Muscular (EEG-EMG) Coherence in non-primary motor area.

The cortico-muscular coherence spectra, expressed in $-\log_{10}(p)$, show the synchrony between the EEG electrodes (over the frontal area, Fz, and parietal area, Pz) and EMG (First Dorsal Interosseous, FDI; and Flexor Pollicis Brevis, FPB, muscles) in different frequency bands. The lower values show less synchrony, whereas the higher value show higher EEG-EMG synchrony. The shaded area corresponds to the non-significant values at $\alpha=0.05$ threshold for p -values (corrected for multiple comparison using FDR at $q = 0.05$). Notice the dominant abnormal coherence in PPS coherence in low gamma-band (30 - 47Hz) which is present in both muscles and in both Pz and Fz electrodes.

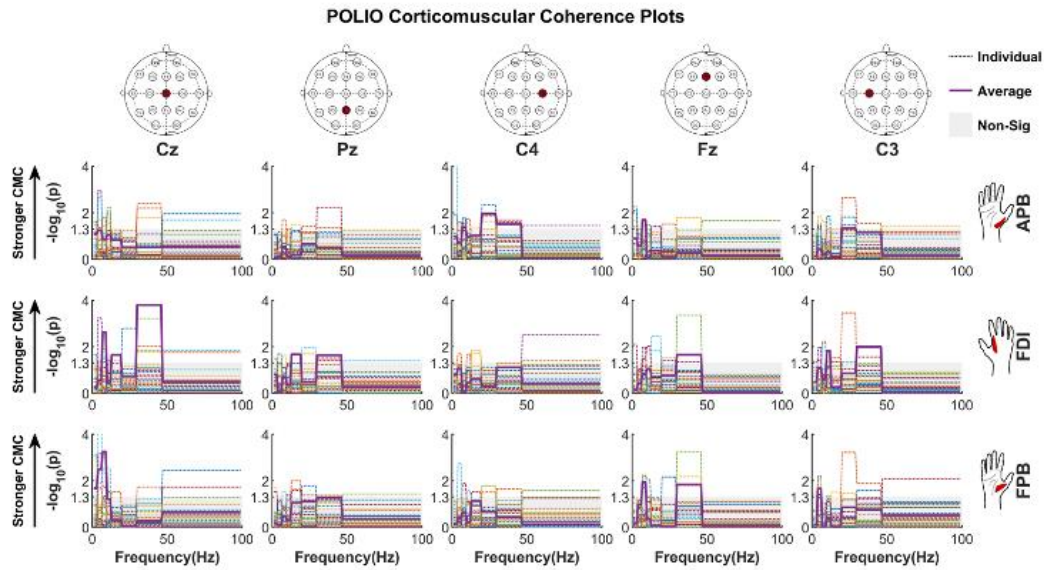


Figure 6-4 The cortico-muscular coherence spectra in the polio patient group, showing the significant values at individual and group-level.

6.1.3.2. Polio Sub-groups

The analysis of CMC in separate patient subgroups, revealed that those affected by polio virus in infancy (define as <24 months), showed different CMC patterns (Fig. 6-5) compared with those who developed paralysis in childhood or adolescence(>24 months). The significant CMC patterns ($p < 0.05$, $q = 0.05$) observed at the group level (especially the increased gamma-band CMC over Pz and Fz) was also observed in the childhood and infant groups, at comparable levels of strengths or with only one subgroup showing a predominant abnormal pattern. Furthermore, abnormal increases in alpha and low-beta frequency band (over frontal areas) were observed in the infant grouping, which was not observed at the group level.

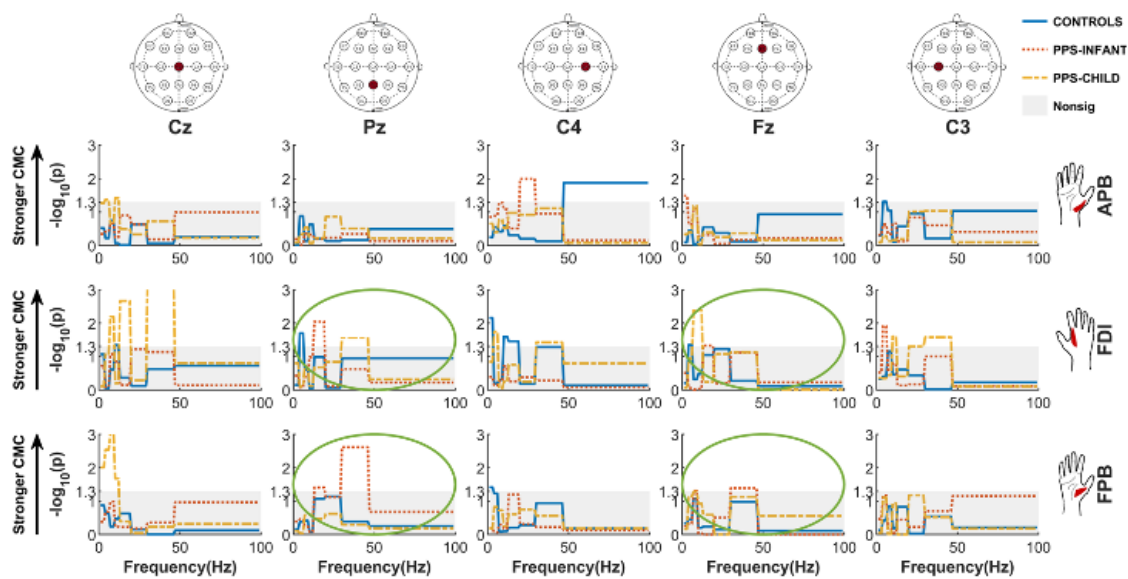


Figure 6-5 Patient subgroups with onsets in infancy (<24 months) vs. childhood (>24 months) show different abnormal Cortico-Muscular (EEG-EMG) Coherence patterns. The subgroups show subgroup-specific abnormal patterns. Notice the similarity to the group-level findings as well as differential trends in the 2 subgroups. The shaded area corresponds to the non-significant values at $\alpha=0.05$ threshold for p-values (corrected for multiple comparison using FDR at $q=0.05$).

6.1.4. Discussion

This study provides robust neurophysiologic evidence of extensive supraspinal changes in those affected by poliomyelitis in childhood. Frequency bands and their physiological correlates have been described in literature. Beta band coherence appears during weak tonic contraction especially when directed towards a motor task²⁹⁴. Physiologically Gamma band coherence is more apparent on strong muscle contraction²⁹⁵, while Alpha band coherence has been described in resting physiological tremor³⁰². The presence of all of these frequency bands in unexpected cortical areas within the patient group implies the presence of a disrupted central-peripheral network.

These abnormal patterns, with increases in the gamma band, were consistent at group level in 24 patients despite major differences and heterogeneity in clinical disability (affected limb), and age of first polio diagnosis. These changes most likely reflect functional re-organisation of the central-peripheral network, possibly as a compensatory response to continuous remodelling of the motor units⁴²².

Our observations are consistent with recent TMS studies which show enlarged motor maps in the cortical areas of Motor Evoked Potentials (MEPs) in adult patients who

contracted polio prior to reaching 18 months of age ⁴²². Our findings are also congruent with existing knowledge of cortical neurophysiology ⁴²³. The role of sensory feedback loops in regulating the motor cortex output has been well described ^{424, 425}, and it is plausible that changes in the normal proprioceptive feedback due to muscle spindle dysfunction (as a consequent of both alpha and gamma motor neuron degeneration) contribute to the observed CMC changes ⁴²². The observed CMC pattern may also be influenced by functional changes in projections from cortical layers to spinal cord, and transcortical and the corticofugal pathways, although multi-modal or source analysis studies will be required to further elucidate the underlying neuroanatomical and neurophysiological origins of the abnormal CMC.

The observed trend toward differences in CMC patterns between infancy and childhood onset patients are likely to reflect differences in compensatory patterns that occur as the neuroaxis matures. During development, the human cortex is closely linked to the spinal motor centre, reflecting the uniquely dominant role of the corticomotoneuronal system in human control of movement ^{416, 426, 427}. Early assaults to the anterior horn cell are more likely to radically alter motor circuitry, as formation and elimination of synapses occurs during infancy and childhood ⁴¹⁴. This “heterochronous synaptogenesis” ⁴²⁰ could differentially influence the development of a compensatory processes following anterior horn cell injury in infancy and later in life respectively.

These patterns imply an age differentiation between the infant vs childhood group with emerging CMC patterns seen. The infant subgroup showed evidence of increased connectivity patterns in the frontal region along the beta and gamma frequency bands when compared to the childhood group which showed higher beta-band frequencies in the parietal region. It was once believed, before the widespread use of MRI, that biological development of the human brain was essentially complete by 6 years of age ⁴²⁸. Since the onset of MRI as a safe technique in paediatric imaging, it has revealed dramatic changes in the brain tissue of the developing infant, often noting a postnatal brain growth spurt ⁴²⁸. These MRI signal changes have been shown to reflect alterations in tissue chemistry that mark the proliferation of oligodendrocytes and the deposition of myelin ^{429, 430}, while also exposing the timing and anatomical distribution of this process. It has been noted that even visual

appearance alone of the brain on MRI, changes a lot over the first 2 years of life, mirroring an orderly pattern of myelination in white matter regions ⁴²⁸. As such when considering cut-off ages for the sub-groups in-terms of “age of onset” evidence suggested that using 24months would best be used to assess potential adaptations the cortex may undergo due to a downstream assault.

This observation of alterations in corticomotor circuitry following poliomyelitis has implications for other conditions, notably spinal muscular atrophy (SMA), for which quantitative biomarkers of drug efficacy are urgently required. In conditions such as SMA, for example, a consistent abnormal CMC measure in (adult) SMA, could be used as a potential biomarker and used to track abnormal network function. Such biomarkers can show network-level changes on the lower motor neurons that may come about over time after the administration of a disease modifying agent and be a marker for efficacy in clinical trials. Indeed, recent imaging studies have suggested the presence of altered cortical connectivity in SMA patients ³²⁸, implying that the CMC changes observed may not be specific to polio patients, but rather a more generic compensatory physiologic reorganization of cortical circuitry following damage to the lower motor neuron.

6.2. Resting State in poliomyelitis

6.2.1. Introduction

The pathophysiology of many neurodegenerative conditions are often linked to neurophysiological impairments which can be detected before the clinical manifestation of symptoms ⁴³¹. With improvements in computational power in neurophysiological techniques, we continue to gain more knowledge into how the brain functions in health, and how function is disrupted in the face of disease. Electroencephalography (EEG) is a powerful method that can also be used to examine network activity across the cortex in health and disease. EEG is inexpensive, and enables non-invasive assessment of neural activity resulting from both local and long range neural coordination ⁴³². In addition to low cost, EEG has millisecond temporal resolution, which is finer than other neuroimaging modalities such as fMRI.

Electroencephalography (EEG) allows us to follow the neural dynamics with accurate temporal resolution, and as such is the most suited to non-invasively track brain interactions ⁴³³.

Resting state EEG are often used to explore functional brain connectivity in the absence of specified tasks ⁴³¹. Resting state studies in various neurodegenerative conditions have shown distinguishing features between various disease conditions. In ALS increased connectivity throughout the cortex has been shown, including increased median absolute coherence in theta and gamma band frequencies over prefrontal areas, accompanied by decreased gamma band synchrony for some prefrontal electrodes ¹⁵¹. While in Parkinson's disease decreased connectivity in frontoparietal coherence in alpha bands has been described with an association with early impairment of executive function ^{434, 435}. A number of studies have shown the usefulness of combining resting state EEG and connectivity measures for differential diagnosis of dementias and other neurodegenerative conditions ^{436, 437}.

After a review of the literature this is the first study of its kind to measure resting state EEG in Polio patients. EEG oscillatory activity is not just an epiphenomenological marker for interneuron function, but also directly measures cortical computation ⁴³⁸⁻⁴⁴⁰. We therefore intended to examine further the CMC changes noted in Polio patients and examine the role of potential oscillatory changes in Polio in altered cortical processing. This aim was achieved by undertaking resting state studies in the same cohort of patients to further explore potential cortical changes previously detected. While Polio primarily affects the anterior horn cells of the spinal cord, we have shown in the previous section that upstream changes occur in the setting of neurodegeneration.

6.2.2. Methods

6.2.2.1. Experimental design

EEG data was collected from 26 Polio patients and 81 healthy controls (m: 43.2%; mean age: 60.93 +/- 11.3).

The EEG acquisition and the experimental paradigm used are described in section 4.4.

6.2.2.2. Pre-processing

Using Matlab R2019b software ⁴⁴¹, the EyeBallGUI toolbox ⁴⁴² and the FieldTrip toolbox 20190905 ³⁴⁰.

Pipeline:

Automatic artefact rejection method was employed to reject bad epochs based on a statistical thresholding³⁷¹. The EEG signal was resampled at 256 Hz with a band-pass ([1-97Hz]) and notch filtered (at 50Hz). Baseline correction was used on the signals. An automatic algorithm, evaluating the removal of noisy channels was based on both the PREP pipeline and the work of Kohe et al.^{344, 345}. The average number of channels removed was 2.7 for controls and 4.4 for patients. If more than 19 channels were marked as noisy, the subject's data was excluded from the study (Subject 23 was excluded for this reason). Channels removed were regenerated by interpolating from the remaining electrodes using spline interpolation³⁴⁰. Channels were referenced to the common average.

6.2.2.3. Processing

The EEG signals were processed using the methods described by Dukic et al.³⁷¹.

6.2.3. Results

Resting State EEG was analysed in 26 polio patients (1 patient excluded as described in section 6.1.3 above) as well as in 81 healthy controls. Analysis using spectral power, average connectivity and imaginary coherence revealed significant changes in the polio group.

Polio patients showed significant spectral power increases between θ and β frequency bands, especially in the parietal, occipital and subcortical regions (Fig 6-6.). Statistical difference between healthy controls (n = 81) and Polio patients (n = 25) was assessed in the six defined frequency bands (δ (2–4 Hz), θ (5–7 Hz), α (8–13 Hz), β (14–30 Hz) and γ_l (31–47 Hz), γ_h (53–97 Hz)) using empirical Bayesian inference (EBI). False discovery rate (FDR) was set to 10%, yielding an estimated statistical power of $1 - \beta = .81$ and posterior probability of $P1 = .56$ (across all frequency bands). AUC means area under the receiver operating characteristic curve. No changes were detected in the frequency bands not shown.

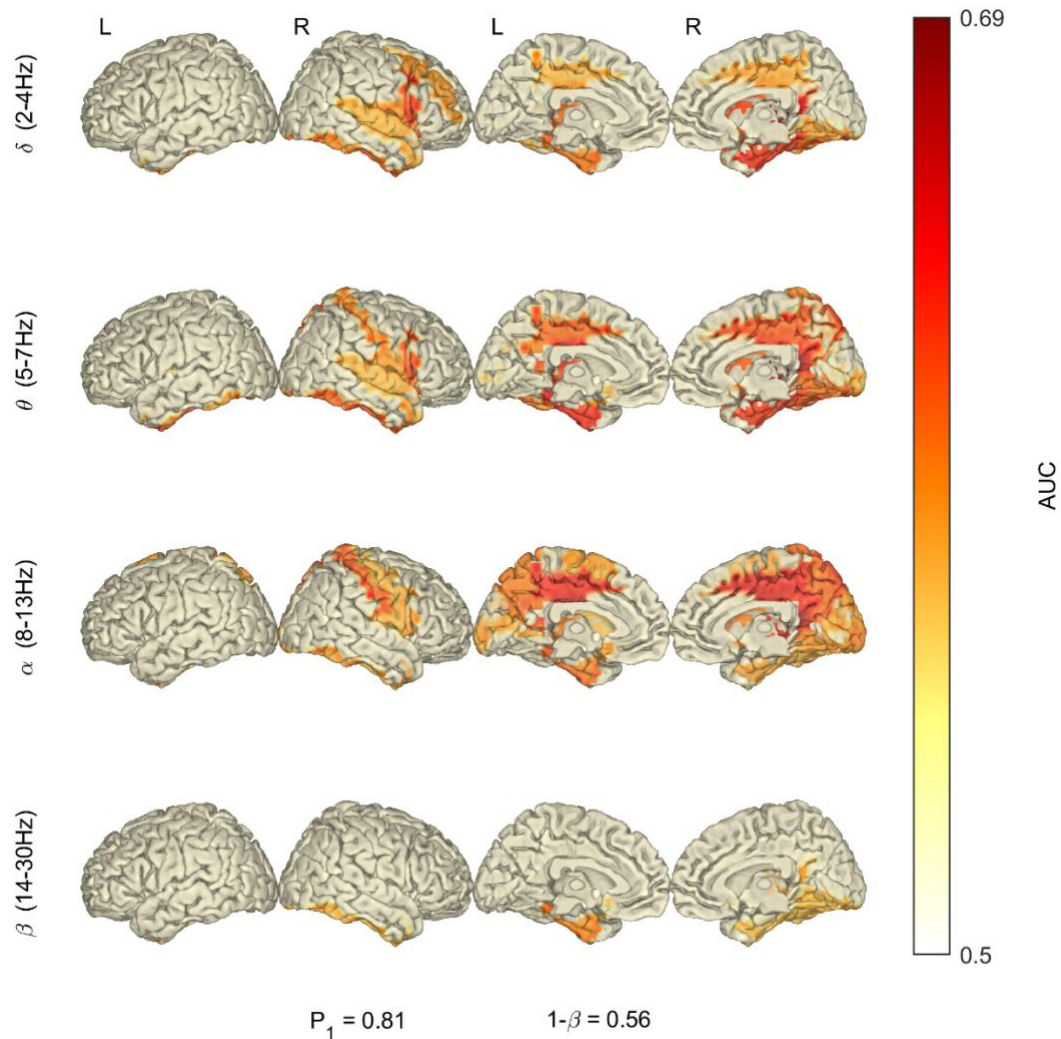


Figure 6-6 In Polio patients, spectral power is significantly increased between θ and β frequency bands, especially in the parietal, occipital and subcortical regions.

Widespread co-modulation decreases between healthy controls and Polio patients was noted in α , β and γ_h frequency bands. (Fig 6-7.) Statistical difference between healthy controls ($n = 81$) and Polio patients ($n = 25$) was assessed in the six defined frequency bands (δ (2–4 Hz), θ (5–7 Hz), α (8–13 Hz), β (14–30 Hz) and γ_l (31–47 Hz), γ_h (53–97 Hz)) using empirical Bayesian inference (EBI). False discovery rate (FDR) was set to 10%, yielding an estimated statistical power of $1 - \beta = .84$ and posterior probability of $P_1 = .43$ (across all frequency bands). AUC means area under the receiver operating characteristic curve. No changes were detected in the frequency bands not shown.

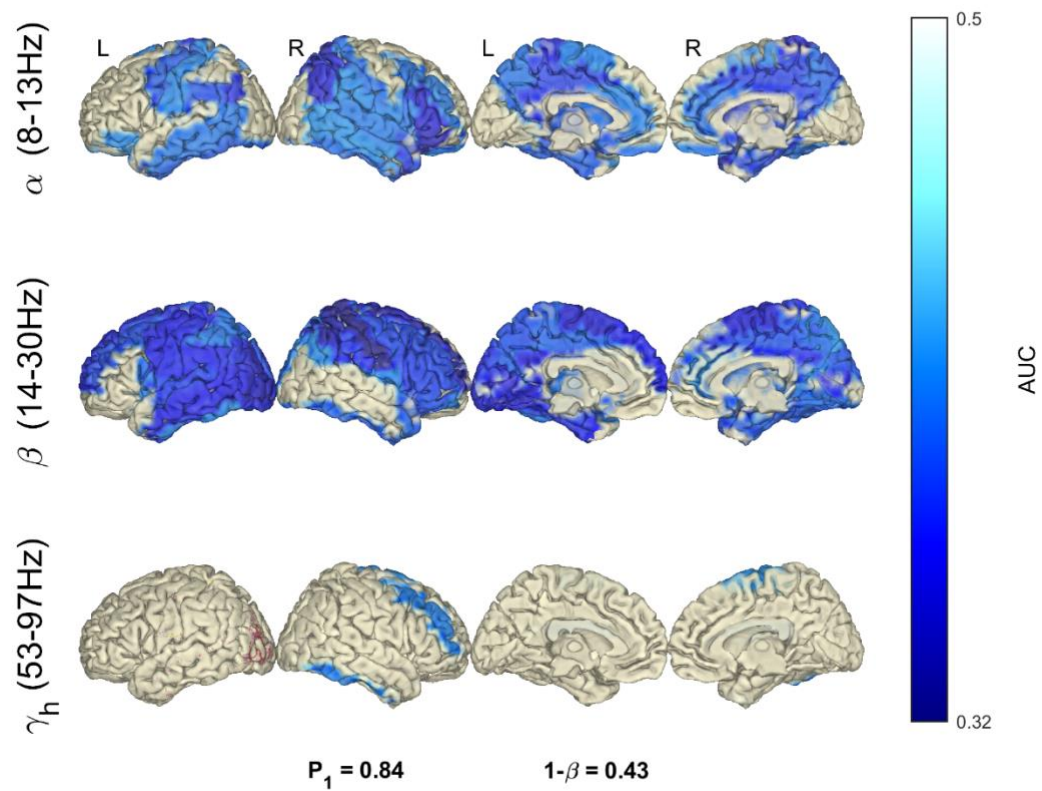


Figure 6-7 Observed widespread co-modulation decrease between healthy controls and Polio patients in α , β and γ_h frequency bands.

Comparisons (Fig. 6-8) show the differences between healthy controls and Polio patients in the areas highlighted by the brain maps (Figures 6-6 and 6-7). Statistical differences between healthy controls and Polio patients were assessed using Mann–Whitney U test in the two measures, each in the two frequency bands with the most prominent changes (see Figures 6-6 and 6-7).

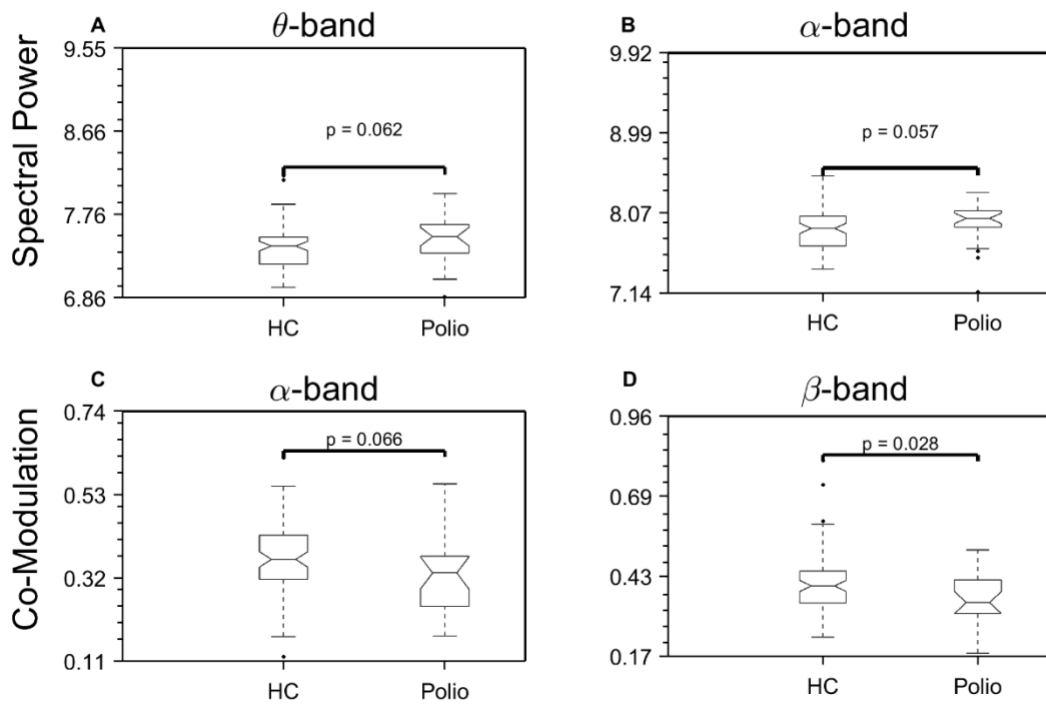


Figure 6-8 Comparisons between co-modulation and spectral power

6.2.4. Discussion

This study showed that neuro-signal analysis can quantify and capture changes occurring in the functional networks of Polio survivors. By using spectral power as well as two different means of connectivity measures (co-modulation and synchrony) we have shown statistically robust evidence of a widespread disruption of networks in Polio patients. These disruptions fall in line with patterned changes noted on CMC.

Central beta oscillations have been shown to synchronize after voluntary as well as after passive movements⁴⁴³. Post-movement beta synchronization is thought to reflect, at least in part, active inhibition of the motor cortex by somatosensory afferents⁴⁴³. Different frequency bands are mediated by complex neurochemistry with oscillations of frequencies 12–80 Hz linked to pyramidal neurons, regulated by GABA_A inhibitory interneurons³⁸³. The loss of Betz cells (giant pyramidal cells) in the motor cortex has been confirmed in autopsy studies of polio survivors⁴⁴⁴. We postulate that the loss of GABAergic interneurons, together with pyramidal neurons

in Polio survivors, along with the increase in the lower frequency spectral power can be attributed to structural regeneration/reorganisation of cortical networks of pyramidal cells and/or the interneurons that entrain them.

Our study shows for the first-time neurophysiological changes associated with increased theta and beta band spectral power on resting state EEG in polio patients. This supports the notion that much like other neurodegenerative conditions, polio is a network-based disorder. Despite our traditional views of polio being a lower motor neuron disorder, we have shown on two different neurophysiological modalities that after primary infection with the polio virus, longer term survivors have produced a network based compensatory changes to a downstream assault. This study is the largest dedicated neurophysiological Polio study of its kind. We show that both CMC and Resting state EEG is a robust means to assess the entire functional brain network. The unmasking of activity in other brain regions outside the primary motor cortex suggests a plasticity of the brain network especially when disruptions occur over time.

6.2.5. Limitations

This study is not without limitations, which include a small sample size which precluded detailed analysis of subgroups. This is a function of the relative rarity of polio survivors in European countries. Accordingly, while the overall group sample size provides robust statistical results, interpretation of the subsequent subcategorizations is preliminary. Source analysis of brain sources, increased sample sizes, and multivariate spectral analysis will be instrumental in further elucidating the patterned changes of motor circuitry in future studies.

Notwithstanding, our study demonstrates that CMC is a powerful tool that can evaluate the function of the motor circuits as an entire connected network. The unmasking of activity in networks upstream to the anterior horn suggests plasticity of motor circuitry especially when these disruptions occur at a younger age.

6.2.6. Conclusion

CMC is a powerful tool that can evaluate the function of the motor circuit as a whole connected network in. The unmasking of compensatory networks upstream to the

anterior horn suggests plasticity of -motor circuitry especially when these disruptions occur at lower age.

Although further investigation is needed for a more complete understanding of whether modulation of cortical circuitry could be harnessed as a biomarker of target engagement and therapeutic efficacy in the PPS patient group these findings provide a proof of concept that warrants further investigation using additional neurophysiological strategies including source localization.

7. Chapter 7: SMA Results

7.1. Corticomuscular Coherence in SMA

7.1.1. Introduction

Spinal Muscular atrophy (SMA) is the most common inherited neurodegenerative condition with a prevalence estimated at 1/11,000 births and a carrier frequency of between 1/40- 1/60 ¹⁹¹.

SMA is characterised by atrophy and muscle weakness giving rise to a progressive degeneration and loss of the anterior horn cells of the spinal cord and brain stem nuclei ⁴⁴⁵. Weakness onset can start from birth to early adulthood. Weakness is symmetric and progressive. Before the onset of greater genetic understanding in SMA, it was often classed into clinical subtypes based on maximum motor function achieved, however it is evident that phenotype of SMN1- associated SMA crosses a continuum with clear cut-offs for subtypes. Historically care was only in the way of supportive management of failure to thrive, restrictive type lung disease, and scoliosis, however with the advancement of targeted treatment options the natural history of SMA is changing ⁴⁴⁵.

A systematic analysis of two SMA mouse models has showed an age and tissue dependent expression of the SMN protein in normal mice ⁴⁴⁶. The SMN protein depletion level varied among tissues in SMA mice, with decreases shown in the central nervous system being related to disease onset and progression ^{446, 447}. A broad variation of SMN gene expression has also been reported in human prenatal spinal cord and skeletal muscles ⁴⁴⁸. SMN expression levels have been shown to decline after birth in humans ⁴⁴⁹. These observations imply that the timing of initiation of targeted treatment plays a critical role in the clinical outcome of patients⁴⁴⁷. While skeletal muscle atrophy in SMA is a direct result of motor neuron degeneration, some defects highlight the intrinsic muscle abnormalities such as altered differentiation during myogenesis, histological developmental defects and impaired progenitor cells ⁴⁵⁰⁻⁴⁵². These observations suggest that a full functional recovery of the motor unit requires SMN in both motor neurons and muscles ⁴⁴⁷.

While it is possible to assess children with SMA using clinical instruments that account both for developmental progress and muscle strength, these instruments are at best semi-quantitative. Moreover, for adults with SMA, there is a very significant

floor effect for most measurements. According, there are increasingly compelling reasons to use advanced neurophysiology-based signal analysis to provide clinical correlates. Although imaging-based assessment tools have shown promise for network-level assessment⁴⁵³ in SMA, they are expensive, suffer from poor temporal resolution and a significant proportion of patients do not tolerate MRI scanning.

Emerging preliminary evidence suggests that interruption of one component in the motor circuit can also lead to compensatory changes both up and down stream, as demonstrated by recent MRI observations of cortical reorganisation in SMA³²⁸ and our evidence of alterations in cortical connectivity in PPS (Results Chp 6). This compensatory change has potential as a marker both of disease, and of target engagement of novel therapeutics, where evidence of modulation of cortical circuitry in a pure LMN disease would provide compelling evidence of target engagement.

Emerging neurophysiology tools¹⁵ can reliably quantify progression in the lower (EMG-based MUNE, MUNIX⁴⁵⁴) or upper motor system (TT-TMS²⁰, surface EMG²⁹²), however these techniques do not effectively assess specific motor networks. By contrast, recent studies^{151, 327} provide strong evidence that it is possible to use *spectral analysis of EEG/EMG* to quantitatively mark the motor and non-motor brain network impairment in MND conditions.

7.1.2. Methods

7.1.2.1. Ethics

The study was approved by the *Tallaght University Hospital / St. James's Hospital Joint Research Ethics Committee - Dublin* [REC Reference: 2019-05 List 17 (01)] as described in section 4.1.

Inclusion Criteria

All patients over the age of 18 with a verified diagnosis of SMA, and all had supportive clinical and electromyographic findings.

Exclusion Criteria

Patients with a history of major head trauma or other neurological conditions that could affect cognition, alcohol dependence syndrome, current use of neuroleptic medications or high-dose psychoactive medication were excluded. Those with

diabetes mellitus, a history of cerebrovascular disease, and those with neuropathy from other causes were also excluded.

7.1.2.2. Clinical assessment

12 Adult SMA Patients were prospectively recruited in this cross-sectional study between July 2019- September 2020 through the SMA clinic at Beaumont Hospital. Due to the Covid-19 Pandemic 2 patients were not available for EEG/EMG recording. 10 Patients were included for statistical analysis.

On the day of EEG recording all patients underwent an extensive clinical assessment. Disease duration from symptom onset and site of disease onset were recorded. Muscle strength was assessed using the Medical Research Council (MRC) score in 9 bilateral (i.e., 18) upper limb muscles, including deltoid, triceps, biceps, wrist flexors and extensors, fingers flexors and extensors, and abductors of the index fingers and thumbs. The degree of clinical lower motor neuron (LMN) involvement in the upper limbs was graded by a LMN score ³⁶⁶ from these MRC scores, which ranged from 90 (absent LMN signs) to 0 (severe LMN signs). Handedness was assessed in all participants with the Edinburgh Handedness Inventory ⁴²¹.

7.1.2.3. Experimental Paradigm and Data acquisition

Assessment was conducted in the same manner for the patients and control groups. EEG/EMG acquisition and the employed experimental paradigm are described in detail in the methods section 4.4.

7.1.2.4. Data Analysis

EEG/EMG data analysis was blinded to clinical assessments. Five EEG channels (Cz, Pz, C4, Fz, C3) and 3 EMG channels (APB, FDI, FPB) were chosen prior to the analysis of cortico-muscular coherence (CMC). This selection was based on the biomechanical involvement of the muscles in the pincer grip task ³⁶⁷, and the suitability of the EEG and EMG channels for assessing CMC ³⁶⁸. A time window/epoch duration of 4s (starting 1s after the visual cue) was chosen for analysis; data epochs where the target force was not correctly achieved were excluded. The raw EEG data was (re-)referenced using (small) surface Laplacian spatial filter ^{369, 370} which is a spatial filter for removing spurious signal components in EEG channels, and EMG data (signal amplitude) were normalized with respect to the EMG amplitude during 100 % MVC. EEG/EMG data were filtered between 1-100Hz

using a dual-pass 4th order Butterworth bandpass filter. This was followed by a 50Hz Discrete Fourier Transform (DFT) filter in the FieldTrip toolbox to remove power line noise. An automatic artefact detection and rejection was performed using FieldTrip toolbox ³⁴⁰ to remove eyeblinks, muscle, and electrode jump artefacts from EEG signals. The auto-spectrum of each EEG/EMG signal, and cross-spectrum between all combinations of EEG-EMG signals (frequency resolution 1Hz, bandwidth 2-100Hz) was calculated using FieldTrip toolbox (Hanning taper and frequency smoothing at 1Hz). The auto- and cross-spectra at each frequency (2-100Hz) was converted into 8 band values- delta (2-4Hz), theta (5-7Hz), lower alpha (8-10Hz), higher alpha (11-13Hz), lower beta (14-20Hz), higher beta (21-30Hz), lower gamma (31-47Hz), and higher gamma (53-97Hz), excluding the 48-52Hz range to avoid mains power noise. The formation of band-specific values was carried out by taking Spatial Median (a variation of the median operator for complex-valued spectra, chosen and preferred over the algebraic averaging to provide robustness against outlier values) ^{346, 347} of the spectra at corresponding frequencies. The spectral coherence (cortico-muscular coherence in this study) was obtained by normalizing the cross-spectrum by the respective auto-spectra ³⁴⁷.

The selection of the parameters and methods for signal processing (e.g. band-specific analysis and the use of non-parametric methods) was based on our previous EEG studies ^{150, 151} that provided robust estimations not sensitive to outliers or observations in individual subjects ³⁷¹.

7.1.2.5. *Statistics*

Participant-level statistics were calculated using one-sample non-parametric rank statistics for spectral coherence ³⁴⁷. This method gave individual p-values for spectral cortico-muscular coherence in each frequency band for both patients and control groups (Fig. S3). Stouffer's method ^{352, 353} was used to combine individual p values to derive group average p value. This procedure is equivalent to the pooled coherence analysis ³⁵⁴.

Correction for multiple comparisons was performed using the adaptive false discovery rate at $q = 0.05$ ³⁵⁵, which was applied by correcting the p-values in the coherence spectra. Negative logarithm of p-values, i.e. $-\log_{10}(p)$, was used to visualize cortico-muscular coherence. The band-specific values of coherence,

expressed in $-\log_{10}(p)$, was used to represent the values for all the frequencies in that frequency band.

7.1.3. Results

A total of 12 SMA patients were recruited, due to the Covid-19 Pandemic 2 patients were not available for EEG/EMG recording and 2 were excluded due to multiple co-morbidities. 8 SMA Patients in total were included for statistical analysis. Patients were recruited from a cohort SMA clinic based in Beaumont hospital, Dublin (see Tables 7-1 and 7-2).

The analysed patient group included 5 female and 3 male patients (mean age of 35.75 ± 12.5 years (Standard deviation), 6 right hand dominant). From this group, 5 patients were clinically classed as SMA type 2 with 3 classed as SMA type 3. Muscle weakness was graded by the LMN score, was 44.7 ± 26.4 points. A total of 11 healthy controls were successfully recruited (mean age of 61.09 ± 14.8 standard deviation).

Table 7-1 Clinical and demographic data of analysed SMA patients.

	Patient Group	Controls
Gender (F/M)	5/3	4/7
Average age at recording (F/M)	32.6± 8.4 /41 ± 18.5	64.7± 4.2 /59 ± 18.5
SMA Type 2/3	5/3	-
LMN score (max 90)	44.7 ± 26.4	90

Table 7-2 Clinical and demographic data of SMA patient sub-groups

	Type 2	Type 3
Number of Patients	5	3
LMN score (Max 90)	45.4 ± 21.9	67.3 ± 8.08

Table 7-3 Individual clinical LMN scores of analysed patients.

SMA Patient	LMN Score	Gender
1	18	M
2	72	F
3	53	F
4	72	F
5	56	F
6	72	M
7	58	F
8	28	M

7.1.3.1. *Abnormal cortico-muscular coherence in the SMA patient group*

Cortico-muscular coherence (CMC), during steady low force isometric pincer grip (Fig. 7-1), was calculated between intrinsic hand muscles (using bipolar EMG) and surface EEG electrodes over scalp. In this context, patterns of CMC were identified in the SMA group that differed from those of the controls. Specifically, the CMC in the patient group did not show the typical beta-band CMC over contralateral motor area (as intended by the task selection), and the CMC across the 5 tested EEG electrodes, 3 muscles, and the frequency bands were scattered and inconsistent (Fig.7-1). These null findings were in accordance with expectations from our pilot study in controls, as well as the anticipation of typical beta-band cortico-muscular coherence primarily over primary motor cortex, C3 electrode (seen in low force sustained muscle contraction ^{297, 298, 303}).

In the SMA group the CMC was statistically significant ($p < 0.05$, $q = 0.05$) at the group level (Fig. 1) and appeared at frequencies different from the commonly observed and expected beta (14- 30Hz) frequency bands. Instead, the significant CMC appeared consistently in the low gamma (31-47Hz) frequency band across several EEG-EMG channels (see Fig.7-1). Importantly, this abnormal low gamma-band coherence was observed at the ipsilateral motor area as well as (C4) area, but also over central and frontal areas (Fz and Pz). The abnormal CMC appeared in other (e.g., alpha) frequency bands but was less consistently across EEG-EMG channels. These CMC patterns differed from the control cohort in frequency (beta vs. low gamma) and location (contralateral motor C3 vs Frontal Fz and Parietal Pz) ^{303, 372}, (Fig.7-2).

The pathological gamma-band CMC was a consistent finding and was observed at both frontal and parietal electrodes and in both FDI and APB muscles (Fig. 7-2).

There are frequency bands lower than the beta range, in which some SMA patients showed significant CMC. However, due to the similarity of this frequency bands and locations to those in healthy controls (where CMC approached significance) these findings were not further assessed. The gamma band significant coherence will be discussed further in the context of the other conditions with LMN dysfunction (PPS) in the discussion.

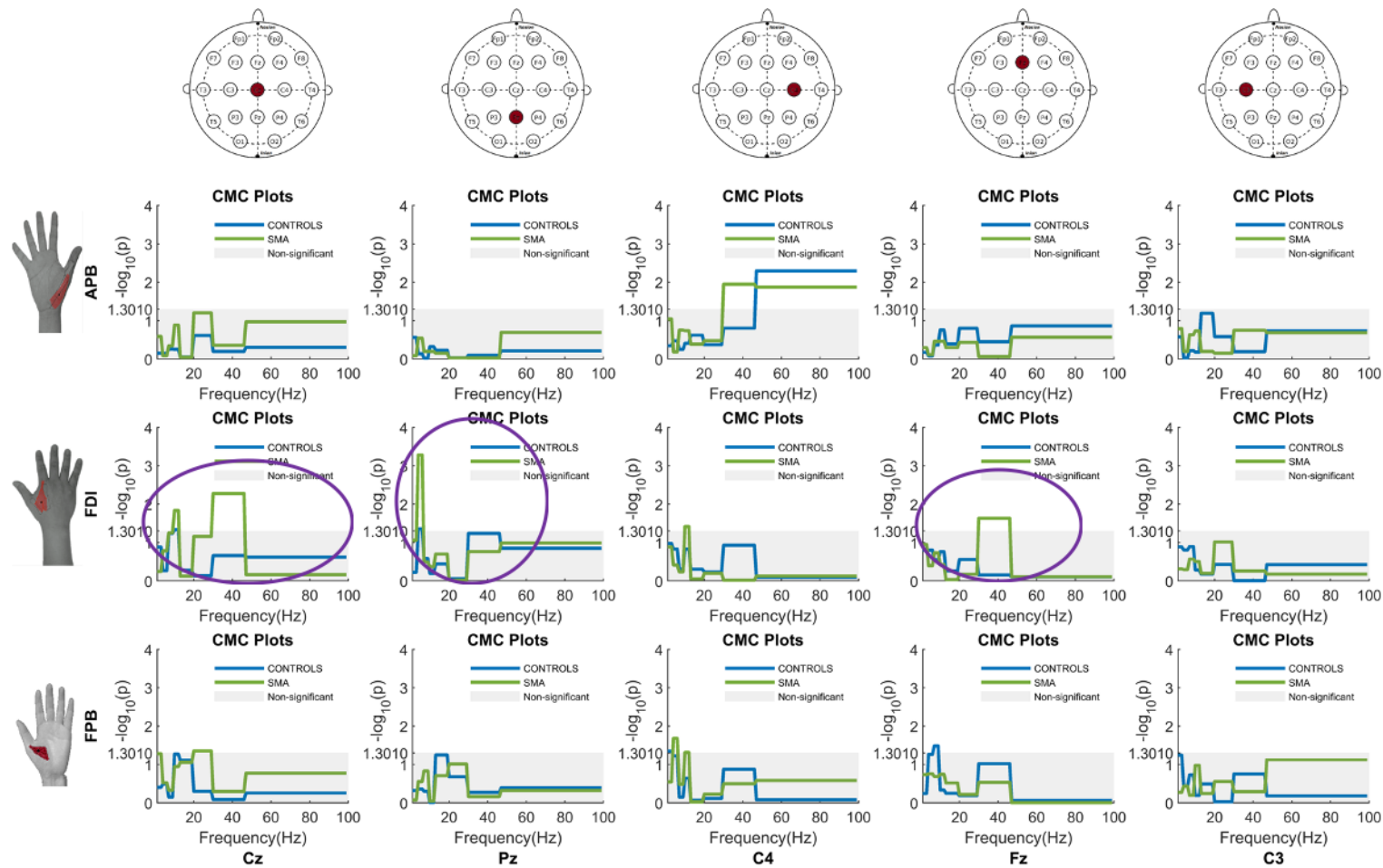


Figure 7-1 Patients with SMA show abnormal group level CMC in non primary motor areas.

The cortico-muscular coherence spectra, expressed in $-\log_{10}(p)$, show the synchrony between the EEG electrodes (over the frontal area, Fz, Central, Cz, and parietal areas, Pz) and EMG (First Dorsal Interosseous, FDI, muscle) in different frequency bands. The lower values show less synchrony, whereas the higher value show higher EEG-EMG synchrony. The shaded area corresponds to the non-significant values at $\alpha=0.05$ threshold for p -values (corrected for multiple comparison using FDR at $q = 0.05$). Notice the dominant abnormal coherence in SMA in low gamma-band (30 - 47Hz) which is present in FDI muscle and in both Cz and Fz electrodes, as well as alpha- band noted between Pz electrode and FDI muscle.

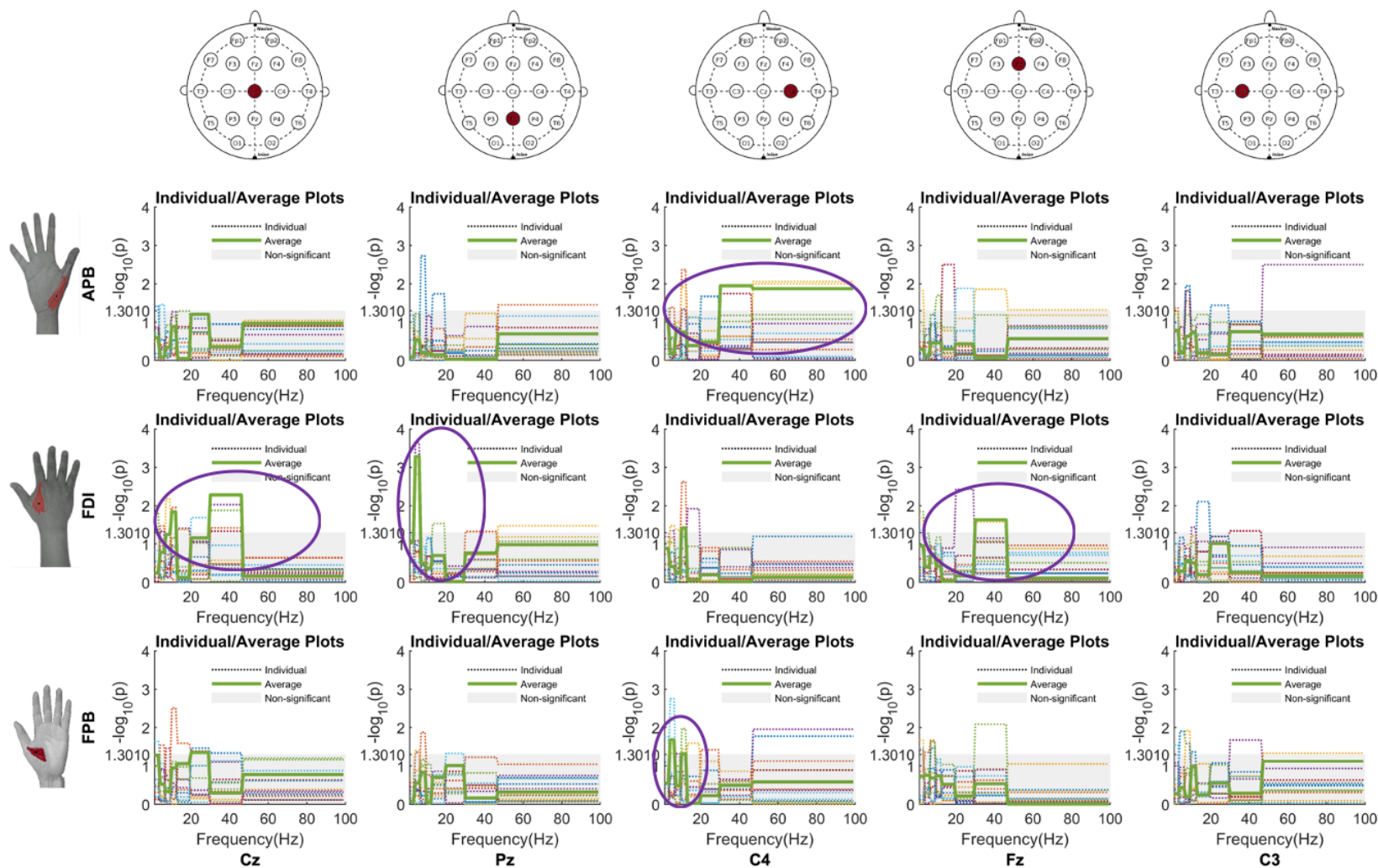


Figure 7-2 Significant pathological increases in CMC seen at individual as well as group level.

Analysis of SMA patients showed abnormal frequency band changes -namely alpha band increases over central (Cz) region. Abnormal coherence patterns noted between FDI muscles and the frontal (Fz), parietal (Pz) and central (Cz) regions. Abnormal coherence patterns noted between FPB and APB muscles and ipsilateral (C4) regions.

7.1.4. Discussion

As improvements in targeted medicine continue to show benefits with respect to survival and motor function ⁴⁵⁵, there remains an increasing need for reliable outcome measures in SMA progression to harness the full benefit or identify accurately the limitations of a potential therapy and reduce the patient burden.

The main obstacle with current clinical outcome measures used in SMA trials is the slow progression of SMA type 2 and 3, making decline difficult to detect over short periods of time compared to SMA type 1 ⁴⁵⁶. Neurophysiology based outcome measures aim to overcome some of the obstacles faced using current clinical measures.

EEG-EMG coherence during functional motor tasks shows pathological changes in the central-peripheral communication in SMA Patients. Pathological locations of CMC suggest compensatory changes in the motor cortex, which involves broader cortical regions with synchronous activity to muscles, in those affected with SMA. The presence of wider cortical regions is consistent with previous studies ³²⁸ demonstrating cortical hypertrophy in young adults with SMA. . Although the relatively low numbers merit careful interpretation, our observations of increases in pathological CMC patterns can be interpreted as a compensatory mechanism in the context of progressive LMN degeneration and discussed from a plasticity perspective.

It is well recognised that the reorganisation of motor regions can occur in response to repetitive tasks ⁴⁵⁷, as well as in patients with perinatal brain injuries. In such instances, increased grey matter density and cortical thickness can be identified in the contralateral hemisphere on MRI ⁴⁵⁸. One of the better known examples of cortical hypertrophy in relation to a repetitive cognitive task is the increased hippocampal volume of taxi drivers in London as described by Maguire et al.⁴⁵⁸.

These compensatory mechanisms have been shown to be more distinct in children, as there may be a critical period for effective brain reorganisation taking place ⁴⁵⁹.

SMA Type 3 and 4 both start to develop LMN degeneration early in life and accordingly could be assumed to be likely to show effective cortical adaptation.

Interestingly, similar such mechanisms have been proposed previously to explain

exceptional language skills in SMA, which was also interpreted as a compensatory response ⁴⁶⁰.

These findings are similar to those I have reported in the Polio patient group (Chp 6), suggestive of compensatory changes that happen in a network based disease.

Although both conditions are traditionally seen as progressive LMN degenerative diseases, my observations and findings are very suggestive of motor neuron diseases being seen as a network based disorder, rather than a discrete disorder affecting a single specific neuroanatomical region.

The cellular mechanisms underpinning the cortical changes seen in the SMA group remain to be established. Dendritic spine density has been previously linked to specific MRI measures in a study of neural plasticity in mice ⁴⁶¹. The study showed that increased voxel based morphometry signalling was associated with an increase in dendritic spine density⁴⁶¹. Based on our results, we hypothesize that adaptive compensatory mechanisms may take place in LMN disorders, like SMA, which may be driven by dendrite density modifications.

7.1.5. Limitations

This study is not without limitations. Larger cohort samples with more robust stratification of SMA type 2 and 3 patients would have been optimal. A longitudinal study would be the natural extension of our proof-of concept findings.

Notwithstanding, this study provides a proof of concept demonstrating that interrogation of CMC patterns could be developed as a reliable marker of therapeutic efficacy in LMN conditions such SMA, where current quantitative clinical outcome measurements are limited by severe motor disability.

While I acknowledge that these results should be cautiously interpreted because of the small sample size of SMA patients, the abnormal CMC patterns may represent compensatory mechanisms of cortical reorganisation which is more evident in patients with slower progression rates and longer disease duration. This opens the opportunity to consider CMC as a potential biomarker of disease, and a possible longer outcome measure when assessing disease modifying therapies.

7.1.6. Conclusion

During the lifetime of this PhD SMA has for the first time gained approval for a disease modifying therapy in the form of an antisense oligonucleotide. Nusinersen was approved in Ireland in 2019 for SMA patients with type I-III to all children under

the age of 18 years. Current age based treatment restrictions has meant that the adult SMA population have had very limited access to treatment, a lot of whom many have function to preserve. The potential of a neurophysiological based approach in the application of CMC as a clinical marker to demonstrate disease stabilisation or even improvement at a sub-clinical level is life changing for those with a life limiting condition such as SMA. While the pathological signatures are a compensatory mechanism, perhaps reflecting years of network dysfunction, it is conceivable that in the setting of ASOs and network recovery, these signatures could be tracked and recorded before any clinical improvements noticed. This opens the possibility that CMC could prove useful as a marker of disease progression or indeed sub clinical change improvement.

8. Chapter 8: PLS Results

8.1. Introduction

Primary lateral sclerosis (PLS) is a slowly progressive disorder of upper motor neuron degeneration, and is regarded as a sub-phenotype within the amyotrophic lateral sclerosis (ALS) spectrum⁴⁶². Diagnostic certainty for PLS requires a disease duration of at least 3 years, the absence of evidence of lower motor neuron dysfunction, and the exclusion of other possible causes of progressive upper motor neuron dysfunction.

The most common clinical presentations of PLS are of leg weakness, spasticity and bulbar weakness. Although minor EMG abnormalities can be detected in some patients with PLS, ⁴⁶³ motor unit estimation (MUNE) and indices (MUNIX) have shown either normal or very mildly reduced motor unit numbers in hand muscles ¹³⁰. Neither method is of benefit as a diagnostic indicator.

There remains an ongoing need for the development of reliable diagnostic biomarkers for PLS, both for early diagnosis, and as a potential quantitative outcome measure in clinical trials. Various potential markers directed at interrogation of the changes in the corticospinal tract have been proposed, including neuroimaging and transcranial magnetic stimulation (TMS). Neuroimaging provides useful insights into the structural changes of established PLS, but is of lesser utility in clinical diagnosis or as a measure of disease progression. TMS assesses the excitability of the motor cortex in PLS patients, but this technique is limited by slowed central motor conduction time, or unrecordable motor evoked potentials from muscles of affected limbs ^{464, 465}.

EEG is a useful non-invasive technique to measure neurophysiological cortical activity. EEG is unique in its combined ability to represent neuronal activity directly and in real time. It is also non-invasive and comparatively inexpensive when compared to other imaging techniques ²⁸⁵. EEG is known to have very good temporal as well as spatial resolution through the use of source localisation methods at lower costs compared to fMRI⁴⁶⁶.

Spectral analysis of EEG recordings involves the calculation of waves in a set of sequenced data ⁴⁶⁷. Spectral analysis can be used to assess rhythmic activity at differing frequencies in terms of oscillating network activity⁴⁶⁸. Spectral EEG is the

most widely used form of computerized analysis of digitized EEG. Spectral analysis is based on the Fourier theorem; stating that any wave form can be broken down into a sum of sine waves at differing frequencies with differing amplitudes and differing phase relationships ⁴⁶⁹.

Previous resting state EEG studies using spectral analysis in ALS patients have shown changes in connectivity patterns, such as increased frontoparietal connectivity ^{149, 151, 470}, but these technologies have not been used to date to explore connectivity in PLS.

As PLS is a motor system degeneration, a possible approach to characterize the disease is the measurement of both spatial and temporal changes in corticospinal function using corticomuscular coherence (CMC).

This study uses both resting state EEG and CMC to further our understanding of the neuropathology in PLS as well as evaluate its potential to harness these approaches for future clinical value.

By using both these techniques, the aim of the study is to explore different aspects of the functional neural network to provide better insight into the effects of PLS on central as well as peripheral brain network function.

We hypothesize that in PLS, there is an adaptive cortical network change that reflects the progressive degeneration of the corticospinal pathways, and that this can be harnessed as a quantitative measure of network change in PLS.

8.2. Methods

8.2.1.1. Ethics

The study was approved by the *Tallaght University Hospital / St. James's Hospital Joint Research Ethics Committee - Dublin* [REC Reference: 2019-05 List 17 (01)] as described in section 4.1.

8.2.1.2. Patient Cohort

Patients were prospectively recruited in this cross-sectional study between June 2017-August 2019 through the national ALS clinic at Beaumont Hospital. All patients fulfilled the clinical criteria for PLS ¹⁶⁵. Healthy controls, age-matched to patients, were recruited from database of healthy controls interested in taking part in research.

Subjects with a history of major head trauma or other neurological conditions that could affect cognition, alcohol dependence syndrome, current use of neuroleptic

medications or high-dose psychoactive medication were excluded. Those with diabetes mellitus, a history of cerebrovascular disease, and those with neuropathy from other causes were also excluded. All patients underwent nerve conduction studies and electromyography to exclude other concurrent peripheral nerve disorders that could interfere with CMC analyses.

8.2.1.3. *Clinical assessment*

On the day of EEG recording all patients underwent an extensive clinical assessment. Disease duration from symptom onset and site of disease onset were recorded. Muscle strength was assessed using the Medical Research Council (*MRC*) score in 9 bilateral (i.e., 18) upper limb muscles, including deltoid, triceps, biceps, wrist flexors and extensors, fingers flexors and extensors, and abductors of the index fingers and thumbs. The degree of clinical upper motor neuron (UMN) involvement in the upper limbs was graded by an UMN score ³⁶⁶. Reflexes were assessed at 3 sites in the upper limbs (biceps, triceps and brachioradialis). The UMN-score ranges from 0 (normal) to 16 (reflecting hyperreflexia, hypertonia, clonus, Babinski and Hoffmann sign).

All participants underwent both resting state EEG and CMC recordings.

8.2.1.4. *Experimental Paradigm-Resting State EEG*

Experimental setup was resting state EEG with eyes open. This was divided into three 2-min recording blocks, allowing for rest between blocks. All participants were seated in a comfortably, and asked “let their mind wander”, while fixing their gaze at the letter X ($6 \times 8 \text{ cm}^2$) printed on an A4 sheet of paper placed approximately 1 m in front of them.

EEG data were recorded in a special purpose laboratory, using 128-channel scalp electrode cap, filtered over the range 0–400 Hz and digitized at 2048 Hz using the BioSemi® ActiveTwo system (BioSemi B.V, Amsterdam, Netherlands). Each participant was fitted with an appropriately sized EEG cap.

8.2.1.5. *Data Analysis*

The EEG acquisition and the experimental paradigm used are described in section 4.4.

Pre-processing

Pre-processing steps are described in the Methodology Chapter (section 4.7.2).

Processing

The EEG signals were processed using the methods described by Dukic et al.³⁷¹.

8.2.1.6. *Experimental Paradigm- CMC*

Assessment was conducted in the same manner for the patients and control groups, similar to previous work carried out by this group and described in detail in the paper by Coffey et al.³⁹⁶ with additional notes in supplementary files. All participants were asked to minimise their eye movements and attempted maximum voluntary contraction (MVC) of the pincer grip between the thumb and the index finger of their right hand. MVC was taken as the average peak force of the three trials which were within 10% of each other.

8.2.1.7. *Data Analysis*

EEG/EMG data analysis was performed and described in detail in a previous study³⁹⁶. Briefly, Five EEG channels (Cz, Pz, C4, Fz, C3) and 3 EMG channels (APB, FDI, FPB) were chosen prior to the analysis of cortico-muscular coherence (CMC). This selection was based on the biomechanical involvement of the muscles in the pincer grip task⁴⁷¹, and the suitability of the EEG and EMG channels for assessing CMC⁴⁷². A time window/epoch duration of 4s (starting 1s after the visual cue) was chosen for analysis; data epochs where the target force was not correctly achieved were excluded. The raw EEG data was (re-)referenced using surface Laplacian spatial filter,^{341, 342} and EMG data (signal amplitude) were normalized with respect to the EMG amplitude during 100 % MVC. EEG/EMG data were filtered between 1-100Hz using a dual-pass 4th order Butterworth bandpass filter. The auto-spectrum of each EEG/EMG signal, and cross-spectrum between all combinations of EEG-EMG signals (frequency resolution 1Hz, bandwidth 2-100Hz) was calculated using FieldTrip toolbox (Hanning taper and frequency smoothing at 1Hz). The auto- and cross-spectra at each frequency (2-100Hz) was converted into 8 band values- delta (2-4Hz), theta (5-7Hz), lower alpha (8-10Hz), higher alpha (11-13Hz), lower beta (14-20Hz), higher beta (21-30Hz), lower gamma (31-47Hz), and higher gamma (53-97Hz), excluding the 48-52Hz range to avoid mains power noise. The spectral coherence (cortico-muscular coherence in this study) was obtained by normalizing the cross-spectrum by the respective auto-spectra³⁴⁷.

8.2.1.8. Statistics

Participant-level statistics were calculated using one-sample non-parametric rank statistics for spectral coherence ³⁴⁷. This method gave individual p-values for spectral cortico-muscular coherence in each frequency band for both patients and control groups. Stouffer's method ^{352, 353} was used to combine individual p values to derive group average p value. This procedure is equivalent to the pooled coherence analysis ³⁵⁴.

Correction for multiple comparisons was performed using the adaptive false discovery rate at $q = 0.05$,³⁵⁵, which was applied by correcting the p-values in the coherence spectra. Negative logarithm of p-values, i.e. $-\log_{10}(p)$, was used to visualize cortico-muscular coherence. The band-specific values of coherence, expressed in $-\log_{10}(p)$, was used to represent the values for all of the frequencies in that frequency band.

8.3. Results

16 patients diagnosed with PLS were prospectively recruited from the national ALS Clinic based in Beaumont hospital, Dublin. 7 Female and 9 male patients took part in the study with a mean age of 62. All Patients were diagnosed with definite PLS fulfilling the consensus criteria ¹⁶⁵ defined as the absence of LMN degeneration 4 or more years from symptom onset. 19 healthy controls were recruited, with a mean age 58.3. An adapted UMN score was calculated using reflex and UMN signs assessment.

Table 8-1 Clinical and demographic data of analysed patients.

	Patient Group	Controls
Gender (F/M)	7/9	10/9
Average Age at Recording (F/M)	62.6± 9.8 /61.5 ± 16.9	58.2± 9.6 /58.5 ± 19.5
Disease Duration (yrs)	7.6± 6.01	-
UMN Score* (Max 16)	12.8 ± 2.3	0
Spasticity (Upper Limb)	3.5±1.09	
MRC (Upper Limb) (max 100)	71.6±4.08	
ECAS Total Abnormal Score n (%)	4 (28%)	

Language	1 (7%)
Verbal Fluency	2 (14%)
Memory	2 (14%)
Visuospatial	1 (7%)

*UMN(Upper Motor Neuron Score)

*MRC (Medical Research Council Scale for Muscle Strength)

8.3.1. Corticomuscular Coherence

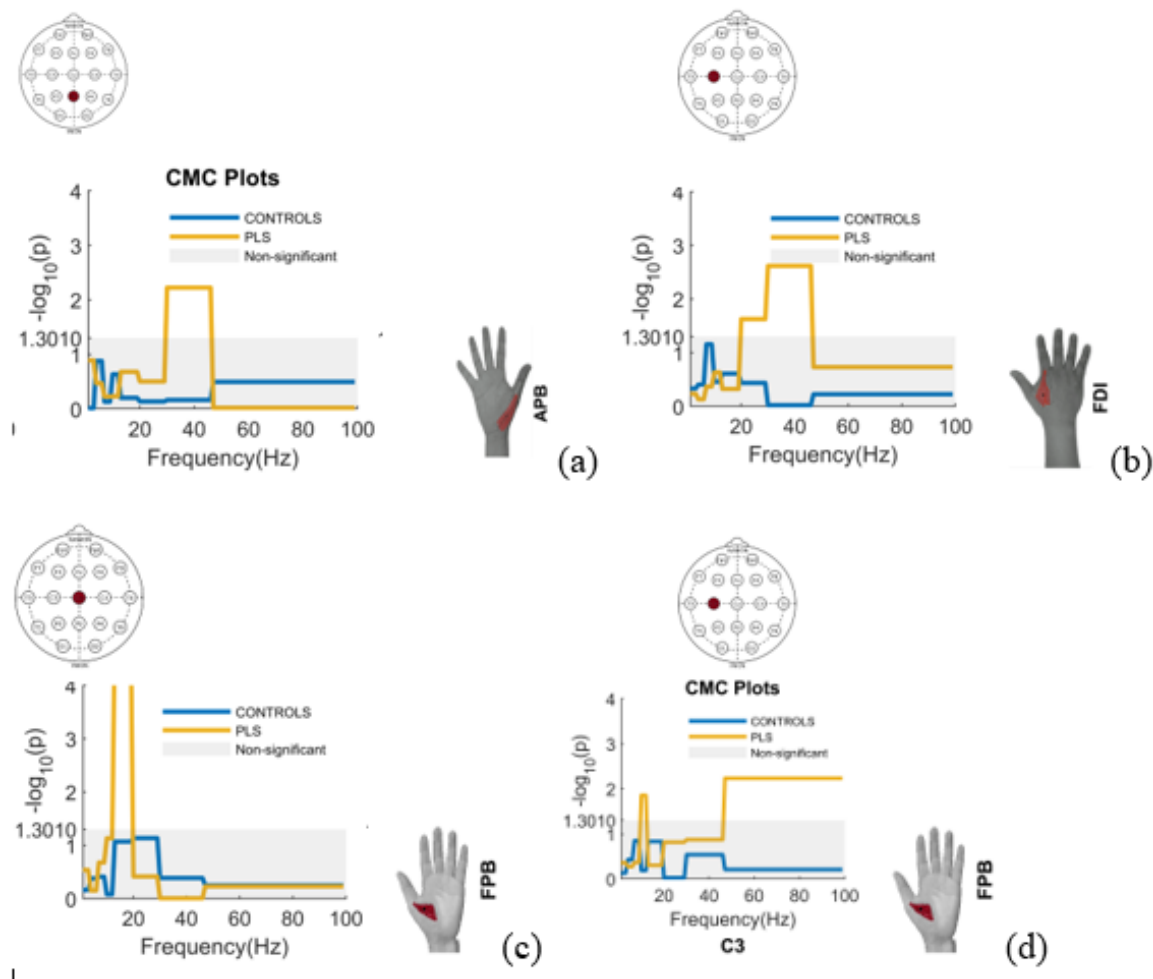


Figure 8-1 Patients with PLS show abnormal Cortico-Muscular (EEG-EMG) Coherence in non-primary motor areas.

The cortico-muscular coherence spectra, expressed in $-\log_{10}(p)$, show the synchrony between the EEG electrodes (over the Central, Cz (c) and C3 (d), and parietal areas, Pz (a)) and EMG (First Dorsal Interosseous, FDI; and Flexor Pollicis Brevis, FPB, muscles) in different frequency bands. The lower values show less synchrony, whereas the higher value show higher EEG-EMG synchrony. The shaded area corresponds to the non-significant values at $\alpha=0.05$ threshold for p-values (corrected for multiple comparison using FDR at $q=0.05$). Notice the dominant abnormal coherence in PLS coherence in low gamma-band (30 - 47Hz) which is present in both muscles and in both Pz and C3 electrode.

Coherence was measured between intrinsic hand muscles (using bipolar EMG) and surface EEG electrodes over scalp. The Expected frequency and contralateral location of (Fig.8-1.b) show evidence of a functional descending pathway, however in the context of the presence of pathological cortico-muscular coherence (CMC) patterns seen, provide evidence of alternate compensatory patterns in the PLS group compared to controls. Abnormal CMC patterns involving the parietal (Fig.8-1.a) and central regions (Fig.8-1.c), with abnormal frequency bands are noted in (Fig.8-1.d) showing high gamma band despite a low force experimental setup. These

findings are based on the isometric force exertion experiments using a pincher grip. The differences are statistically significant ($p < 0.05$) both at individual subject level, as well as at the group level (Fig.8-1), and shows that the frequency, location and intensity specific features of CMC can reliably distinguish between healthy controls and the PLS patient group.

We found a strong, positive partial correlation between CMC and UMN score at β frequency ($r = 0.765$, $p = 0.004$) involving APB muscle and the parietal region on EEG recording (Fig. 8-2).

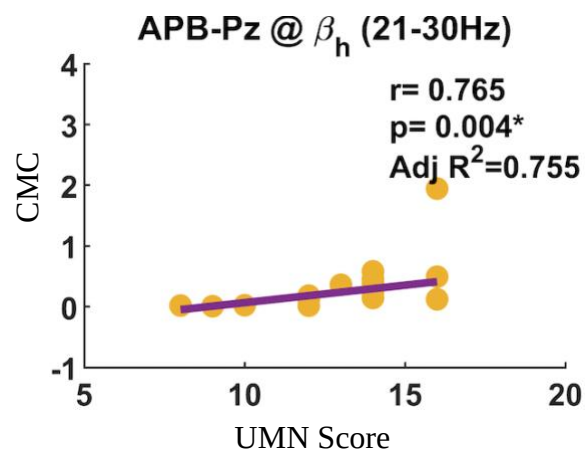


Figure 8-2 CMC shows a partial positive correlation with UMN score between APB muscle and Pz region

Negative partial correlation between CMC and the UMN score (Figure 8-3) ($r = -0.721$, $p = 0.013$) involving APB muscle and contralateral motor region at alpha frequency.

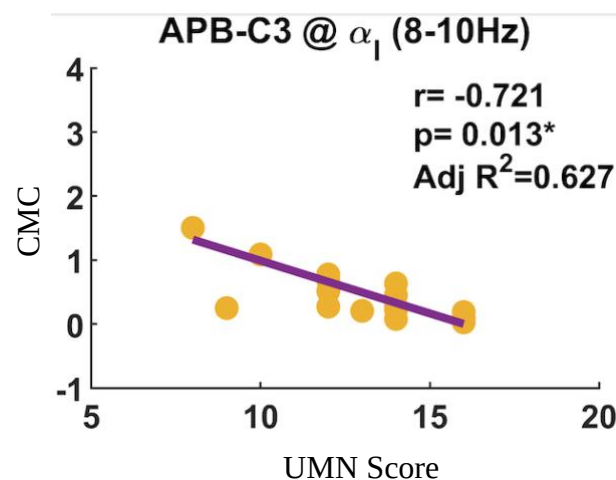


Figure 8-3 CMC shows a negative partial correlation with UMN Score between APB muscle and C3 region

We found a negative partial correlation between CMC and the UMN score (*Figure 8-4*) ($r = -0.616$, $p = 0.045$) involving APB muscle and contralateral motor region at high Gamma frequency. Similarly, negative partial correlation between CMC and the UMN score (*Figure 8-5*) was noted between APB and ipsilateral motor region ($r = -0.668$, $p = 0.037$)

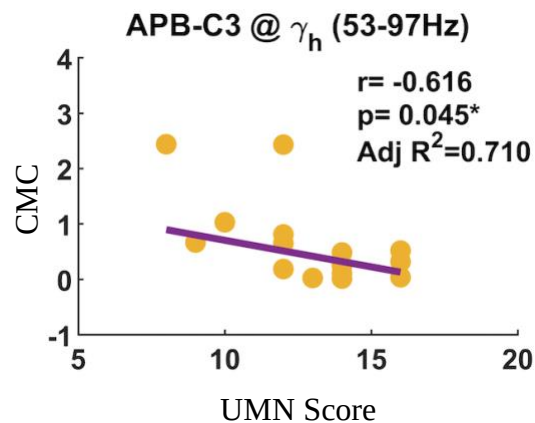


Figure 8-4 CMC shows a negative partial correlation with UMN Score between APB muscle and C3 region

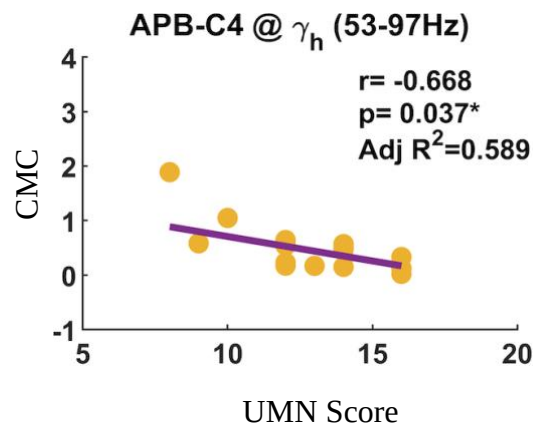


Figure 8-5 CMC shows a partial negative correlation with UMN Score between APB muscle and C4 region

8.3.2. Resting State

Resting state EEG was analysed in 15 PLS patients as well as 18 healthy controls. Analysis using spectral power, average connectivity and imaginary coherence revealed significant changes in the PLS group.

PLS patients showed widespread significant increases in AEC connectivity (a functional connectivity measure in EEG) when compared to healthy controls (*Fig.8-*

6(a)). The most noteworthy changes are seen in the frontal and central regions in the γ frequency band.

PLS patients showed significant decrease in synchrony at β band frequency.

Changes were noted to be in the motor/premotor cortex region (Fig.8-6 (b)).

Spectral power in PLS was significantly decreased across low to mid frequency bands (from δ to β band). The most notable changes were seen in the frontal, temporal and occipital regions. (Fig.8-6 (c)) Significant increased spectral power noted in the γ frequency bands, with a dominance over the frontal regions

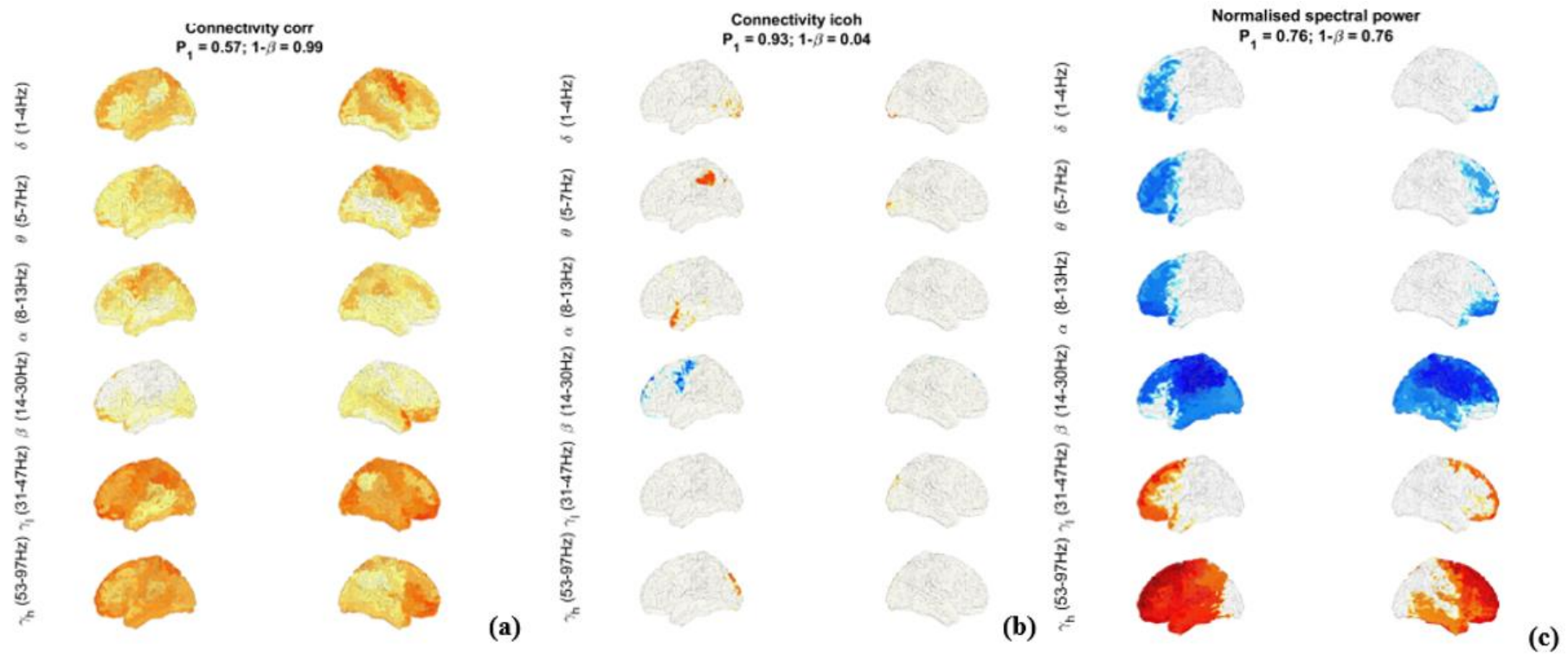


Figure 8-6 Functional network changes on resting state EEG in PLS.

- (a) In PLS the co-modulation is significantly increased in the γ frequency bands. Note the increase of amplitude envelope correlation (AEC) in the frontal regions.
- (b) In PLS the average synchrony is significantly decreased in the β band frequency. Note the decrease of imaginary coherence (iCoh) in the motor/premotor region.
- (c) In PLS spectral power is significantly decreased between δ and β frequency bands. Note the dominant decrease in the temporoparietal region. Significant increased spectral power noted in the γ frequency bands, with a dominance over the frontal region.

8.3.3. Source analysis CMC

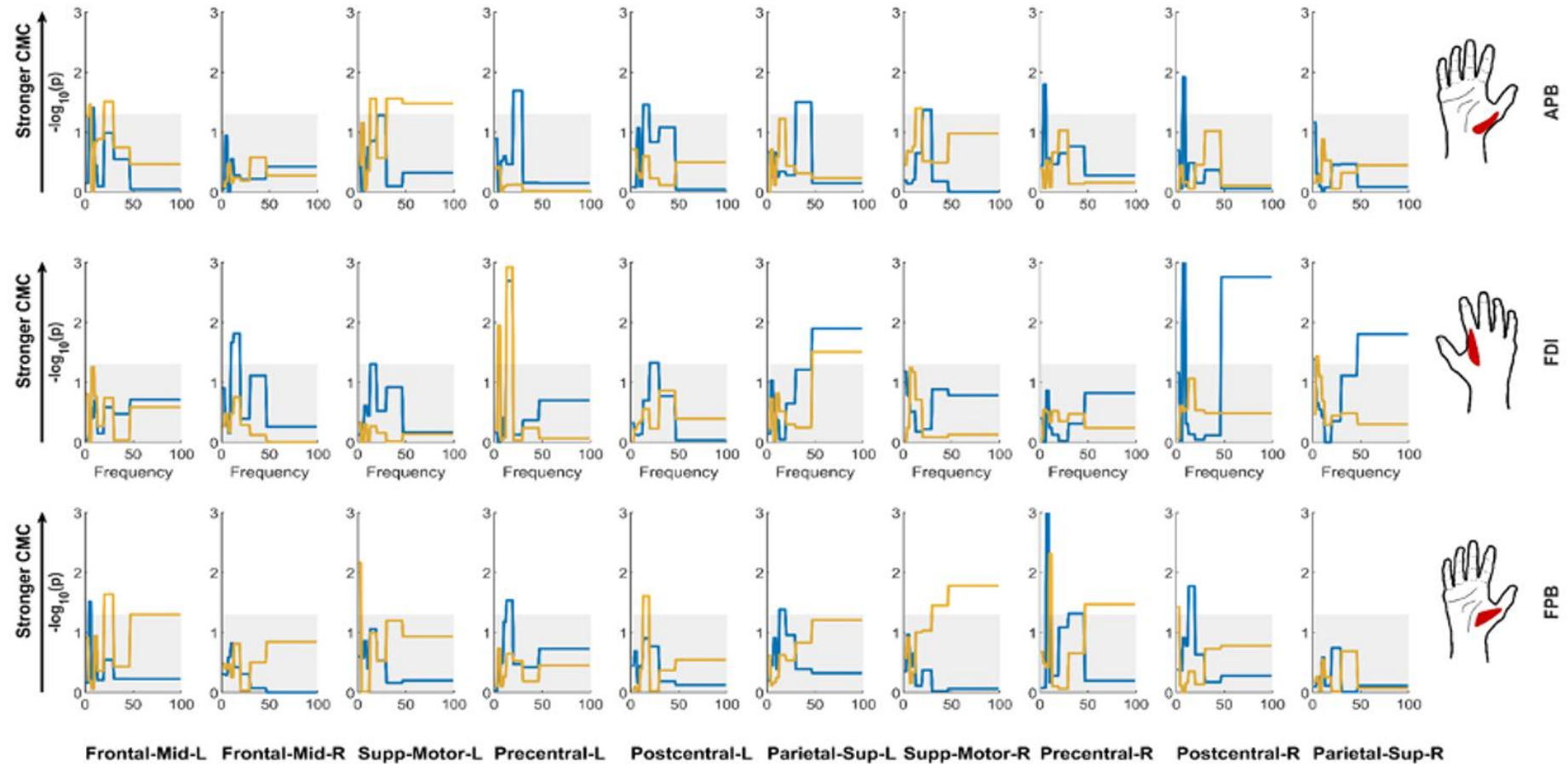


Figure 8-7 Cortico-muscular coherence (CMC) plots of PLS patients and healthy controls

Between 10 cortical regions of interest (5 on each side of brain) and three muscles (APB, FDI, FPB). The presence of significant beta cortico-muscular coherence between left Precentral region (primary motor) and all three muscles (APB, FDI, FPB) in Controls validates that the methods used effectively detects CMC.

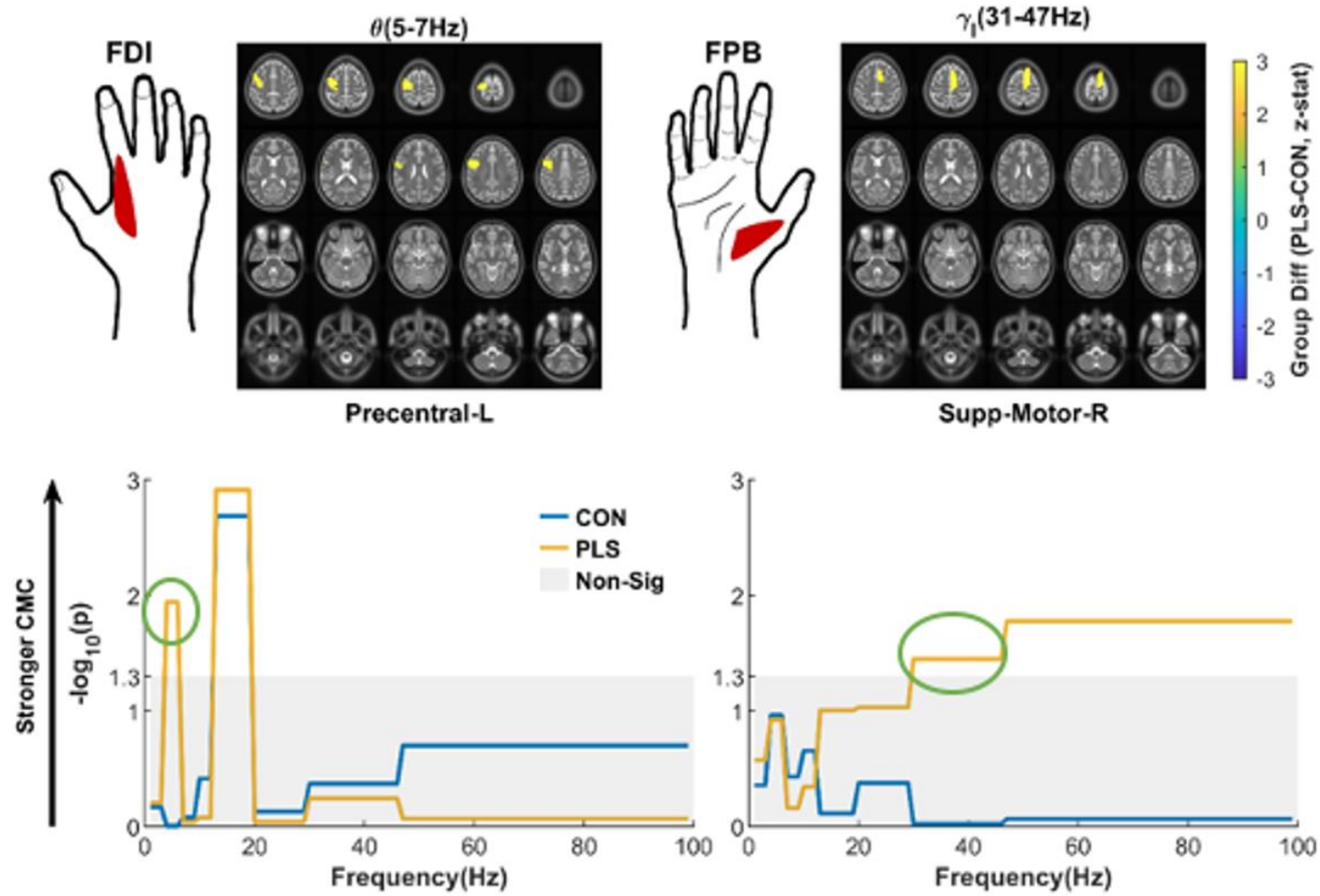


Figure 8-8 Cortico-muscular coherence (CMC) plot of PLS patients and healthy control group

Where significant group difference was observed after correction for multiple comparison using adaptive FDR at $q=0.05$. A significantly higher CMC was observed in PLS between FDI muscle and left Precentral region (primary motor region) at theta band (Left figures). Similarly, a significantly higher CMC was observed in PLS between FPB muscle and right Supplementary motor region at lower gamma band (Right figures).

Figure 8-8 shows the source-level cortico-muscular coherence. It can be seen that the sensor level decrease in the normal frequency bands and primary motor areas (seen in controls), is also observed in the PLS group at source level. Furthermore, there are additional abnormal CMC patterns in non-motor regions such as frontal areas, but also at abnormal (e.g., gamma) frequency bands in the premotor and other non-primary motor areas. Importantly, the localisation of significant differences between the source level CMC in Figure 8-8, points to left pre-central regions as well as supplementary motor areas, similarly confirming the presence of abnormal patterns in both primary motor areas and other areas that are not typically recognised for normal CMC in healthy individuals.

8.4. Discussion

Since its first clinical description by Charcot in 1874, PLS remains poorly understood with very few disease-specific clinical trials. Previous studies have provided evidence that the underlying pathology in PLS is not confined to the motor cortices and corticospinal tracts ^{473, 474}

These data show that neurophysiological signals can reliably capture changes in functional networks in PLS. Our data suggest that sensorimotor regions bilaterally has increased connectivity. The CMC findings has shown pathological increases in patterns of corticomuscular coherence, most notably over the parietal (Pz) and central (Cz) areas.

Changes in connectivity in PLS has been identified previously using functional neuroimaging. ^{475, 476}. However, these methods are limited by low temporal resolution, which limit the detection of dynamic compensatory pathways in PLS central and peripheral pathways.

Here, I have shown that EEG resting state can detect significant widespread frontal increases in connectivity patterns. The EEG resting state study demonstrate significant increases in β and γ frequency bands, with increased AEC in the frontal and parietal regions. These findings recapitulate those reported in imaging studies. These findings are congruent with previous resting state EEG studies in ALS, which demonstrate changes in the sensorimotor

cortex, such as decreased alpha-band power ^{477, 478}, with increased connectivity throughout the cortex ¹⁵¹.

The corticomuscular coherence study, capturing in real time cortical-peripheral dynamics compared to imaging techniques, has shown changes in time based functional networks during specific movement tasks ^{308, 330}. It is generally accepted that while many of the low-frequency oscillations have been associated with functional inhibition, faster gamma-band oscillations are believed to reflect cortical activation ⁴⁷⁹. Depending on cortical region, γ -oscillations are closely related to attentive processing, memory maintenance and perception ⁴⁸⁰⁻⁴⁸². This raises the possibility that there may be altered informational processing associated with PLS.

Despite limited autopsy data involvement of descending corticospinal pathways as a common feature in PLS ¹⁵⁷. ALS and PLS patients exhibit considerable brainstem atrophy compared to healthy controls ⁴⁷⁴. PLS Imaging studies have shown marked medullar, pontine and mesencephalic atrophy, ⁴⁷⁴ reflecting its distinct clinical presentation compared to ALS.

Other neurophysiology based studies have inferred loss of functional cortical motor neurons. Loss of fast conducting corticospinal axons can result in slowed voluntary movements in PLS that are likely to utilize slower conducting or indirect descending cortical projections or to be relayed through more primitive motor pathways ^{162, 483}. We hypothesize that the strong CMC patterns seen in our study reflects these alternative pathways in the face of the degeneration of the fast corticospinal axons. This is evidenced by the significant negative correlation seen between disease burden (aka increased spasticity) and decreased CMC between the APB muscle and the contralateral (C3) motor region on EEG.

The ECAS data in this study supports the presence a cognitive domain in PLS. Several previous studies have also described. impaired verbal fluency, executive function and memory impairment in PLS patients ^{473, 484}, PLS is associated with widespread neurophysiological cortical changes as well as previously documented subcortical pathology and neuropsychological deficits ¹⁶⁰. While it was not possible to characterize a neurophysiologic signal

associated with these cognitive changes in this study , as the numbers were too few, cortical gamma band oscillations have been linked to higher cognitive functions such as intermodal selective attention and perception ⁴⁸⁵.

Neuroimaging studies support these findings, with white matter changes in the limbic and interhemispheric regions⁴⁸⁶⁻⁴⁸⁸. This suggests that spectral EEG, along with neuroimaging have potential as a quantitative measure for detecting early cognitive impairment in both ALS and PLS. . As such, PLS should no longer be regarded as a pure upper motor neuron disorder, in light of these extra motor findings.

8.5. Limitations

Limitations in this study include a small sample size. This is a function of the relatively rare nature of PLS. However, despite this the overall group sample size provides robust statistical results. Source analysis of brain regions, increased sample sizes, and multivariate spectral analysis will be instrumental in further elucidating the patterned changes of motor circuitry in future studies. Future use of source localisation methods may be able to associate the identified abnormal patterns to more specific cortical areas. Corticomuscular coherence studies have a unique potential to provide insights into the neurophysiology of this disease.

Further analysis is required to provide a more in-depth understanding of how changes of the cortical circuitry occur in chronic neurodegenerative conditions, demonstrating the value of interrogating motor circuits using cortico-muscular coherence. The reliability of our CMC studies to date demonstrates the potential for CMC as a possible diagnostic biomarker.

PLS patients currently have long periods of diagnostic uncertainty, the period of time from symptom onset to meeting diagnostic criteria. Even with a diagnosis patients are very often excluded from therapeutic clinical trials. However, with an effective clinical biomarker, trial design maybe enhanced. Corticomuscular coherence lends itself to potentially filling the gap that currently exists.

8.6. Conclusion

Our study shows for the first time neurophysiological changes associated with increased gamma band spectral power on resting state EEG in PLS.

This study is the largest dedicated PLS neurophysiological study of its kind. Our study shows that CMC is a robust means to assess the function of motor circuits as an entire connected network. The unmasking of activity in other brain regions outside the primary motor cortex suggests a plasticity of the motor circuitry especially when these disruptions occur in the setting of chronic disease.

9. Chapter 9: Discussion and Conclusion

The aim of this thesis was to examine and further characterize the dysfunction in corticospinal associated networks in four clinically related conditions – namely ALS, PLS, SMA and post-polio syndrome. The objective was examined cortical brain networks using non-invasive methods based on neurophysiological and signal analysis, and to was to improve our understanding of the neurophysiology of degeneration within the corticospinal system in the context of these 4 different but related motor system degenerations.

My experiments were designed to determine how measurement of the neurophysiological disruption might help to develop novel neurophysiological markers associated with the various patterns of motor system dysfunction, and to provide quantitative data that can support sub-phenotyping, stratification, as well as network-specific prognostic biomarkers of the disease, in clinical settings. My main focus was on cortico-muscular coherence (CMC), which measure the linked neurophysiological signals in the context of specific motor tasks.

One of the central themes that underlies this work has been informed by the debate within the ALS world about whether the disease is best characterized as a cortical

degeneration with secondary anterior horn cell degeneration (“the dying forward hypothesis”) or a disease that begins in the anterior horn cell with spread and secondary degeneration of the corticospinal system. As ALS is a disease for which there is no naturally occurring animal model, the debate has been informed primarily by pathology studies, rodent models and more recently neurophysiological studies using transcranial magnetic stimulation (TMS).

The prevailing evidence in neurodegenerative conditions points to disease spread in a transsynaptic rather than contiguous manner, indicating the importance of neural networks.

Neural networks may be seen as a series of interconnected neural nodes that determine a physiological function. A neural network will typically be described as having synchronous baseline activity, a unified corticotrophic fate, and selective vulnerability to neurodegenerative illnesses.⁴⁸⁹ At a cellular level we know that in ALS mitochondria are essential for calcium buffering and energy production. This is maintained independently within the pre and postsynaptic areas⁴⁹⁰. This type of autonomy allows for the efficient long-distance communication of neurons, however there are concessions; the lysosomal intracellular upkeep processes responsible for recycling organelles and cellular debris function less effectively. As a result, abnormal structural changes in prion like proteins can propagate uncontrolled, and dysfunctional mitochondria accumulate⁴⁹⁰. These pathways on a molecular basis, can be viewed as being linked together with a shared purpose. When these molecular underpinnings degenerate, the neural networks follow suit, giving rise to progressive clinical manifestations, which are often varied e.g., cortical/subcortical, motor, frontal etc...

This is where CMC studies may shed more light on the subject matter of better defining and delineating the onset of ALS. CMC, as a tool can comparatively assess the functional abnormalities seen in the motor cortex and considers them in relation to both EMG and clinical changes. One could potentially finally answer the question of where ALS starts and when?

The motor cortex is traditionally thought of, as meeting the task of generating patterns of movement, with columns of motor cells within the spinal cord generating triggers of action to the individual muscles. Increasing our

understanding of what is a most complex area becomes even more pressing as we enter an era of gene therapy and its implication as a disease modifier. Given the delay between biological onset of disease and initiation of disease-modifying therapy, earlier detection of corticomotoneuronal abnormalities would allow for earlier interventions and potentially better clinical outcomes.

When considering the “Dying back vs the Dying forward” models of neurodegeneration, one has to reconcile what we know already in models already described in literature. A number of basic features have to be addressed. We can speculate that the reason for ALS complexity goes as far as to combine, the “dying forward” and “dying back” theories, which I reason as being not entirely mutually exclusive but rather likely to be a combination of both.

Firstly, degenerative changes are known to occur largely in anterior horn cells and brainstem motor neurons that receive monosynaptic connections from the motor cortex ¹⁶⁴ as well as in the CST neurons within the primary motor cortex. This has been established both by pathological examination and using neurophysiological investigations including TMS.

Secondly, in some forms of ALS, the disease only affects the corticospinal tract neurons ⁴⁹¹ while in other forms, it only affects anterior horn cells, or affects corticospinal tract neurons only very late in the disease. This is established by clinical examination, demonstrating various combinations of upper and lower motor neuron changes (spasticity, pathologically brisk deep tendon reflexes, the emergence of new pathological reflexes including plantar response and pectoral reflexes; the loss of dexterity, and the presence of wasting and fasciculations respectively). Thirdly, ALS progresses persistently between spinal, brainstem and cortical regions, in what has been labelled a ‘prion-like’ arrangement ²⁰⁶. This has been established by previous imaging and neurophysiological studies. Fourthly, cortical regions that have become affected late in the disease are linked via long-range synaptic connections ^{492, 493}. This has been established by previous neuroimaging and neuropsychological studies. .

Lastly, humans are the only species affected by sporadic ALS, and only nonhuman primate models of ALS have been able to show features of the disease seen in humans ⁴⁹⁴.

The corticomotoneuronal synapse sets primates apart from other mammalian species, while the number of these synapses and length of the axons within the corticospinal tract distinguish humans from nonhuman primates ¹¹².

With respect to the proposed hypothesis relating to disease spread, the axonal transport hypothesis ⁴⁹³ speaks to both synaptic integrity and the underlying importance of long-range axonal networks in disease spread. The developing synapse functions independently, while there is evidence this synaptic autonomy can continue into adulthood ⁴⁹⁵. Synaptic prion-like proteins, for example, have been shown to maintain activity-dependent changes in synaptic efficacy independently of nuclear transcription within neuronal soma ¹³⁹.

Abnormal adaptive changes in these prions like proteins can replicate uncontrolled, while dysfunctional mitochondria accumulate ^{139, 490}.

The larger the synapse and the longer the axon, the more likely this process is to malfunction, which may in part drive the vulnerability of the monosynaptic corticomotoneuronal synapse arises, with the onset of ALS in humans.

From different animal studies, we can infer that the human CST originates from a wide variety of cortical areas, including, the primary motor cortex (M1), the dorsal and ventral premotor cortices, supplementary motor area, and cingulate motor areas ^{112, 119}. The origins of the CST from many different cortical areas make it less likely that it fulfils a single role. Notably, the CST terminates broadly within the spinal gray matter, possibly reflecting control of somatosensory, reflex, autonomic, and somatic motor functions.

CST projections to the dorsal horn are an important source of presynaptic inhibition of primary sensory afferent fibers ¹²⁰, and this mechanism could allow for the removal of predictable sources of afferent input associated with feedforward motor commands for voluntary movement. We also know from both animal and human studies (based on trauma and vascular events that lesions involving CST cause a breakdown in fine sensorimotor control, implying a

deterioration not only in motor function, but also in the capacity to interrogate correctly the sensory feedback particularly with respect to fine /dexterous movements involving hand function. ¹¹².

Notwithstanding the wealth of knowledge regarding the complexity of motor control, there remains a perception that ALS as a disease of independent degeneration of the upper and lower motor neurons, which references the original clinicopathological studies of Charcot, in which he described amyotrophy (wasting due to denervation) and sclerosis of the lateral tracts of the spinal cord which decussate in the brainstem and, which include fibres from the motor cortex , thus classified anatomically as the corticospinal tract.

My work, when combined with our knowledge of the complexity of human motor circuitry, suggests that there is a logical error in the construct of the debate as to “where does ALS start”. There is an assumption, which has evolved from the perspective of the clinicopathological studies of the early 20th century, that the neuroaxis can be viewed as a static entity that neatly divides into “upper” and “lower motor” motor neuron systems, and that traffic flows in a linear and unidirectional manner. While the importance of the afferent pathways and the mechanisms or circuit modulation are understood in the context of motor control and disorders thereof, until recently, there has been limited interest in considering the motor circuitry spanning from the pre-motor cortex to the neuromuscular junction as a single network system. Rather than debating the point of whether disease origin starts as being in the cortex or at the neuromuscular junction /anterior horn cell, a more nuanced argument should be that a dynamic pattern of network activity renders the system vulnerable at multiple points, affecting the cortex or the LMN and its associated motor neuron pools. Thus, regardless of origin, we must acknowledge that both scenarios describe a network based system in which disequilibrium in one affects the other.

My work has applied modern neurophysiological tools to seek to deconstruct the dynamic pathways within the corticomotor system (in the context of voluntary movement) by interrogating conditions that are clinically characterized by the

predominance of either upper or lower motor neuron loss (PLS, and PPS and SMA respectively, and by the presence of mixed degeneration (ALS).

I formulated a series of hypotheses based around the disruption of motor subsystems during isometric motor tasks. In doing so, I have attempted to interrogate specific alteration within the motor networks and have sought to identify compensatory mechanisms in different conditions. With specific reference to ALS, I have developed a series of experimental paradigms that I have used to help uncover neurophysiologic correlates of disease heterogeneity, and that complemented the resting state and activation studies performed by other members of the research group to which I belong¹⁴⁹⁻¹⁵¹. I have explored this disruption in the context of what is known about the distinct pattern of motor neuron loss, as defined by clinical and pathological studies, and informed by animal models. With the knowledge that circuitry changes in ALS disrupt the “normal homeostatic” mechanisms , I have sought to explore the hypothesis that network disruptions are likely to form a spectrum in ALS, based on the relative distribution of upper and lower motor neuron involvement ,

I chose cortico-muscular coherence (CMC) between EEG-EMG to explore the specific alterations in motor networks, based on the hypothesis that cortico-muscular Coherence (CMC) between EEG and EMG provides valuable insights into the descending motor drive, and that interrogation of the cross correlation between two different signals (i.e. EEG/EMG) can be undertaken in both frequency domains²⁹⁷ using spectral coherence³²⁹.

The technique enables simultaneous recordings of multi-channel EEG and EMG for time-series This was determined by the observation that the linear correlation to quantify the strength of corticospinal input to motor neurons can be based on the frequency domain between EMG and concurrently recorded EEG³³⁰.

As a practicing clinician, not only did I seek to explore the underlying physiology of the clinical presentation of disease, I also sought to develop better quantitative measures that could be used to better characterize the disease state. I based my theoretical construct on the assumption that cortico-muscular coherence between EEG-EMG can quantify the network-specific impairments which are clinically valid. And on this basis, I sought to make a clinical correlate to the neurophysiologic measures.

My entire project was based on previous work performed by the team with whom I work. The group has previously demonstrated that MND/ALS and ALS/FTD encompasses a wide range of clinically distinct phenotypes with various degrees of motor and extra-motor ^{151, 327} involvement, driven by alterations in specific functional networks ^{38, 202}. We already know that one aspect of clinical heterogeneity in these conditions is driven by the varying degree of upper and lower motor neuron dysfunction, as manifest by the spectrum spanning in the MNDs ranging from PLS to Progressive Muscular Atrophy (PMA) and SMA ⁴⁹⁶, and the involvement of frontotemporal and frontostriatal networks in those with cognitive and behavioural change.

I determined that further characterization of the heterogeneity requires a network-based approach that recognizes the complex relationships between transcortical and corticofugal pathways and spinal cord circuitry ³²⁶.

My work has shown that on the CMC patterns in ALS/Polio/PLS/SMA are associated with a dynamic compensatory process.

My experiments have recorded extensive supraspinal changes in neurodegenerative diseases. The abnormal patterns of CMC recorded likely reflect functional re-organisation of the central-peripheral network, which could be plausibly related to a compensatory response to continuous remodelling of the motor units ⁴²². My findings are consistent with a rearrangement of the motor networks involved with specific muscles affected by motor neuron lesions. The results are consistent with the reports that have shown, in the context of motor nerve lesions, de-efferent portions of motor cortex can be enough of a trigger to give rise to reorganization. ⁴⁹⁷. This possibly reflects the compensatory non-specific recruitment of alternate motor circuits in the presence of α - motor neuron loss.

This remodeling of the motor unit also affects the upstream physiology, as demonstrated by the presence of abnormal cortical signalling may reflect compensatory cortical physiology. This compensatory function is expected (and previously described in other neurodegenerative conditions ^{498, 499} due to the plastic nature of neural networks.

9.1. Summary of Results

9.1.1. ALS Corticomuscular Coherence

Previous EEG studies have shown changes in network patterns, such as increased frontoparietal connectivity in ALS ^{149, 151}. I have postulated that the characterisation of the disease heterogeneity in terms of the underlying network disruptions requires alternative tools to explore these network changes and how they apply to clinical presentations. Most of the neurophysiological methods to date, have a primary focus on quantifying the structural degeneration of either upper or lower motor neurons, and imaging studies focus on the characterisation of the general features of disruption in the brain as a whole ^{149, 151} or in the cognitive domain ³²⁷. As a result, the motor decline, as the main feature of ALS, has never been fully quantified at a network level using modern tools of neurophysiology. We sought to investigate potential network changes during motor tasks on EEG/EMG recordings based on abnormalities characterised in resting state EEG analysis by the wider team from previous work carried out.

Comparison of CMC patterns in ALS and healthy controls revealed a significant pathological presence of alpha frequency bands (8-12Hz) in the ALS cohort. . In the ALS group the CMC was statistically significant ($p < 0.05$, $q = 0.05$) at group level and appeared at frequencies different from the commonly expected beta (14- 30Hz) frequency bands. Instead, the significant CMC appeared consistently in the alpha (8-12Hz) frequency band across several EEG-EMG channels. Importantly, this abnormal alpha-band coherence was observed at the ipsilateral motor area as well as (C4) area, but also over parietal and frontal areas (Fz and Pz). The abnormal CMC appeared in other (e.g., beta) frequency bands but was less consistent across EEG-EMG channels.

These findings show that there is a non-primary motor region connectivity detected on motor task involvement and demonstrated the presence of modulation of cortical circuitry in ALS.

As they were not observed in controls, these abnormal frequency bands most likely infer that this is a mechanism to compensate for primary motor (M1) corticospinal projections, considering the M1 degeneration in ALS ¹⁵¹. The

increased connectivity patterns seen in the frontal region supports previous MRI findings of cortical atrophy patterns and inter-connected cortical-subcortical grey matter regions ³⁷⁵.

Further analysis is required to fully characterize the pathologic CMC patterns in the ALS patient group.

The experimental work has demonstrated that the distinct signatures shown in ALS patients could be harnessed. The methodology may provide a means of unmasking altered neural communication in ALS and can help to provide new patient stratification perspectives in ALS in the future.

9.1.2. Resting State Beta-band Measures in EEG and ALS Motor Symptoms

The aim of these experiments was to study possible utility of source-reconstructed resting-state EEG measures, that reflect cortical motor network function, in quantifying the severity and progression of ALS motor symptoms. Previous resting-state EEG studies in ALS showed the capacity of this technique as a sensitive tool in to study different aspects of ALS . Differences between ALS patients and healthy controls have been described in different brain regions: a significant decrease in θ frequency spectral power over bilateral motor areas (spreading to δ - and α -bands and to adjacent scalp locations) was shown in ALS patients and it correlated with MRI measures of gray and white matter degeneration in motor cortical regions and cortico-spinal tracts ³⁸¹. Another study within our group expanded these findings further by showing a decrease in the β -band power source-localized in the sensorimotor network ¹⁵⁰. Considering this previous work, my focus was on the β -band power in the motor network as a measure of the overall activity of the cortico-spinal projections from the motor system to muscles ³⁸². This frequency band is expressed in the somatosensory and motor cortices ³⁸³ and it is mainly the result of electrical activity in layer V^o of cortical networks ³⁸⁴. Additionally, previous studies indicated that the EEG β -band power over the sensorimotor network differs in ALS patients when compared with healthy controls ³⁷¹.

9.1.3. *Motor β -band power and Clinical Scores*

A strong negative correlation was noted between beta-power values in the motor network and the fine motor functions sub-score of the ALSFRS-R. That is to say, that with high levels of β -power there is an associated higher clinical impairment. When analyzing the progression rate and motor β -power, I discovered a positive partial correlation between β -band power in the motor and the fine motor functions. When considering correlation between LMN score in ALS and β -band power, there was a strong correlation with the burden of LMN impairment. I also found a strong negative partial correlation between β -synchrony and the LMN score.

These findings expand the previously described correlations between connectivity changes in cortical networks and patients' neuropsychological impairment, highlighting the use of EEG as a multi-dimensional approach that can capture different aspects of the disease ¹⁵⁰. The findings regarding the correlation between EEG β -power and patients' motor symptoms-progression rate suggest that this measure might also have a prognostic value. Although longitudinal data was not carried out, in a clinical trial setting the EEG β -band would be an ideal outcome measure as it seems to modify with advanced motor impairment (as assessed by the fine motor function sub-score and LMN score). Consequently, an experimental therapeutic that stabilizes β -band power in the motor network over time would provide evidence of an underlying biological effect of the treatment. This work therefore shows that EEG-based measures have the potential to be developed as accessible and quantifiable biomarkers for severity and progression of motor disability, which remains a key unmet need of ALS therapeutic trials.

9.1.4. *Polio Corticomuscular Coherence*

Clinically, ALS is a condition characterized by a combination of both U/LMN dysfunction. In the lower motor neurons, the degeneration of the α motor system occurs prior to the γ - system, thereby changing the relative contribution of the α - γ , which distorts the patterns of neuromuscular communication in movement. This is evident clinically by the preservation of deep tendon reflexes in the context of marked muscle wasting. My objective was to distinguish and dissociate the electrophysiological signatures that arose from CMC recordings,

to allow for possible interpretation of the sensorimotor network communication patterns as they apply to each sub-system. By using specific patient subgroups, I sought to assess for specific network changes in communication patterns. I therefore deconstructed the motor system from a clinical perspective, by studying patients who had been affected by poliomyelitis, as a representation of a purely LMN condition. I also studied patients with SMA, representing an infant onset and genetically mediated form of LMN degeneration.

With respect to those previously affected by poliomyelitis, I sought to investigate by CMC the motor pathway disruption and the possible presence of (upstream) cortical reorganisation in adult patients with a history of paralytic poliomyelitis (a lower motor neuron group). My proposal was to explore the neurophysiological characteristics of long-term reorganisation of cortical networks as a consequence of a direct (virally mediated) insult to the anterior horn cell.

I have identified patterns of CMC in the Polio group that differed from those of the healthy controls. Specifically, the CMC in the Polio group did not show the typical beta-band CMC over contralateral motor area (as intended by the task selection). These null findings were anticipated in accordance with expectations from our pilot study in controls, as well as the anticipation of typical beta-band cortico-muscular coherence primarily over primary motor cortex, C3 electrode (seen in low force sustained muscle contraction^{297, 298, 303}). Significant CMC appeared consistently in the low gamma (31-47Hz) frequency band across several EEG-EMG channels in the polio cohort. Importantly, this abnormal low gamma-band coherence was observed not only over primary motor (C3 and Cz, and C4) areas, but also over parietal and frontal areas (Fz and Pz). The abnormal CMC appeared in other (e.g., alpha) frequency bands but was less consistent across EEG-EMG channels. These CMC patterns differed from the control cohort in frequency (beta vs. low-gamma) and location (contralateral motor C3 vs Frontal Fz and Parietal Pz)^{303, 372}. The pathological gamma-band CMC was a consistent finding and was observed at both frontal and parietal electrodes and in both FDI and FPB muscles.

This study provides robust neurophysiological evidence of extensive supraspinal changes in those affected by poliomyelitis in childhood, thus

confirming the hypothesis that an ictus to one part of the neuroaxis (in the form of isolated injury to anterior horn cells) leads to subsequent re-modelling of other parts of the motor network. The presence of altered frequency bands in unexpected cortical areas within the patient group implies the presence of a disrupted central-peripheral network. These changes most likely reflect functional re-organisation of the central-peripheral network, most likely as a compensatory response to continuous remodelling of the motor units ⁴²². This observation was most apparent in the cohort who experienced polio at a younger age, suggesting that although re-modelling can occur throughout life, if the ictus occurs early in life at a time of increased synaptic plasticity, the remodeling is likely to be more extensive.

9.1.5. *Polio - Resting State Study*

A number of studies have shown the usefulness of combining resting state EEG and connectivity measures for differential diagnosis of dementias and other neurodegenerative conditions ^{436, 437}. While noting our CMC findings, I sought to determine whether changes could be detected on resting state EEG in the same patient cohort. After a review of the literature, I noted that this is the first study of its kind to measure resting state EEG in Polio patients. I intended to examine further the CMC changes noted in Polio patients and examine the role of potential oscillatory changes in Polio in altered cortical processing. This aim was achieved by undertaking resting state studies in the same cohort of patients to further explore potential cortical changes previously detected. Analysis using spectral power, average connectivity and imaginary coherence showed significant changes in the polio group. Polio patients showed significant spectral power increases between θ and β frequency bands, especially in the parietal, occipital and subcortical regions. Widespread co-modulation decreases between healthy controls and Polio patients was noted in α , β and γ_h frequency bands. This study showed that neuroelectric signal analysis can quantify and capture changes occurring in the functional networks of Polio survivors. By using spectral power as well as two different means of connectivity measures (co-modulation and synchrony) I have shown statistically robust evidence of a widespread disruption of networks in Polio patients. These disruptions are consistent with patterned changes noted on CMC. Central beta oscillations

have been shown to synchronized after voluntary as well as after passive movements ⁴⁴³. Post-movement beta synchronization is thought to reflect, at least in part, active inhibition of the motor cortex by somatosensory afferents ⁴⁴³. Different frequency bands are mediated by complex neurochemistry with oscillations of frequencies 12–80 Hz linked to pyramidal neurons, regulated by GABA_A inhibitory interneurons ³⁸³. I have postulated that the loss of GABAergic interneurons, together with functional change of pyramidal neurons in Polio survivors (as demonstrate by increase in the lower frequency spectral power), can be attributed to structural regeneration/reorganisation of cortical networks of pyramidal cells and/or the interneurons that entrain them.

9.1.6. SMA Corticomuscular Coherence

The observed alterations in corticomotor circuitry following poliomyelitis have implications for other conditions, notably spinal muscular atrophy (SMA), a pure LMN condition caused by recessive mutations in the SMN gene. The findings within Polio group suggest that the CMC changes are due to an upstream effect of LMN injury, as such, one would expect the same/similar findings in the SMA group.

Significant CMC appeared consistently in the low gamma (31-47Hz) frequency band across several EEG-EMG channels. Importantly, this abnormal low gamma-band coherence was observed at the ipsilateral motor area as well as (C4) area, but also over central and frontal areas (Fz and Pz). The abnormal CMC appeared in other (e.g., alpha) frequency bands but was less consistently across EEG-EMG channels.

EEG-EMG coherence during functional motor tasks shows pathological changes in the central-peripheral communication in SMA Patients. Pathological locations of CMC suggest compensatory changes in the motor cortex, which involves broader cortical regions with synchronous activity to muscles, in those affected with SMA. The presence of wider cortical regions being recorded is consistent with previous MRI studies ³²⁸ where cortical hypertrophy was noted in older children / adults with SMA. Our results are similar to the Polio patient group, supporting the concept of upstream network changes when the injury is limited to the anterior horn cells. network based disease.

These findings have clinical relevance. Both conditions are traditionally viewed by clinicians as pure LMN disorders. Poliomyelitis is a monophasic illness, but there is strong evidence of ongoing remodelling at anterior horn cell / neuromuscular junction level throughout life. My data has shown the upstream remodelling also occurs. SMA is a progressive illness, with ongoing degeneration of anterior horn cells and progressive loss of function. In this instance, I have also shown evidence of upstream remodelling . Findings from both conditions support the concept of motor neuron diseases presenting as disorders that can be viewed through the prism of dynamic network disruption, in addition to a more static neuropathologic perspective that segregates the neuroaxis into discrete anatomic units.

9.1.7. PLS Corticomuscular Coherence

PLS is clinically considered to be a purely UMN disorder. The aim of this study was to explore different aspects of the functional neural network to provide better insight into the effects of PLS on central as well as peripheral brain network function.

Despite limited autopsy data, involvement of descending corticospinal pathways is considered to be a common feature in PLS ¹⁵⁷. ALS and PLS patients exhibit considerable brainstem atrophy compared to healthy controls ⁴⁷⁴. PLS Imaging studies have shown marked medullar, pontine and mesencephalic atrophy, ⁴⁷⁴ reflecting its distinct clinical presentation compared to ALS.

Other neurophysiology based studies have inferred loss of functional cortical motor neurons Loss of fast conducting corticospinal axons can result in slowed voluntary movements in PLS that are likely to utilize slower conducting or indirect descending cortical projections or to be relayed through more primitive motor pathways ^{162, 483}.

I have hypothesized that in PLS, there is an adaptive cortical network change that reflects the progressive degeneration of the corticospinal pathways, and that this can be harnessed as a quantitative measure of network change in PLS .

In this experimental paradigm, coherence was measured between intrinsic hand muscles (using bipolar EMG) and surface EEG electrodes over scalp. Abnormal CMC patterns were noted involving the parietal and central regions, with

abnormal frequency bands showing high gamma band despite a low force experimental setup. The differences were statistically significant ($p < 0.05$) both at individual subject level, as well as at the group level, and shows that the frequency, location and intensity specific features of CMC can reliably distinguish between healthy controls and the PLS patient group. I found a strong, positive partial correlation between CMC and UMN score at β frequency involving the APB muscle and the parietal region on EEG recording. I identified negative partial correlation between CMC and the UMN score involving APB muscle and contralateral motor region at alpha frequency. I have postulated that the strong CMC patterns seen in our study reflects alternative pathways in the context of degeneration of the fast corticospinal axons. This is evidenced by the significant negative correlation seen between disease burden (as measured clinically by increased spasticity) and decreased CMC between the APB muscle and the contralateral (C3) motor region on EEG.

9.1.8. Resting State in PLS

To further examine cortical network changes in PLS, EEG resting state was also employed to compare any abnormal findings found on CMC.

Analysis using spectral power, average connectivity and imaginary coherence revealed significant changes in the PLS group.

PLS patients showed widespread significant increases in AEC connectivity when compared to healthy controls. The most noteworthy changes are seen in the frontal and central regions in the γ frequency band. PLS patients showed significant decrease in synchrony at β band frequency. Changes were noted to be in the motor/premotor cortex region. Spectral power in PLS was significantly decreased across low to mid frequency bands (from δ to β band). The most notable changes were seen in the frontal, temporal and occipital regions. Significant increased spectral power noted in the γ frequency bands, with a dominance over the frontal regions.

These data show that neurophysiological signals can reliably capture changes in functional networks in PLS, indicating that sensorimotor regions bilaterally have increased connectivity. The CMC findings has shown pathological increases in patterns of corticomuscular coherence, most notably over the parietal (Pz) and central (Cz) areas. Additional analysis of resting state has

detected detect significant widespread frontal increases in connectivity patterns. These findings recapitulate those reported in imaging studies. These findings are congruent with previous resting state EEG studies in ALS, which demonstrate changes in the sensorimotor cortex, such as decreased alpha-band power ^{477, 478}, with increased connectivity throughout the cortex ¹⁵¹.

9.2. Proposed Neuronal Selection model in Motor Neuron Disease

From paediatric medicine, we know that controlled movement evolves as the nervous/muscular system develops. The concept of motor control allows for the idea of an integrated circuit of iterative communication from premotor cortex to the neuromuscular junction leading to muscle contraction, with multiple levels of feedback that permit smooth navigation of movement. As the motor system matures during infancy and early childhood, the requirements for motor control evolve. The Neuronal Group Selection theory ⁵⁰⁰ proposes that motor skill development arises from the continued dynamic interaction between the musculoskeletal system and structure/functions of the brain. In other words, “the brain's structures are changed by how the body is moved” ⁵⁰⁰ – indeed this is the conceptual framework that underpins practice for both elite athleticism and other motor skills such as those developed by professional musicians. . In infancy, neural networks are primed to enable development of efficient and precise movements. As the neuronal system matures, three input measures are needed for this “Neuronal Darwinism” ⁵⁰¹. In the first instance, the infant masters a basic portfolio of movement. Secondly, the establishment of sensory input to allow for select and precise forms of movement. Lastly, with repetitive voluntary controlled movement, motor pathways are established and refined ⁵⁰⁰. The human genetic blueprint and initial infant activity is the driving force behind the laying down of the immature neuronal networks ⁵⁰². This initial blueprint of neuronal networks is a form of maturing self-organization. The selection of one pathway over another enhances the synaptic efficacy needed in establishing robust neural circuits ⁵⁰². This constant pruning and adjusting neuronal circuits in early infant stages in the context of learning and adapting controlled voluntary movement allows for the selective organization of patterned networks in movement.

I propose that our findings in neurodegenerative disorders reflect and validate the neuronal group selection theory. I hypothesise that in the setting of neurodegenerative disease the motor system must re-engineer alternate pathways. As an established network is disrupted, I propose a model that allows for the disrupted communications to form and re-establish the less efficient networks that may have been “pruned” in early infancy (Fig 9-1).

While this model remains speculative, it is supported by reference to multiple studies that have found networking changes beyond the primary motor cortex ^{149-151, 371, 375}. The findings from my experiments illustrate that highly connected areas operate as a network that can be identified using neurophysiologic based tools. And regardless of the point of origin of a lesion (U/LMN), my findings support the idea that neuronal circuitry adapts, and communication pathways are re-modelled as individual neurons within the motor circuit degenerate. This remodelling occurs both “upstream” and “downstream” of the neuronal injury. This is supported by my experimental findings. despite the varying types of motor neuron involvement at differing levels (SMA/Polio/PLS/ALS) cortical connectivity changes. Whether these changes occur on previously established vestigial pathways remains to be determined. However, the emergence of “primitive reflexes “ in some neurodegenerations (e.g., snout, grasp, and rooting) supports this conjecture.

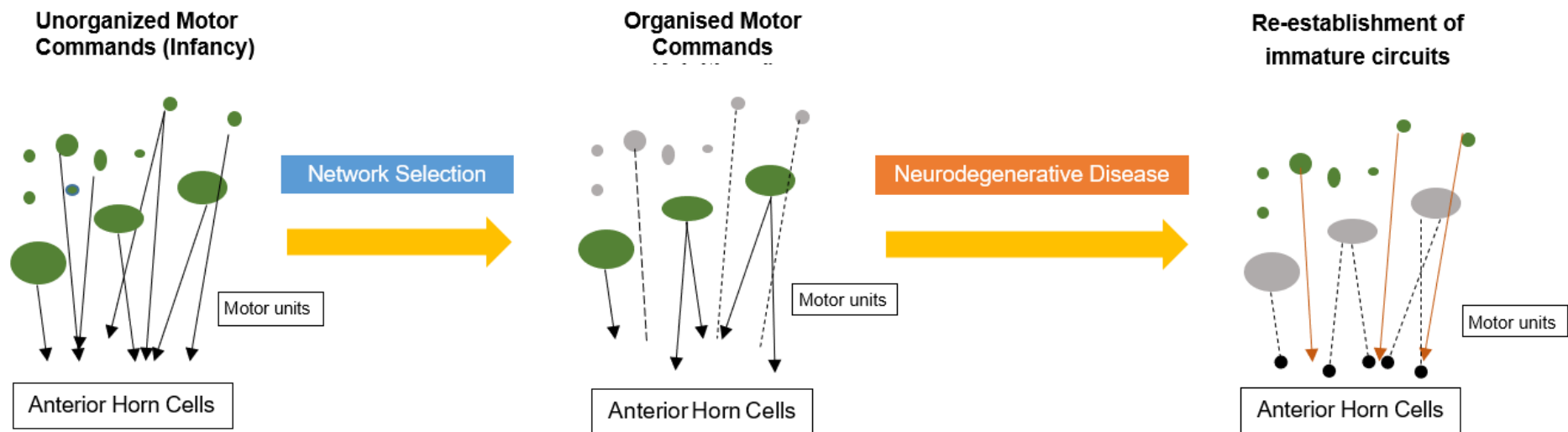


Figure 9-1 The establishment of the developmental process from infancy to adulthood of 'neuronal selection'.

Multiple inefficient pathways present in infancy are pruned to the selected few motor networks in adulthood. In the setting of disease, where these established pathways are disrupted the re-establishment of the immature network prevails over time during the course of the disease.

In graph theory (a mathematical application of using pairwise relations between regions) these networks are illustrated as nodes and edges (brain regions and connections). Given my ALS findings, one can theorise that the changes seen on CMC relate to the wider changes the neocortex undergoes in the face of progressing disease. If we speculate that the motor/premotor cortex maybe the centre point and considered a “local” network node from which disease fans out into the larger neocortex connectome.

Note that in the CMC ALS patient group the alpha band predominates, I hypothesize that this may reflect the relatively shorter time frame of disease onset and CMC recording. Contrast this to the Polio/SMA group, where the findings are strikingly similar (both noting changes in the frontal/parietal regions- with similar signatures CMC signatures of low gamma bands). The abnormal CMC patterns may represent compensatory mechanisms of cortical reorganisation which is more evident in patients with slower progression rates and longer disease duration.

The maturation of the neuroaxis in humans occurs at differing stages for different cortical regions ⁴¹⁴, and this may account for the observed differences I identified between those who experienced polio in infancy, and those who developed the disease in childhood or early adulthood. For those who developed the disease in infancy, there was greater capacity for the developing cortex to reorganise, result in a greater degree of compensatory neuronal network changes when compared to those affected later in life. ⁴¹⁵. These synaptic connections from the motor cortex to spinal α -motor neurons and interneurons are generally established early in humans compared to primates. Early corticospinal innervation was demonstrated by a study published in 2000 ⁴¹⁶, showing that in humans the cortex is intimately involved in the development of the spinal motor centre reflecting the uniquely dominant role of the corticomotoneuronal system in human control of movement ⁴¹⁶. This is likely to explain the contrasting patterns of CMC between the adult affected ALS/PLS group (central/parietal) regions and Polio/SMA (frontal/parietal) region.

Polio and SMA are both LMN conditions of childhood onset, while the former is acquired and latter genetic, both groups of patients were seen in adulthood for this study, years after this initial assault.

We know that LMN dysfunction does not occur in isolation, but rather maintains links with its UMN (cortical) counterparts that may indeed be reactive to the ongoing changes distally. This brings us to a pattern often described as the “dying-back” phenomenon. There have been varying mechanisms proposed in the context of ALS. While SMA and Polio differ in their pathogenesis, the progressive loss of motor neurons is leads to an insufficient maintenance of the distal axon, resulting in axonal abnormalities and eventual motor neuron loss. The precise cellular mechanisms underpinning the cortical changes in both SMA and old polio remain to be established Dendritic spine density has been previously linked to specific MRI measures in a study of neural plasticity in mice⁴⁶¹. The study showed that increased voxel based morphometry signalling was associated with an increase in dendritic spine density⁴⁶¹. Based on my findings, I hypothesize that adaptive compensatory mechanisms may take place in LMN disorders such as SMA, which may be driven by dendrite density modifications.

9.3. Advantages of Using Neurophysiological Measures

9.3.1. CMC- A Means to unlocking Neurodegeneration

ALS onset remains undefined. ALS as an example of a neurodegenerative disease, may be seen as a network disorder. As a neurodegenerative condition it has long been understood to start before the onset of clinical symptoms⁴², whether this be at the motor cortex or distally at the lower motor neurons. TMS and MRI studies showed changes in the motor cortex of pre-symptomatic carriers prior to the onset of clinical symptoms^{503, 504}. This raises the possibility that UMN dysfunction precedes clinical weakness.

9.3.2. Relevance of a Networked Based Approach

The failure of clinical trials to produce disease modifying drugs in neurodegeneration can be attributed to a failure to appreciate this network - based aspect of disease, the presence of clinical and biological heterogeneity, the absence of reliable biomarkers³⁸ and variance in currently-employed clinical

outcome measures; all pointing to an urgent need for quantitative biomarkers of both disease subtype and progression ⁵⁰⁵.

It is striking that there are no naturally occurring animal models of ALS, giving fuel to the idea that the enlarged neo-cortex in humans is integral to the disease process. The direct corticospinal projections is the most recent in the evolutionary process, with the least well conserved genes, thereby making it the most vulnerable to change ¹⁰¹. While most of the neocortex is involved in ALS, the motor and pre-motor cortices are likely the point of network node from which disease spreads out into the larger neocortical connectome ¹⁰¹. Identifying the region of origin of ALS is not just for academic interest, but rather essential. With a long pre-clinical period in ALS, the search for early markers of disease is essential to rescue dysfunctional but not recoverable neurons ⁵⁰⁶. Markers, such as CMC, have been shown in this study to be cognisant of the cortical events in ALS and neurodegenerative diseases at large.

The corticomotoneuronal synapse is a vital link between the CST and anterior horn cells, because of this vulnerability, it is also makes for the potentially weakest point for neurodegeneration to occur. Therefore, biomarkers like CMC, that can show changes in the integrity of this network should be able to identify the very earliest stages of ALS, allowing for early disease-modifying therapeutic interventions at a point when we can make a significant impact on survival.

9.4. Impact and Clinical Applications of CMC

9.4.1. CMC as a Therapeutic Outcome Measure in Neurodegenerative Diseases

There have been great strides made in the world of genetics, which has provided considerable insights into the pathology and mechanisms of many neurodegenerative and neuromuscular disorders. The translation of this insight into clinical practice has been challenging at times, and, until recently effective treatment approaches were rare. This all changed in 2016 with the onset and approval by the FDA of an antisense oligonucleotide first for Duchenne muscular dystrophy and then later the same year for SMA, in the form of nusinersen ²⁰¹. When first trialled nusinersen was investigated in SMA type 2 and 3 children before being trialled on SMA type1 infants. The largest gains

were noted in the infant group, one presumes as they had greater function to preserve. The question arises though is the clinical outcome measures used in these types of trials enough? While we continue to use the standard MRC power scoring system and functional rating scales, in the real terms are these enough?

As ASOs continue to be explored in other conditions , including genetic forms of ALS as well as now being offered to adult SMA, there is an urgent need both to identify disease at an earlier stage, and to provide outcome measure that are more sensitive than the standard clinical measurements.

Should we be at a stage to now consider neurophysiological biomarkers as potential outcome measures? My experiments suggest that CMC has considerable potential . While my experiments were limited in numbers, I have noted significant abnormal cortical signatures in all 4 diseases. I have postulated that these pathological signatures are a compensatory mechanism, perhaps reflecting years of network dysfunction. CMC could conceivably be used in the setting of ASOs and network recovery. With refinement, these signatures could be tracked and recorded before any clinical improvements noticed. This lends itself to the possibility that CMC, in addition to being a possible diagnostic tool could also prove useful as a marker of disease progression or indeed change (improvement).

CMC has been used in other neurodegenerations. Early studies have pointed to CMC as a possible therapeutic indicator in Parkinson's disease ⁵⁰⁷, both as an indicator of dopamine efficacy, and following deep brain stimulation of the subthalamic nucleus, the latter showing improvement of motor symptoms and normalizing pathologically altered oscillations ⁵⁰⁸.

CMC may potentially be used in identifying early network disruption in risk asymptomatic carriers of certain genetic variations of ALS. CMC can be used to track any abnormal changes during an asymptomatic period, and where any detected the risk of corticospinal tract degeneration assessed and a timely diagnosis is potentially reached in what is increasingly optimistically becoming a possible curable disease.

9.5. Limitations

In this section, general limitations which influenced the design of this project or limited the analyses/interpretation of results within this project are discussed. Limitations pertaining specifically to each analysis performed within this project are described within chapters 5-8.

The rapid rate of ALS progression⁵⁸ presented a challenge in the recruitment of patients to this hospital-based research. Participants had to be sufficiently mobile (at minimum, by wheelchair) to attend the research facility, and in the case of those no longer able to take public transport or drive due to decline in motor function, participants needed to be accompanied by someone who could transport them to and from the research location. This was particularly the case for adult SMA group. Furthermore, in the case of the motor component (e.g., grip task), participants required sufficient motor function to respond.

Recruitment was further limited by the exclusion criteria of the studies performed here. Each study required that participants not have any co-morbid neuromuscular or psychiatric diagnosis which might influence the electrophysiological measures under investigation, a typical exclusion criteria in such studies¹⁵¹.

Unfortunately, the pausing of Data collection in 2020 due to the Covid-19 pandemic, resulted in a slowing of recruitment, especially affecting the SMA patient group. Therefore, while a preliminary analysis was performed at individual level for the SMA group and described in chapter 7. CMC data collection remains ongoing for further analysis.

9.6. Future Work

9.6.1. Longitudinal CMC Studies

Longitudinal recording of CMC measures may provide insight into the ever changing nature of ALS. In broad terms, varying frequency bands in CMC represent different modes of neural communication between cerebral cortex and spinal cord. The challenge that faces us is the future focus on expanding our depth of knowledge in understanding the relationship between cortical and muscular activities and realising its potential applications in the clinical field. The motor signatures gained by in-depth study of the functional mechanism using CMC is more accurate than using EEG only⁵⁰⁹.

If CMC was to become the standard of motor decoding, it will eventually help exploit a longitudinal protocol. It would also benefit the longer term fruition of CMC to expand this study to include adult SMA patients that have newly commenced an ASO and to track them longitudinally over time to assess in depth out neurophysiological changes in the face of a disease modifying agent.

9.7. Overall Conclusion

This project has shown complex patterns of cortical networking changes across various neurodegenerative conditions. These changes definitively demonstrate that ALS, as an example of a neurodegenerative condition, should be thought of as a network based disorder. I have shown that regardless of site of onset (U/LMN), a pattern of dysfunction during a degenerative process is merely manifest by the end organ (i.e., the brain). My experiments challenge the prevailing view of ALS, PLS, SMA and polio. My work demonstrates that we could evaluate such conditions as a series of neural networks, while understanding that these compensatory mechanisms are insufficient to compensate for the loss of motor units over time. My experiments also point to the possibility of using CMC to track network changes in the setting of disease modifying therapies.

My work confirms that ALS is a complex disorder and cannot be explained by the occurrence of a single event. There is established evidence that the disorder is more likely to be the result of a multistep process, leading to motor neuron degeneration and clinical symptoms. Subtle changes at the NMJ may produce subtle changes in connectivity which may in turn render the system more vulnerable to subsequent genetic or environmental insults⁵¹⁰, and subtle change in cortical networking (early loss of inhibitory pathways) can also lead to downstream effect.

I have shown that compensatory mechanisms may take place even in “pure” LMN disorders, like SMA and Polio, which may be driven by dendrite density modifications. I propose that early assaults to the anterior horn cell are more likely to radically alter motor circuitry, as formation and elimination of synapses

occurs during infancy and childhood ⁴¹⁴. I propose that this “heterochronous synaptogenesis” ⁴²⁰ could differentially influence the development of a compensatory processes following anterior horn cell injury in infancy and later in life. Source analysis of CMC would be the ideal to better quantify the regions giving rise to abnormal signalling.

Finally, my work suggests that rather than recognizing that CMC is best placed as a diagnostic marker, CMC has the potential for development as a marker of disease progression within individuals. A future longitudinal design could advantageously also define the reliability of CMC within subjects.

10. Bibliography

1. Charcot J-M. Deux cas d'atrophie musculaire progressive avec lesions de la substance grise et faisceaux anterolateraux de la moelle epiniere. Arch Physiol Norm Pathol. 1869;2.
2. Moore C, McDermott CJ, Shaw PJ. Clinical aspects of motor neurone disease. Medicine. 2008;36(12):640-5.
3. McDermott CJ, Shaw PJ. Diagnosis and management of motor neurone disease. BMJ (Clinical research ed). 2008;336(7645):658-62.
4. Tiriyaki E, Horak HA. ALS and other motor neuron diseases. Continuum (Minneapolis, Minn). 2014;20(5 Peripheral Nervous System Disorders):1185-207.
5. Phukan J, Elamin M, Bede P, Jordan N, Gallagher L, Byrne S, et al. The syndrome of cognitive impairment in amyotrophic lateral sclerosis: a population-based study. J Neurol Neurosurg Psychiatry. 2012;83(1):102-8.
6. Burke T, Elamin M, Galvin M, Hardiman O, Pender N. Caregiver burden in amyotrophic lateral sclerosis: a cross-sectional investigation of predictors. J Neurol. 2015;262(6):1526-32.
7. Burke T, Pinto-Grau M, Loneragan K, Bede P, O'Sullivan M, Heverin M, et al. A Cross-sectional population-based investigation into behavioral change in amyotrophic lateral sclerosis: subphenotypes, staging, cognitive predictors, and survival. Ann Clin Transl Neurol. 2017;4(5):305-17.
8. Elamin M, Bede P, Byrne S, Jordan N, Gallagher L, Wynne B, et al. Cognitive changes predict functional decline in ALS: a population-based longitudinal study. Neurology. 2013;80(17):1590-7.
9. Pinto-Grau M, Hardiman O, Pender N. The Study of Language in the Amyotrophic Lateral Sclerosis - Frontotemporal Spectrum Disorder: a Systematic Review of Findings and New Perspectives. Neuropsychol Rev. 2018;28(2):251-68.
10. Gordon PH, Delgadillo D, Piquard A, Bruneteau G, Pradat PF, Salachas F, et al. The range and clinical impact of cognitive impairment in French patients with ALS: a cross-sectional study of neuropsychological test performance. Amyotroph Lateral Scler. 2011;12(5):372-8.
11. Brooks BR. El Escorial World Federation of Neurology criteria for the diagnosis of amyotrophic lateral sclerosis. Subcommittee on Motor Neuron Diseases/Amyotrophic Lateral Sclerosis of the World Federation of Neurology Research Group on Neuromuscular Diseases and the El Escorial "Clinical limits of amyotrophic lateral sclerosis" workshop contributors. Journal of the neurological sciences. 1994;124 Suppl:96-107.
12. Brooks BR, Miller RG, Swash M, Munsat TL. El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. Amyotroph Lateral Scler Other Motor Neuron Disord. 2000;1(5):293-9.
13. Agosta F, Al-Chalabi A, Filippi M, Hardiman O, Kaji R, Meininger V, et al. The El Escorial criteria: strengths and weaknesses. Amyotrophic lateral sclerosis & frontotemporal degeneration. 2015;16(1-2):1-7.
14. Hannaford A, Pavey N, van den Bos M, Geevasinga N, Menon P, Shefner JM, et al. Diagnostic Utility of Gold Coast Criteria in Amyotrophic Lateral Sclerosis. Ann Neurol. 2021;89(5):979-86.
15. Turner MR, Kiernan MC, Leigh PN, Talbot K. Biomarkers in amyotrophic lateral sclerosis. Lancet Neurol. 2009;8(1):94-109.
16. Chiò A. ISIS Survey: an international study on the diagnostic process and its implications in amyotrophic lateral sclerosis. J Neurol. 1999;246 Suppl 3:iii1-5.

17. de Carvalho M, Dengler R, Eisen A, England JD, Kaji R, Kimura J, et al. Electrodiagnostic criteria for diagnosis of ALS. *Clin Neurophysiol.* 2008;119(3):497-503.
18. Johnsen B, Pugdahl K, Fuglsang-Frederiksen A, Kollewe K, Paracka L, Dengler R, et al. Diagnostic criteria for amyotrophic lateral sclerosis: A multicentre study of inter-rater variation and sensitivity. *Clin Neurophysiol.* 2019;130(2):307-14.
19. Shefner JM, Al-Chalabi A, Baker MR, Cui LY, de Carvalho M, Eisen A, et al. A proposal for new diagnostic criteria for ALS. *Clin Neurophysiol.* 2020;131(8):1975-8.
20. Menon P, Geevasinga N, Yiannikas C, Howells J, Kiernan MC, Vucic S. Sensitivity and specificity of threshold tracking transcranial magnetic stimulation for diagnosis of amyotrophic lateral sclerosis: a prospective study. *Lancet Neurol.* 2015;14(5):478-84.
21. Strong MJ, Abrahams S, Goldstein LH, Woolley S, McLaughlin P, Snowden J, et al. Amyotrophic lateral sclerosis - frontotemporal spectrum disorder (ALS-FTSD): Revised diagnostic criteria. *Amyotroph Lateral Scler Frontotemporal Degener.* 2017;18(3-4):153-74.
22. Rascovsky K, Hodges JR, Knopman D, Mendez MF, Kramer JH, Neuhaus J, et al. Sensitivity of revised diagnostic criteria for the behavioural variant of frontotemporal dementia. *Brain : a journal of neurology.* 2011;134(Pt 9):2456-77.
23. Al-Chalabi A, Hardiman O. The epidemiology of ALS: a conspiracy of genes, environment and time. *Nat Rev Neurol.* 2013;9(11):617-28.
24. Donaghy C, O'Toole O, Sheehan C, Kee F, Hardiman O, Patterson V. An all-Ireland epidemiological study of MND, 2004-2005. *Eur J Neurol.* 2009;16(1):148-53.
25. Abhinav K, Stanton B, Johnston C, Hardstaff J, Orrell RW, Howard R, et al. Amyotrophic lateral sclerosis in South-East England: a population-based study. The South-East England register for amyotrophic lateral sclerosis (SEALS Registry). *Neuroepidemiology.* 2007;29(1-2):44-8.
26. Pugliatti M, Parish LD, Cossu P, Leoni S, Ticca A, Saddi MV, et al. Amyotrophic lateral sclerosis in Sardinia, insular Italy, 1995-2009. *J Neurol.* 2013;260(2):572-9.
27. Johnston CA, Stanton BR, Turner MR, Gray R, Blunt AH, Butt D, et al. Amyotrophic lateral sclerosis in an urban setting: a population based study of inner city London. *J Neurol.* 2006;253(12):1642-3.
28. Gordon PH, Mehal JM, Holman RC, Rowland LP, Rowland AS, Cheek JE. Incidence of amyotrophic lateral sclerosis among American Indians and Alaska natives. *JAMA Neurol.* 2013;70(4):476-80.
29. Gil J, Vazquez MC, Ketzoian C, Perna A, Marin B, Preux PM, et al. Prognosis of ALS: comparing data from the Limousin referral centre, France, and a Uruguayan population. *Amyotroph Lateral Scler.* 2009;10(5-6):355-60.
30. Zaldivar T, Gutierrez J, Lara G, Carbonara M, Logroscino G, Hardiman O. Reduced frequency of ALS in an ethnically mixed population: a population-based mortality study. *Neurology.* 2009;72(19):1640-5.
31. Ryan M, Zaldivar Vaillant T, McLaughlin RL, Doherty MA, Rooney J, Heverin M, et al. Comparison of the clinical and genetic features of amyotrophic lateral sclerosis across Cuban, Uruguayan and Irish clinic-based populations. *J Neurol Neurosurg Psychiatry.* 2019;90(6):659-65.
32. Cronin S, Hardiman O, Traynor BJ. Ethnic variation in the incidence of ALS: a systematic review. *Neurology.* 2007;68(13):1002-7.

33. Global, regional, and national burden of motor neuron diseases 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol.* 2018;17(12):1083-97.
34. Pinto-Grau M, Burke T, Lonergan K, McHugh C, Mays I, Madden C, et al. Screening for cognitive dysfunction in ALS: validation of the Edinburgh Cognitive and Behavioural ALS Screen (ECAS) using age and education adjusted normative data. *Amyotroph Lateral Scler Frontotemporal Degener.* 2017;18(1-2):99-106.
35. Byrne S, Elamin M, Bede P, Shatunov A, Walsh C, Corr B, et al. Cognitive and clinical characteristics of patients with amyotrophic lateral sclerosis carrying a C9orf72 repeat expansion: a population-based cohort study. *Lancet Neurol.* 2012;11(3):232-40.
36. O'Brien M, Burke T, Heverin M, Vajda A, McLaughlin R, Gibbons J, et al. Clustering of Neuropsychiatric Disease in First-Degree and Second-Degree Relatives of Patients With Amyotrophic Lateral Sclerosis. *JAMA Neurol.* 2017;74(12):1425-30.
37. Byrne S, Heverin M, Elamin M, Bede P, Lynch C, Kenna K, et al. Aggregation of neurologic and neuropsychiatric disease in amyotrophic lateral sclerosis kindreds: a population-based case-control cohort study of familial and sporadic amyotrophic lateral sclerosis. *Ann Neurol.* 2013;74(5):699-708.
38. Al-Chalabi A, Hardiman O, Kiernan MC, Chio A, Rix-Brooks B, van den Berg LH. Amyotrophic lateral sclerosis: moving towards a new classification system. *Lancet Neurol.* 2016;15(11):1182-94.
39. Zoccolella S, Beghi E, Palagano G, Fraddosio A, Samarelli V, Lamberti P, et al. Predictors of delay in the diagnosis and clinical trial entry of amyotrophic lateral sclerosis patients: a population-based study. *J Neurol Sci.* 2006;250(1-2):45-9.
40. Swash M. Why are upper motor neuron signs difficult to elicit in amyotrophic lateral sclerosis? *J Neurol Neurosurg Psychiatry.* 2012;83(6):659-62.
41. Huynh W, Simon NG, Grosskreutz J, Turner MR, Vucic S, Kiernan MC. Assessment of the upper motor neuron in amyotrophic lateral sclerosis. *Clin Neurophysiol.* 2016;127(7):2643-60.
42. Swash M, Ingram D. Preclinical and subclinical events in motor neuron disease. *J Neurol Neurosurg Psychiatry.* 1988;51(2):165-8.
43. Chio A, Hammond ER, Mora G, Bonito V, Filippini G. Development and evaluation of a clinical staging system for amyotrophic lateral sclerosis. *Journal of neurology, neurosurgery, and psychiatry.* 2015;86(1):38-44.
44. Roche JC, Rojas-Garcia R, Scott KM, Scotton W, Ellis CE, Burman R, et al. A proposed staging system for amyotrophic lateral sclerosis. *Brain.* 2012;135(Pt 3):847-52.
45. Fang T, Al Khleifat A, Stahl DR, Lazo La Torre C, Murphy C, Young C, et al. Comparison of the King's and MiToS staging systems for ALS. *Amyotrophic lateral sclerosis & frontotemporal degeneration.* 2017;18(3-4):227-32.
46. Balendra R, Al Khleifat A, Fang T, Al-Chalabi A. A standard operating procedure for King's ALS clinical staging. *Amyotrophic lateral sclerosis & frontotemporal degeneration.* 2019;20(3-4):159-64.
47. Elamin M, Phukan J, Bede P, Jordan N, Byrne S, Pender N, et al. Executive dysfunction is a negative prognostic indicator in patients with ALS without dementia. *Neurology.* 2011;76(14):1263-9.
48. Ryan M, Heverin M, McLaughlin RL, Hardiman O. Lifetime Risk and Heritability of Amyotrophic Lateral Sclerosis. *JAMA Neurology.* 2019;76(11):1367-74.
49. Zou ZY, Zhou ZR, Che CH, Liu CY, He RL, Huang HP. Genetic epidemiology of amyotrophic lateral sclerosis: a systematic review and meta-analysis. *J Neurol Neurosurg Psychiatry.* 2017;88(7):540-9.

50. Al-Chalabi A, van den Berg LH, Veldink J. Gene discovery in amyotrophic lateral sclerosis: implications for clinical management. *Nat Rev Neurol*. 2017;13(2):96-104.
51. Cady J, Allred P, Bali T, Pestronk A, Goate A, Miller TM, et al. Amyotrophic lateral sclerosis onset is influenced by the burden of rare variants in known amyotrophic lateral sclerosis genes. *Ann Neurol*. 2015;77(1):100-13.
52. Chiò A, Calvo A, Mazzini L, Cantello R, Mora G, Moglia C, et al. Extensive genetics of ALS: a population-based study in Italy. *Neurology*. 2012;79(19):1983-9.
53. Kenna KP, McLaughlin RL, Byrne S, Elamin M, Heverin M, Kenny EM, et al. Delineating the genetic heterogeneity of ALS using targeted high-throughput sequencing. *J Med Genet*. 2013;50(11):776-83.
54. Özoğuz A, Uyan Ö, Birdal G, Iskender C, Kartal E, Lahut S, et al. The distinct genetic pattern of ALS in Turkey and novel mutations. *Neurobiol Aging*. 2015;36(4):1764.e9-.e18.
55. van Rheenen W, van der Spek RAA, Bakker MK, van Vugt J, Hop PJ, Zwamborn RAJ, et al. Common and rare variant association analyses in amyotrophic lateral sclerosis identify 15 risk loci with distinct genetic architectures and neuron-specific biology. *Nat Genet*. 2021;53(12):1636-48.
56. Alsultan AA, Waller R, Heath PR, Kirby J. The genetics of amyotrophic lateral sclerosis: current insights. *Degener Neurol Neuromuscul Dis*. 2016;6:49-64.
57. Turner MR, Kiernan MC. Does interneuronal dysfunction contribute to neurodegeneration in amyotrophic lateral sclerosis? *Amyotroph Lateral Scler*. 2012;13(3):245-50.
58. Bonafede R, Mariotti R. ALS Pathogenesis and Therapeutic Approaches: The Role of Mesenchymal Stem Cells and Extracellular Vesicles. *Front Cell Neurosci*. 2017;11:80.
59. Zufiría M, Gil-Bea FJ, Fernández-Torrón R, Poza JJ, Muñoz-Blanco JL, Rojas-García R, et al. ALS: A bucket of genes, environment, metabolism and unknown ingredients. *Prog Neurobiol*. 2016;142:104-29.
60. Chen S, Sayana P, Zhang X, Le W. Genetics of amyotrophic lateral sclerosis: an update. *Mol Neurodegener*. 2013;8:28.
61. Renton AE, Chiò A, Traynor BJ. State of play in amyotrophic lateral sclerosis genetics. *Nat Neurosci*. 2014;17(1):17-23.
62. van Blitterswijk M, van Es MA, Hennekam EA, Dooijes D, van Rheenen W, Medic J, et al. Evidence for an oligogenic basis of amyotrophic lateral sclerosis. *Hum Mol Genet*. 2012;21(17):3776-84.
63. Hardiman O, Al-Chalabi A, Chio A, Corr EM, Logroscino G, Robberecht W, et al. Amyotrophic lateral sclerosis. *Nat Rev Dis Primers*. 2017;3:17071.
64. Boillée S, Vande Velde C, Cleveland DW. ALS: a disease of motor neurons and their nonneuronal neighbors. *Neuron*. 2006;52(1):39-59.
65. Wong PC, Cai H, Borchelt DR, Price DL. Genetically engineered mouse models of neurodegenerative diseases. *Nat Neurosci*. 2002;5(7):633-9.
66. Papadimitriou D, Le Verche V, Jacquier A, Ikiz B, Przedborski S, Re DB. Inflammation in ALS and SMA: sorting out the good from the evil. *Neurobiol Dis*. 2010;37(3):493-502.
67. Lalancette-Hebert M, Sharma A, Lyashchenko AK, Shneider NA. Gamma motor neurons survive and exacerbate alpha motor neuron degeneration in ALS. *Proc Natl Acad Sci U S A*. 2016;113(51):E8316-e25.
68. Kaur SJ, McKeown SR, Rashid S. Mutant SOD1 mediated pathogenesis of Amyotrophic Lateral Sclerosis. *Gene*. 2016;577(2):109-18.

69. Beckman JS, Estévez AG, Crow JP, Barbeito L. Superoxide dismutase and the death of motoneurons in ALS. *Trends Neurosci.* 2001;24(11 Suppl):S15-20.
70. Liochev SI, Fridovich I. Mutant Cu,Zn superoxide dismutases and familial amyotrophic lateral sclerosis: evaluation of oxidative hypotheses. *Free Radic Biol Med.* 2003;34(11):1383-9.
71. Zarei S, Carr K, Reiley L, Diaz K, Guerra O, Altamirano PF, et al. A comprehensive review of amyotrophic lateral sclerosis. *Surg Neurol Int.* 2015;6:171.
72. Bendotti C, Carri MT. Lessons from models of SOD1-linked familial ALS. *Trends Mol Med.* 2004;10(8):393-400.
73. Lin CL, Bristol LA, Jin L, Dykes-Hoberg M, Crawford T, Clawson L, et al. Aberrant RNA processing in a neurodegenerative disease: the cause for absent EAAT2, a glutamate transporter, in amyotrophic lateral sclerosis. *Neuron.* 1998;20(3):589-602.
74. Guo H, Lai L, Butchbach ME, Stockinger MP, Shan X, Bishop GA, et al. Increased expression of the glial glutamate transporter EAAT2 modulates excitotoxicity and delays the onset but not the outcome of ALS in mice. *Hum Mol Genet.* 2003;12(19):2519-32.
75. Kjældgaard AL, Pilely K, Olsen KS, Pedersen SW, Lauritsen A, Møller K, et al. Amyotrophic lateral sclerosis: The complement and inflammatory hypothesis. *Mol Immunol.* 2018;102:14-25.
76. Appel SH, Zhao W, Beers DR, Henkel JS. The microglial-motoneuron dialogue in ALS. *Acta Myol.* 2011;30(1):4-8.
77. Zhao W, Beers DR, Appel SH. Immune-mediated mechanisms in the pathoprosession of amyotrophic lateral sclerosis. *J Neuroimmune Pharmacol.* 2013;8(4):888-99.
78. Moisse K, Strong MJ. Innate immunity in amyotrophic lateral sclerosis. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease.* 2006;1762(11):1083-93.
79. Turner MR, Cagnin A, Turkheimer FE, Miller CC, Shaw CE, Brooks DJ, et al. Evidence of widespread cerebral microglial activation in amyotrophic lateral sclerosis: an [11C](R)-PK11195 positron emission tomography study. *Neurobiol Dis.* 2004;15(3):601-9.
80. Jara JH, Genç B, Stanford MJ, Pytel P, Roos RP, Weintraub S, et al. Evidence for an early innate immune response in the motor cortex of ALS. *J Neuroinflammation.* 2017;14(1):129.
81. Dupuis L, Loeffler J-P. Neuromuscular junction destruction during amyotrophic lateral sclerosis: insights from transgenic models. *Curr Opin Pharmacol.* 2009;9(3):341-6.
82. Musarò A. State of the art and the dark side of amyotrophic lateral sclerosis. *World J Biol Chem.* 2010;1(5):62-8.
83. Howland DS, Liu J, She Y, Goad B, Maragakis NJ, Kim B, et al. Focal loss of the glutamate transporter EAAT2 in a transgenic rat model of SOD1 mutant-mediated amyotrophic lateral sclerosis (ALS). *Proc Natl Acad Sci U S A.* 2002;99(3):1604-9.
84. Komine O, Yamanaka K. Neuroinflammation in motor neuron disease. *Nagoya J Med Sci.* 2015;77(4):537-49.
85. Johnson BS, Snead D, Lee JJ, McCaffery JM, Shorter J, Gitler AD. TDP-43 is intrinsically aggregation-prone, and amyotrophic lateral sclerosis-linked mutations accelerate aggregation and increase toxicity. *J Biol Chem.* 2009;284(30):20329-39.
86. Swash M. How does ALS spread between neurones in the CNS? *Journal of Neurology, Neurosurgery & Psychiatry.* 2013;84(1):116.

87. Prusiner SB. Cell biology. A unifying role for prions in neurodegenerative diseases. *Science*. 2012;336(6088):1511-3.
88. Veldink JH, Van den Berg LH, Wokke JH. The future of motor neuron disease: the challenge is in the genes. *J Neurol*. 2004;251(4):491-500.
89. Bensimon G, Lacomblez L, Meininger V. A controlled trial of riluzole in amyotrophic lateral sclerosis. ALS/Riluzole Study Group. *The New England journal of medicine*. 1994;330(9):585-91.
90. Lacomblez L, Bensimon G, Leigh PN, Guillet P, Meininger V. Dose-ranging study of riluzole in amyotrophic lateral sclerosis. Amyotrophic Lateral Sclerosis/Riluzole Study Group II. *Lancet (London, England)*. 1996;347(9013):1425-31.
91. Miller RG, Mitchell JD, Moore DH. Riluzole for amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND). *The Cochrane database of systematic reviews*. 2012(3):Cd001447.
92. National Institute for Health and Care Excellence (NICE) Guidance on the use of Riluzole (Rilutek) for the treatment of Motor Neurone Disease 2001 [Technology appraisal guidance [TA20]]. Available from: <https://www.nice.org.uk/Guidance/TA20>.
93. Miller RG, Jackson CE, Kasarskis EJ, England JD, Forshew D, Johnston W, et al. Practice Parameter update: The care of the patient with amyotrophic lateral sclerosis: Drug, nutritional, and respiratory therapies (an evidence-based review). Report of the Quality Standards Subcommittee of the American Academy of Neurology. 2009;73(15):1218-26.
94. Bellingham MC. A review of the neural mechanisms of action and clinical efficiency of riluzole in treating amyotrophic lateral sclerosis: what have we learned in the last decade? *CNS neuroscience & therapeutics*. 2011;17(1):4-31.
95. Dharmadasa T, Kiernan MC. Riluzole, disease stage and survival in ALS. *The Lancet Neurology*. 2018;17(5):385-6.
96. Safety and efficacy of edaravone in well defined patients with amyotrophic lateral sclerosis: a randomised, double-blind, placebo-controlled trial. *The Lancet Neurology*. 2017;16(7):505-12.
97. Hardiman O, van den Berg LH. Edaravone: a new treatment for ALS on the horizon? *The Lancet Neurology*. 2017;16(7):490-1.
98. Collins FS, Varmus H. A new initiative on precision medicine. *N Engl J Med*. 2015;372(9):793-5.
99. Montine TJ, Montine KS. Precision medicine: Clarity for the clinical and biological complexity of Alzheimer's and Parkinson's diseases. *J Exp Med*. 2015;212(5):601-5.
100. van den Bos MAJ, Geevasinga N, Higashihara M, Menon P, Vucic S. Pathophysiology and Diagnosis of ALS: Insights from Advances in Neurophysiological Techniques. *Int J Mol Sci*. 2019;20(11).
101. Eisen A. The Dying Forward Hypothesis of ALS: Tracing Its History. *Brain Sci*. 2021;11(3).
102. Eisen A. Amyotrophic lateral sclerosis-Evolutionary and other perspectives. *Muscle Nerve*. 2009;40(2):297-304.
103. Eisen A, Turner MR, Lemon R. Tools and talk: An evolutionary perspective on the functional deficits associated with amyotrophic lateral sclerosis. *Muscle Nerve*. 2014;49(4):469-77.
104. Turner MR, Agosta F, Bede P, Govind V, Lulé D, Verstraete E. Neuroimaging in amyotrophic lateral sclerosis. *Biomark Med*. 2012;6(3):319-37.

105. Eisen A, Kim S, Pant B. Amyotrophic lateral sclerosis (ALS): a phylogenetic disease of the corticomotoneuron? *Muscle Nerve*. 1992;15(2):219-24.
106. Williamson TL, Cleveland DW. Slowing of axonal transport is a very early event in the toxicity of ALS-linked SOD1 mutants to motor neurons. *Nat Neurosci*. 1999;2(1):50-6.
107. Fischer LR, Culver DG, Tennant P, Davis AA, Wang M, Castellano-Sanchez A, et al. Amyotrophic lateral sclerosis is a distal axonopathy: evidence in mice and man. *Exp Neurol*. 2004;185(2):232-40.
108. Finkelstein A, Kunis G, Seksenyan A, Ronen A, Berkutzki T, Azoulay D, et al. Abnormal Changes in NKT Cells, the IGF-1 Axis, and Liver Pathology in an Animal Model of ALS. *PLoS One*. 2011;6(8):e22374.
109. Smith MC. Nerve Fibre Degeneration in the Brain in Amyotrophic Lateral Sclerosis. *J Neurol Neurosurg Psychiatry*. 1960;23(4):269-82.
110. de Carvalho M, Eisen A, Krieger C, Swash M. Motoneuron firing in amyotrophic lateral sclerosis (ALS). *Front Hum Neurosci*. 2014;8(719).
111. Baker SN, Olivier E, Lemon RN. Task-related variation in corticospinal output evoked by transcranial magnetic stimulation in the macaque monkey. *J Physiol*. 1995;488 (Pt 3):795-801.
112. Lemon RN. Descending pathways in motor control. *Annu Rev Neurosci*. 2008;31:195-218.
113. Cheema SS, Rustioni A, Whitsel BL. Light and electron microscopic evidence for a direct corticospinal projection to superficial laminae of the dorsal horn in cats and monkeys. *J Comp Neurol*. 1984;225(2):276-90.
114. Lemon R. The Circuitry of the Human Spinal Cord: Its Role in Motor Control and Movement Disorders^[1]. *Brain*. 2006;129(2):551-4.
115. Maier MA, Illert M, Kirkwood PA, Nielsen J, Lemon RN. Does a C3-C4 propriospinal system transmit corticospinal excitation in the primate? An investigation in the macaque monkey. *J Physiol*. 1998;511 (Pt 1)(Pt 1):191-212.
116. Bacon SJ, Smith AD. A monosynaptic pathway from an identified vasomotor centre in the medial prefrontal cortex to an autonomic area in the thoracic spinal cord. *Neuroscience*. 1993;54(3):719-28.
117. Wolpaw JR. The complex structure of a simple memory. *Trends Neurosci*. 1997;20(12):588-94.
118. Martin JH, Kably B, Hacking A. Activity-dependent development of cortical axon terminations in the spinal cord and brain stem. *Exp Brain Res*. 1999;125(2):184-99.
119. Dum RP, Strick PL. Frontal lobe inputs to the digit representations of the motor areas on the lateral surface of the hemisphere. *J Neurosci*. 2005;25(6):1375-86.
120. Canedo A. Primary motor cortex influences on the descending and ascending systems. *Prog Neurobiol*. 1997;51(3):287-335.
121. Kanning KC, Kaplan A, Henderson CE. Motor neuron diversity in development and disease. *Annu Rev Neurosci*. 2010;33:409-40.
122. Burke RE, Levine DN, Tsairis P, Zajac FE, 3rd. Physiological types and histochemical profiles in motor units of the cat gastrocnemius. *J Physiol*. 1973;234(3):723-48.
123. Hunt CC, Kuffler SW. Further study of efferent small-nerve fibers to mammalian muscle spindles; multiple spindle innervation and activity during contraction. *J Physiol*. 1951;113(2-3):283-97.

124. Kuffler SW, Hunt CC, Quilliam JP. Function of medullated small-nerve fibers in mammalian ventral roots; efferent muscle spindle innervation. *J Neurophysiol.* 1951;14(1):29-54.
125. Burke RE, Strick PL, Kanda K, Kim CC, Walmsley B. Anatomy of medial gastrocnemius and soleus motor nuclei in cat spinal cord. *J Neurophysiol.* 1977;40(3):667-80.
126. Shneider NA, Brown MN, Smith CA, Pickel J, Alvarez FJ. Gamma motor neurons express distinct genetic markers at birth and require muscle spindle-derived GDNF for postnatal survival. *Neural Dev.* 2009;4:42.
127. Westbury DR. A comparison of the structures of alpha and gamma-spinal motoneurons of the cat. *J Physiol.* 1982;325:79-91.
128. Eccles JC, Eccles RM, Iggo A, Lundberg A. Electrophysiological studies on gamma motoneurons. *Acta Physiol Scand.* 1960;50:32-40.
129. Friesse A, Kaltschmidt JA, Ladle DR, Sigrist M, Jessell TM, Arber S. Gamma and alpha motor neurons distinguished by expression of transcription factor *Err3*. *Proc Natl Acad Sci U S A.* 2009;106(32):13588-93.
130. Mitsumoto H, Ulug AM, Pullman SL, Gooch CL, Chan S, Tang MX, et al. Quantitative objective markers for upper and lower motor neuron dysfunction in ALS. *Neurology.* 2007;68(17):1402-10.
131. Swash M, Leader M, Brown A, Swettenham KW. Focal loss of anterior horn cells in the cervical cord in motor neuron disease. *Brain.* 1986;109 (Pt 5):939-52.
132. Swash M, Fox KP. The pathology of the human muscle spindle: effect of denervation. *J Neurol Sci.* 1974;22(1):1-24.
133. Stephens B, Guiloff RJ, Navarrete R, Newman P, Nikhar N, Lewis P. Widespread loss of neuronal populations in the spinal ventral horn in sporadic motor neuron disease. A morphometric study. *J Neurol Sci.* 2006;244(1-2):41-58.
134. Dubowitz V. Muscle disorders in childhood. *Major Probl Clin Pediatr.* 1978;16:iii-xiii, 1-282.
135. Kubota M, Sakakihara Y, Uchiyama Y, Nara A, Nagata T, Nitta H, et al. New ocular movement detector system as a communication tool in ventilator-assisted Werdnig-Hoffmann disease. *Dev Med Child Neurol.* 2000;42(1):61-4.
136. Iwata M, Hirano A. Sparing of the Onufrowicz nucleus in sacral anterior horn lesions. *Ann Neurol.* 1978;4(3):245-9.
137. Sung JH, Mastri AR. Spinal autonomic neurons in Werdnig-Hoffmann disease, mannosidosis, and Hurler's syndrome: distribution of autonomic neurons in the sacral spinal cord. *J Neuropathol Exp Neurol.* 1980;39(4):441-51.
138. Baker MR. ALS—dying forward, backward or outward? *Nature Reviews Neurology.* 2014;10(11):660-.
139. Raveendra BL, Siemer AB, Puthanveetil SV, Hendrickson WA, Kandel ER, McDermott AE. Characterization of prion-like conformational changes of the neuronal isoform of Aplysia CPEB. *Nat Struct Mol Biol.* 2013;20(4):495-501.
140. Ramirez-Jarquin UN, Lazo-Gomez R, Tovar YRLB, Tapia R. Spinal inhibitory circuits and their role in motor neuron degeneration. *Neuropharmacology.* 2014;82:101-7.
141. Renshaw B. Influence of Discharge of Motoneurons Upon Excitation of Neighbouring Motoneurons. *J Neurophysiol.* 1941;4(2):167-83.
142. Pierrot-Deseilligny E, Bussel B. Evidence for recurrent inhibition by motoneurons in human subjects. *Brain Res.* 1975;88(1):105-8.
143. Schneider SP, Fyffe RE. Involvement of GABA and glycine in recurrent inhibition of spinal motoneurons. *J Neurophysiol.* 1992;68(2):397-406.

144. Alvarez FJ, Fyffe RE. The continuing case for the Renshaw cell. *J Physiol.* 2007;584(Pt 1):31-45.
145. Chang Q, Martin LJ. Glycinergic innervation of motoneurons is deficient in amyotrophic lateral sclerosis mice: a quantitative confocal analysis. *Am J Pathol.* 2009;174(2):574-85.
146. Mazzocchio R, Rossi A. Role of Renshaw cells in amyotrophic lateral sclerosis. *Muscle Nerve.* 2010;41(4):441-3.
147. Zagoraïou L, Akay T, Martin JF, Brownstone RM, Jessell TM, Miles GB. A cluster of cholinergic premotor interneurons modulates mouse locomotor activity. *Neuron.* 2009;64(5):645-62.
148. Bak TH, Chandran S. What wires together dies together: verbs, actions and neurodegeneration in motor neuron disease. *Cortex.* 2012;48(7):936-44.
149. Iyer PM, Egan C, Pinto-Grau M, Burke T, Elamin M, Nasserolelami B, et al. Functional Connectivity Changes in Resting-State EEG as Potential Biomarker for Amyotrophic Lateral Sclerosis. *PLoS One.* 2015;10(6):e0128682.
150. Dukic S, McMackin R, Buxo T, Fasano A, Chipika R, Pinto-Grau M, et al. Patterned functional network disruption in amyotrophic lateral sclerosis. *Hum Brain Mapp.* 2019;40(16):4827-42.
151. Nasserolelami B, Dukic S, Broderick M, Mohr K, Schuster C, Gavin B, et al. Characteristic Increases in EEG Connectivity Correlate With Changes of Structural MRI in Amyotrophic Lateral Sclerosis. *Cereb Cortex.* 2017:1-15.
152. Hebb DO. The organization of behavior; a neuropsychological theory. Oxford, England: Wiley; 1949. xix, 335-xix, p.
153. Bi G, Poo M. Distributed synaptic modification in neural networks induced by patterned stimulation. *Nature.* 1999;401(6755):792-6.
154. Katz LC, Shatz CJ. Synaptic activity and the construction of cortical circuits. *Science.* 1996;274(5290):1133-8.
155. Statland JM, Barohn RJ, Dimachkie MM, Floeter MK, Mitsumoto H. Primary Lateral Sclerosis. *Neurol Clin.* 2015;33(4):749-60.
156. Gordon PH, Cheng B, Katz IB, Pinto M, Hays AP, Mitsumoto H, et al. The natural history of primary lateral sclerosis. *Neurology.* 2006;66(5):647-53.
157. Singer MA, Statland JM, Wolfe GI, Barohn RJ. Primary lateral sclerosis. *Muscle Nerve.* 2007;35(3):291-302.
158. Erb W. Concerning Spastic and Syphilitic Spinal Paralysis. *Br Med J.* 1902;2(2180):1114-9.
159. Tartaglia MC, Rowe A, Findlater K, Orange JB, Grace G, Strong MJ. Differentiation between primary lateral sclerosis and amyotrophic lateral sclerosis: examination of symptoms and signs at disease onset and during follow-up. *Arch Neurol.* 2007;64(2):232-6.
160. Finegan E, Shing SLH, Chipika RH, Chang KM, McKenna MC, Doherty MA, et al. Extra-motor cerebral changes and manifestations in primary lateral sclerosis. *Brain Imaging Behav.* 2021.
161. Caselli RJ, Smith BE, Osborne D. Primary lateral sclerosis: a neuropsychological study. *Neurology.* 1995;45(11):2005-9.
162. Zhai P, Pagan F, Statland J, Butman JA, Floeter MK. Primary lateral sclerosis: A heterogeneous disorder composed of different subtypes? *Neurology.* 2003;60(8):1258-65.
163. Floeter MK, Mills R. Progression in primary lateral sclerosis: a prospective analysis. *Amyotroph Lateral Scler.* 2009;10(5-6):339-46.

164. Pringle CE, Hudson AJ, Munoz DG, Kiernan JA, Brown WF, Ebers GC. Primary lateral sclerosis. Clinical features, neuropathology and diagnostic criteria. *Brain*. 1992;115 (Pt 2):495-520.
165. Turner MR, Barohn RJ, Corcia P, Fink JK, Harms MB, Kiernan MC, et al. Primary lateral sclerosis: consensus diagnostic criteria. *J Neurol Neurosurg Psychiatry*. 2020;91(4):373-7.
166. Lo JK, Robinson LR. Postpolio syndrome and the late effects of poliomyelitis. Part 1. pathogenesis, biomechanical considerations, diagnosis, and investigations. *Muscle Nerve*. 2018;58(6):751-9.
167. Kidd D, Williams AJ, Howard RS. Poliomyelitis. *Postgrad Med J*. 1996;72(853):641-7.
168. Anderson GW, Rondeau JL. Absence of tonsils as a factor in the development of bulbar poliomyelitis. *J Am Med Assoc*. 1954;155(13):1123-30.
169. Plum F, Swanson AG. Central neurogenic hyperventilation in man. *AMA Arch Neurol Psychiatry*. 1959;81(5):535-49.
170. Muir P, Nicholson F, Jhetam M, Neogi S, Banatvala JE. Rapid diagnosis of enterovirus infection by magnetic bead extraction and polymerase chain reaction detection of enterovirus RNA in clinical specimens. *J Clin Microbiol*. 1993;31(1):31-8.
171. Bodian D. Histopathologic basis of clinical findings in poliomyelitis. *Am J Med*. 1949;6(5):563-78.
172. Gallagher HG. *FDR's splendid deception*. New York: Dodd, Mead; 1985.
173. Bradley WG. Recent views on amyotrophic lateral sclerosis with emphasis on electrophysiological studies. *Muscle Nerve*. 1987;10(6):490-502.
174. Dalakas MC. The post-polio syndrome as an evolved clinical entity. Definition and clinical description. *Ann N Y Acad Sci*. 1995;753:68-80.
175. Halstead LS. Research and clinical aspects of the late effects of poliomyelitis. Second research symposium. Warm Springs, Georgia, September 5-7, 1986. *Proceedings. Birth Defects Orig Artic Ser*. 1987;23(4):1-363.
176. Halstead LS. Assessment and differential diagnosis for post-polio syndrome. *Orthopedics*. 1991;14(11):1209-17.
177. Dimes Mo. Post-polio Syndrome: Identifying Best Practices in Diagnosis and Care. <http://www.post-polio.org/edu/pps.2001> [
178. Dalakas MC, Elder G, Hallett M, Ravits J, Baker M, Papadopoulos N, et al. A long-term follow-up study of patients with post-poliomyelitis neuromuscular symptoms. *N Engl J Med*. 1986;314(15):959-63.
179. Wiechers DO, Hubbell SL. Late changes in the motor unit after acute poliomyelitis. *Muscle Nerve*. 1981;4(6):524-8.
180. Cashman NR, Maselli R, Wollmann RL, Roos R, Simon R, Antel JP. Late denervation in patients with antecedent paralytic poliomyelitis. *N Engl J Med*. 1987;317(1):7-12.
181. Li Hi Shing S, Chipika RH, Finegan E, Murray D, Hardiman O, Bede P. Post-polio Syndrome: More Than Just a Lower Motor Neuron Disease. *Front Neurol*. 2019;10(773).
182. Jubelt B, Cashman NR. Neurological manifestations of the post-polio syndrome. *Crit Rev Neurobiol*. 1987;3(3):199-220.
183. Tomlinson BE, Irving D. The numbers of limb motor neurons in the human lumbosacral cord throughout life. *J Neurol Sci*. 1977;34(2):213-9.
184. Drachman DB, Murphy SR, Nigam MP, Hills JR. "Myopathic" changes in chronically denervated muscle. *Arch Neurol*. 1967;16(1):14-24.

185. Pezeshkpour GH, Dalakas MC. Long-term changes in the spinal cords of patients with old poliomyelitis. Signs of continuous disease activity. *Arch Neurol.* 1988;45(5):505-8.
186. Sharief MK, Hentges R, Ciardi M. Intrathecal immune response in patients with the post-polio syndrome. *N Engl J Med.* 1991;325(11):749-55.
187. Melchers W, de Visser M, Jongen P, van Loon A, Nibbeling R, Oostvogel P, et al. The postpolio syndrome: no evidence for poliovirus persistence. *Ann Neurol.* 1992;32(6):728-32.
188. Roivainen M, Kinnunen E, Hovi T. Twenty-one patients with strictly defined postpoliomyelitis syndrome: no poliovirus-specific IgM antibodies in the cerebrospinal fluid. *Ann Neurol.* 1994;36(1):115-6.
189. Kolb SJ, Kissel JT. Spinal Muscular Atrophy. *Neurol Clin.* 2015;33(4):831-46.
190. Sugarman EA, Nagan N, Zhu H, Akmaev VR, Zhou Z, Rohlfes EM, et al. Pan-ethnic carrier screening and prenatal diagnosis for spinal muscular atrophy: clinical laboratory analysis of >72,400 specimens. *Eur J Hum Genet.* 2012;20(1):27-32.
191. Munsat TL, Davies KE. International SMA consortium meeting. (26-28 June 1992, Bonn, Germany). *Neuromuscul Disord.* 1992;2(5-6):423-8.
192. Barkats M. [SMA: from gene discovery to gene therapy]. *Med Sci (Paris).* 2020;36(2):137-40.
193. Messina S, Sframeli M. New Treatments in Spinal Muscular Atrophy: Positive Results and New Challenges. *J Clin Med.* 2020;9(7).
194. Wang CH, Finkel RS, Bertini ES, Schroth M, Simonds A, Wong B, et al. Consensus statement for standard of care in spinal muscular atrophy. *J Child Neurol.* 2007;22(8):1027-49.
195. D'Amico A, Mercuri E, Tiziano FD, Bertini E. Spinal muscular atrophy. *Orphanet J Rare Dis.* 2011;6:71.
196. Russman BS. Spinal muscular atrophy: clinical classification and disease heterogeneity. *J Child Neurol.* 2007;22(8):946-51.
197. Zerres K, Davies KE. 59th ENMC International Workshop: Spinal Muscular Atrophies: recent progress and revised diagnostic criteria 17-19 April 1998, Soestduinen, The Netherlands. *Neuromuscul Disord.* 1999;9(4):272-8.
198. Farooq F, Holcik M, Mackenzie A. Spinal Muscular Atrophy: Classification, Diagnosis, Background, Molecular Mechanism and Development of Therapeutics. 2013.
199. Lefebvre S, Bürglen L, Reboullet S, Clermont O, Burlet P, Viollet L, et al. Identification and characterization of a spinal muscular atrophy-determining gene. *Cell.* 1995;80(1):155-65.
200. Lefebvre S, Burlet P, Liu Q, Bertrand S, Clermont O, Munnich A, et al. Correlation between severity and SMN protein level in spinal muscular atrophy. *Nat Genet.* 1997;16(3):265-9.
201. Chiriboga CA. Nusinersen for the treatment of spinal muscular atrophy. *Expert Rev Neurother.* 2017;17(10):955-62.
202. Hardiman O, van den Berg LH, Kiernan MC. Clinical diagnosis and management of amyotrophic lateral sclerosis. *Nat Rev Neurol.* 2011;7(11):639-49.
203. Verber NS, Shephard SR, Sassani M, McDonough HE, Moore SA, Alix JJP, et al. Biomarkers in Motor Neuron Disease: A State of the Art Review. *Front Neurol.* 2019;10:291.
204. Mitchell JD, Callaghan P, Gardham J, Mitchell C, Dixon M, Addison-Jones R, et al. Timelines in the diagnostic evaluation of people with suspected amyotrophic lateral

- sclerosis (ALS)/motor neuron disease (MND)--a 20-year review: can we do better? *Amyotroph Lateral Scler*. 2010;11(6):537-41.
205. Fatima M, Tan R, Halliday GM, Kril JJ. Spread of pathology in amyotrophic lateral sclerosis: assessment of phosphorylated TDP-43 along axonal pathways. *Acta Neuropathol Commun*. 2015;3:47.
 206. Kanouchi T, Ohkubo T, Yokota T. Can regional spreading of amyotrophic lateral sclerosis motor symptoms be explained by prion-like propagation? *J Neurol Neurosurg Psychiatry*. 2012;83(7):739-45.
 207. Ravits J, Appel S, Baloh RH, Barohn R, Brooks BR, Elman L, et al. Deciphering amyotrophic lateral sclerosis: what phenotype, neuropathology and genetics are telling us about pathogenesis. *Amyotroph Lateral Scler Frontotemporal Degener*. 2013;14 Suppl 1(0 1):5-18.
 208. Gomeni R, Fava M. Amyotrophic lateral sclerosis disease progression model. *Amyotroph Lateral Scler Frontotemporal Degener*. 2014;15(1-2):119-29.
 209. Rosengren LE, Karlsson JE, Karlsson JO, Persson LI, Wikkelsø C. Patients with amyotrophic lateral sclerosis and other neurodegenerative diseases have increased levels of neurofilament protein in CSF. *J Neurochem*. 1996;67(5):2013-8.
 210. Li D, Shen D, Tai H, Cui L. Neurofilaments in CSF As Diagnostic Biomarkers in Motor Neuron Disease: A Meta-Analysis. *Front Aging Neurosci*. 2016;8:290.
 211. Steinacker P, Feneberg E, Weishaupt J, Brettschneider J, Tumani H, Andersen PM, et al. Neurofilaments in the diagnosis of motoneuron diseases: a prospective study on 455 patients. *J Neurol Neurosurg Psychiatry*. 2016;87(1):12-20.
 212. Oeckl P, Jardel C, Salachas F, Lamari F, Andersen PM, Bowser R, et al. Multicenter validation of CSF neurofilaments as diagnostic biomarkers for ALS. *Amyotroph Lateral Scler Frontotemporal Degener*. 2016;17(5-6):404-13.
 213. Gaiani A, Martinelli I, Bello L, Querin G, Puthenparampil M, Ruggero S, et al. Diagnostic and Prognostic Biomarkers in Amyotrophic Lateral Sclerosis: Neurofilament Light Chain Levels in Definite Subtypes of Disease. *JAMA Neurol*. 2017;74(5):525-32.
 214. Skillbäck T, Mattsson N, Blennow K, Zetterberg H. Cerebrospinal fluid neurofilament light concentration in motor neuron disease and frontotemporal dementia predicts survival. *Amyotroph Lateral Scler Frontotemporal Degener*. 2017;18(5-6):397-403.
 215. Lehmer C, Oeckl P, Weishaupt JH, Volk AE, Diehl-Schmid J, Schroeter ML, et al. Poly-GP in cerebrospinal fluid links C9orf72-associated dipeptide repeat expression to the asymptomatic phase of ALS/FTD. *EMBO Mol Med*. 2017;9(7):859-68.
 216. Gendron TF, Chew J, Stankowski JN, Hayes LR, Zhang YJ, Prudencio M, et al. Poly(GP) proteins are a useful pharmacodynamic marker for C9ORF72-associated amyotrophic lateral sclerosis. *Sci Transl Med*. 2017;9(383).
 217. Cereda C, Leoni E, Milani P, Pansarasa O, Mazzini G, Guareschi S, et al. Altered intracellular localization of SOD1 in leukocytes from patients with sporadic amyotrophic lateral sclerosis. *PLoS One*. 2013;8(10):e75916.
 218. De Marco G, Lupino E, Calvo A, Moglia C, Buccinnà B, Grifoni S, et al. Cytoplasmic accumulation of TDP-43 in circulating lymphomonocytes of ALS patients with and without TARDBP mutations. *Acta Neuropathol*. 2011;121(5):611-22.
 219. Nardo G, Pozzi S, Pignataro M, Lauranzano E, Spano G, Garbelli S, et al. Amyotrophic lateral sclerosis multiprotein biomarkers in peripheral blood mononuclear cells. *PLoS One*. 2011;6(10):e25545.
 220. Charil A, Corbo M, Filippi M, Kesavadas C, Agosta F, Munerati E, et al. Structural and metabolic changes in the brain of patients with upper motor neuron

- disorders: a multiparametric MRI study. *Amyotroph Lateral Scler*. 2009;10(5-6):269-79.
221. Hecht MJ, Fellner F, Fellner C, Hilz MJ, Heuss D, Neundörfer B. MRI-FLAIR images of the head show corticospinal tract alterations in ALS patients more frequently than T2-, T1- and proton-density-weighted images. *J Neurol Sci*. 2001;186(1-2):37-44.
 222. Foerster BR, Dwamena BA, Petrou M, Carlos RC, Callaghan BC, Churchill CL, et al. Diagnostic accuracy of diffusion tensor imaging in amyotrophic lateral sclerosis: a systematic review and individual patient data meta-analysis. *Acad Radiol*. 2013;20(9):1099-106.
 223. Schuster C, Hardiman O, Bede P. Development of an Automated MRI-Based Diagnostic Protocol for Amyotrophic Lateral Sclerosis Using Disease-Specific Pathognomonic Features: A Quantitative Disease-State Classification Study. *PLoS One*. 2016;11(12):e0167331.
 224. Lehnert S, Costa J, de Carvalho M, Kirby J, Kuzma-Kozakiewicz M, Morelli C, et al. Multicentre quality control evaluation of different biomarker candidates for amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Frontotemporal Degener*. 2014;15(5-6):344-50.
 225. Kent-Braun JA, Walker CH, Weiner MW, Miller RG. Functional significance of upper and lower motor neuron impairment in amyotrophic lateral sclerosis. *Muscle Nerve*. 1998;21(6):762-8.
 226. Lomen-Hoerth C, Slawnych MP. Statistical motor unit number estimation: from theory to practice. *Muscle Nerve*. 2003;28(3):263-72.
 227. Drory VE, Kovach I, Groozman GB. Electrophysiologic evaluation of upper motor neuron involvement in amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Other Motor Neuron Disord*. 2001;2(3):147-52.
 228. Koelman JH, Bour LJ, Hilgevoord AA, van Bruggen GJ, Ongerboer de Visser BW. Soleus H-reflex tests and clinical signs of the upper motor neuron syndrome. *J Neurol Neurosurg Psychiatry*. 1993;56(7):776-81.
 229. Bussel B, Pierrot-Deseilligny E. Inhibition of human motoneurons, probably of Renshaw origin, elicited by an orthodromic motor discharge. *J Physiol*. 1977;269(2):319-39.
 230. Abbruzzese M, Minatel C, Faga D, Favale E. Testing for pre-synaptic and post-synaptic changes in the soleus H reflex pathway following selective muscle vibration in humans. *Neurosci Lett*. 1997;231(2):99-102.
 231. Katz R, Pierrot-Deseilligny E. Facilitation of soleus-coupled Renshaw cells during voluntary contraction of pretibial flexor muscles in man. *J Physiol*. 1984;355:587-603.
 232. Maffiuletti NA, Martin A, Babault N, Pensini M, Lucas B, Schieppati M. Electrical and mechanical H(max)-to-M(max) ratio in power- and endurance-trained athletes. *J Appl Physiol* (1985). 2001;90(1):3-9.
 233. Buchthal F, Schmalbruch H. Contraction times of twitches evoked by H-reflexes. *Acta Physiol Scand*. 1970;80(3):378-82.
 234. Burke D, Gandevia SC, McKeon B. Monosynaptic and oligosynaptic contributions to human ankle jerk and H-reflex. *J Neurophysiol*. 1984;52(3):435-48.
 235. Misiaszek JE. The H-reflex as a tool in neurophysiology: its limitations and uses in understanding nervous system function. *Muscle Nerve*. 2003;28(2):144-60.
 236. Raynor EM, Shefner JM. Recurrent inhibition is decreased in patients with amyotrophic lateral sclerosis. *Neurology*. 1994;44(11):2148-53.

237. Mazzini L, Balzarini C, Gareri F, Brigatti M. H-reflex changes in the course of amyotrophic lateral sclerosis. *Electroencephalogr Clin Neurophysiol*. 1997;104(5):411-7.
238. Simon NG, Lin CS, Lee M, Howells J, Vucic S, Burke D, et al. Segmental motoneuronal dysfunction is a feature of amyotrophic lateral sclerosis. *Clin Neurophysiol*. 2015;126(4):828-36.
239. Pierrot-Deseilligny E, Mazevet D. The monosynaptic reflex: a tool to investigate motor control in humans. Interest and limits. *Neurophysiol Clin*. 2000;30(2):67-80.
240. Feiereisen P, Duchateau J, Hainaut K. Motor unit recruitment order during voluntary and electrically induced contractions in the tibialis anterior. *Exp Brain Res*. 1997;114(1):117-23.
241. Wynne MM, Burns JM, Eland DC, Conatser RR, Howell JN. Effect of counterstrain on stretch reflexes, hoffmann reflexes, and clinical outcomes in subjects with plantar fasciitis. *J Am Osteopath Assoc*. 2006;106(9):547-56.
242. Weber F. The diagnostic sensitivity of different F wave parameters. *Journal of Neurology, Neurosurgery & Psychiatry*. 1998;65(4):535-40.
243. Sathya GR, Krishnamurthy N, Veliath S, Arulneyam J, Venkatachalam J. F wave index: A diagnostic tool for peripheral neuropathy. *Indian J Med Res*. 2017;145(3):353-7.
244. Drory VE, Neufeld MY, Korczyn AD. F-wave characteristics following acute and chronic upper motor neuron lesions. *Electromyogr Clin Neurophysiol*. 1993;33(7):441-6.
245. Fisher MA. AAEM Minimonograph #13: H reflexes and F waves: physiology and clinical indications. *Muscle Nerve*. 1992;15(11):1223-33.
246. Fox JE, Hitchcock ER. F wave size as a monitor of motor neuron excitability: the effect of deafferentation. *J Neurol Neurosurg Psychiatry*. 1987;50(4):453-9.
247. Eisen A, Swash M. Clinical neurophysiology of ALS. *Clin Neurophysiol*. 2001;112(12):2190-201.
248. de Carvalho M, Scotto M, Lopes A, Swash M. F-Waves and the corticospinal lesion in amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Other Motor Neuron Disord*. 2002;3(3):131-6.
249. Fisher MA. F/M ratios in polyneuropathy and spastic hyperreflexia. *Muscle Nerve*. 1988;11(3):217-22.
250. McComas AJ, Fawcett PR, Campbell MJ, Sica RE. Electrophysiological estimation of the number of motor units within a human muscle. *J Neurol Neurosurg Psychiatry*. 1971;34(2):121-31.
251. Kadrie HA, Yates SK, Milner-Brown HS, Brown WF. Multiple point electrical stimulation of ulnar and median nerves. *J Neurol Neurosurg Psychiatry*. 1976;39(10):973-85.
252. Stein RB, Yang JF. Methods for estimating the number of motor units in human muscles. *Ann Neurol*. 1990;28(4):487-95.
253. Gooch CL, Doherty TJ, Chan KM, Bromberg MB, Lewis RA, Stashuk DW, et al. Motor unit number estimation: a technology and literature review. *Muscle Nerve*. 2014;50(6):884-93.
254. Doherty TJ, Komori T, Stashuk DW, Kassam A, Brown WF. Physiological properties of single thenar motor units in the F-response of younger and older adults. *Muscle Nerve*. 1994;17(8):860-72.
255. Shefner JM, Watson ML, Simionescu L, Caress JB, Burns TM, Maragakis NJ, et al. Multipoint incremental motor unit number estimation as an outcome measure in ALS. *Neurology*. 2011;77(3):235-41.

256. Jagtap SA, Kuruvilla A, Govind P, Nair MD, Sarada C, Varma RP. Multipoint incremental motor unit number estimation versus amyotrophic lateral sclerosis functional rating scale and the medical research council sum score as an outcome measure in amyotrophic lateral sclerosis. *Ann Indian Acad Neurol.* 2014;17(3):336-9.
257. Brown WF, Strong MJ, Snow R. Methods for estimating numbers of motor units in biceps-brachialis muscles and losses of motor units with aging. *Muscle Nerve.* 1988;11(5):423-32.
258. Doherty TJ, Stashuk DW. Decomposition-based quantitative electromyography: methods and initial normative data in five muscles. *Muscle Nerve.* 2003;28(2):204-11.
259. Henderson RD, McCombe PA. Assessment of Motor Units in Neuromuscular Disease. *Neurotherapeutics.* 2017;14(1):69-77.
260. Nandedkar SD, Barkhaus PE, Stalberg EV. Motor unit number index (MUNIX): principle, method, and findings in healthy subjects and in patients with motor neuron disease. *Muscle Nerve.* 2010;42(5):798-807.
261. Neuwirth C, Barkhaus PE, Burkhardt C, Castro J, Czell D, de Carvalho M, et al. Motor Unit Number Index (MUNIX) detects motor neuron loss in pre-symptomatic muscles in Amyotrophic Lateral Sclerosis. *Clin Neurophysiol.* 2016.
262. Neuwirth C, Barkhaus PE, Burkhardt C, Castro J, Czell D, de Carvalho M, et al. Tracking motor neuron loss in a set of six muscles in amyotrophic lateral sclerosis using the Motor Unit Number Index (MUNIX): a 15-month longitudinal multicentre trial. *J Neurol Neurosurg Psychiatry.* 2015;86(11):1172-9.
263. Bromberg MB, Brownell AA. Motor unit number estimation in the assessment of performance and function in motor neuron disease. *Phys Med Rehabil Clin N Am.* 2008;19(3):509-32, ix.
264. de Carvalho M, Swash M. Fasciculation potentials and earliest changes in motor unit physiology in ALS. *J Neurol Neurosurg Psychiatry.* 2013;84(9):963-8.
265. de Carvalho M, Turkman A, Swash M. Sensitivity of MUP parameters in detecting change in early ALS. *Clin Neurophysiol.* 2014;125(1):166-9.
266. Neuwirth C, Nandedkar S, Stalberg E, Weber M. Motor unit number index (MUNIX): a novel neurophysiological technique to follow disease progression in amyotrophic lateral sclerosis. *Muscle Nerve.* 2010;42(3):379-84.
267. Wohlfart G. Collateral regeneration from residual motor nerve fibers in amyotrophic lateral sclerosis. *Neurology.* 1957;7(2):124-34.
268. Aggarwal A, Nicholson G. Detection of preclinical motor neurone loss in SOD1 mutation carriers using motor unit number estimation. *J Neurol Neurosurg Psychiatry.* 2002;73(2):199-201.
269. Benatar M, Wu J. Presymptomatic studies in ALS: rationale, challenges, and approach. *Neurology.* 2012;79(16):1732-9.
270. Turner MR, Benatar M. Ensuring continued progress in biomarkers for amyotrophic lateral sclerosis. *Muscle Nerve.* 2015;51(1):14-8.
271. Ahn SW, Kim SH, Kim JE, Kim SM, Kim SH, Park KS, et al. Reproducibility of the motor unit number index (MUNIX) in normal controls and amyotrophic lateral sclerosis patients. *Muscle Nerve.* 2010;42(5):808-13.
272. Nandedkar SD, Barkhaus PE, Stalberg EV. Reproducibility of MUNIX in patients with amyotrophic lateral sclerosis. *Muscle Nerve.* 2011;44(6):919-22.
273. Neuwirth C, Nandedkar S, Stalberg E, Barkhaus PE, Carvalho M, Furtula J, et al. Motor Unit Number Index (MUNIX): a novel neurophysiological marker for neuromuscular disorders; test-retest reliability in healthy volunteers. *Clin Neurophysiol.* 2011;122(9):1867-72.

274. Furtula J, Johnsen B, Christensen PB, Pugdahl K, Bisgaard C, Christensen MK, et al. MUNIX and incremental stimulation MUNE in ALS patients and control subjects. *Clin Neurophysiol.* 2013;124(3):610-8.
275. Boekestein WA, Schelhaas HJ, van Putten MJ, Stegeman DF, Zwarts MJ, van Dijk JP. Motor unit number index (MUNIX) versus motor unit number estimation (MUNE): a direct comparison in a longitudinal study of ALS patients. *Clin Neurophysiol.* 2012;123(8):1644-9.
276. Wilbourn AJ. The "split hand syndrome". *Muscle Nerve.* 2000;23(1):138.
277. Bae JS, Sawai S, Misawa S, Kanai K, Iose S, Kuwabara S. Differences in excitability properties of FDI and ADM motor axons. *Muscle Nerve.* 2009;39(3):350-4.
278. <cortical split hand.pdf>.
279. Eisen A, Kuwabara S. The split hand syndrome in amyotrophic lateral sclerosis. *J Neurol Neurosurg Psychiatry.* 2012;83(4):399-403.
280. Voermans NC, Schelhaas HJ, Munneke M, Zwarts MJ. Dissociated small hand muscle atrophy in aging: the 'senile hand' is a split hand. *Eur J Neurol.* 2006;13(12):1381-4.
281. Weber M, Eisen A, Stewart H, Hirota N. The split hand in ALS has a cortical basis. *J Neurol Sci.* 2000;180(1-2):66-70.
282. Lawrence DG, Kuypers HG. The functional organization of the motor system in the monkey. I. The effects of bilateral pyramidal lesions. *Brain.* 1968;91(1):1-14.
283. Menon P, Kiernan MC, Yiannikas C, Stroud J, Vucic S. Split-hand index for the diagnosis of amyotrophic lateral sclerosis. *Clin Neurophysiol.* 2013;124(2):410-6.
284. Kim DG, Hong YH, Shin JY, Park KH, Sohn SY, Lee KW, et al. Split-hand phenomenon in amyotrophic lateral sclerosis: A motor unit number index study. *Muscle Nerve.* 2016;53(6):885-8.
285. Kayser J, Tenke CE. Issues and considerations for using the scalp surface Laplacian in EEG/ERP research: A tutorial review. *Int J Psychophysiol.* 2015;97(3):189-209.
286. Friston KJ. Brain function, nonlinear coupling, and neuronal transients. *Neuroscientist.* 2001;7(5):406-18.
287. Guevara R, Velazquez JL, Nenadovic V, Wennberg R, Senjanovic G, Dominguez LG. Phase synchronization measurements using electroencephalographic recordings: what can we really say about neuronal synchrony? *Neuroinformatics.* 2005;3(4):301-14.
288. McMackin R, Bede P, Pender N, Hardiman O, Nasserroleslami B. Neurophysiological markers of network dysfunction in neurodegenerative diseases. *Neuroimage Clin.* 2019;22:101706.
289. FM G. Physico-Mathesis de Lumine Coloribus, et Iride. Bologna1665
290. McLachlan RS, Leung LW. A movement-associated fast rolandic rhythm. *Can J Neurol Sci.* 1991;18(3):333-6.
291. Grosse P, Cassidy MJ, Brown P. EEG-EMG, MEG-EMG and EMG-EMG frequency analysis: physiological principles and clinical applications. *Clin Neurophysiol.* 2002;113(10):1523-31.
292. Fisher KM, Zaaimi B, Williams TL, Baker SN, Baker MR. Beta-band intermuscular coherence: a novel biomarker of upper motor neuron dysfunction in motor neuron disease. *Brain.* 2012;135(Pt 9):2849-64.
293. Murayama N, Lin YY, Salenius S, Hari R. Oscillatory interaction between human motor cortex and trunk muscles during isometric contraction. *Neuroimage.* 2001;14(5):1206-13.

294. Kristeva-Feige R, Fritsch C, Timmer J, Lucking CH. Effects of attention and precision of exerted force on beta range EEG-EMG synchronization during a maintained motor contraction task. *Clin Neurophysiol.* 2002;113(1):124-31.
295. Kilner JM, Baker SN, Salenius S, Jousmaki V, Hari R, Lemon RN. Task-dependent modulation of 15-30 Hz coherence between rectified EMGs from human hand and forearm muscles. *J Physiol.* 1999;516 (Pt 2):559-70.
296. Farina D, Merletti R, Enoka RM. The extraction of neural strategies from the surface EMG: an update. *J Appl Physiol* (1985). 2014;117(11):1215-30.
297. Mima T, Hallett M. Corticomuscular coherence: a review. *J Clin Neurophysiol.* 1999;16(6):501-11.
298. Salenius S, Portin K, Kajola M, Salmelin R, Hari R. Cortical control of human motoneuron firing during isometric contraction. *J Neurophysiol.* 1997;77(6):3401-5.
299. Sanes JN, Donoghue JP, Thangaraj V, Edelman RR, Warach S. Shared neural substrates controlling hand movements in human motor cortex. *Science.* 1995;268(5218):1775-7.
300. Wassermann EM, McShane LM, Hallett M, Cohen LG. Noninvasive mapping of muscle representations in human motor cortex. *Electroencephalogr Clin Neurophysiol.* 1992;85(1):1-8.
301. de Luca CJ, LeFever RS, McCue MP, Xenakis AP. Behaviour of human motor units in different muscles during linearly varying contractions. *The Journal of Physiology.* 1982;329(1):113-28.
302. McAuley JH, Marsden CD. Physiological and pathological tremors and rhythmic central motor control. *Brain.* 2000;123 (Pt 8):1545-67.
303. Conway BA, Halliday DM, Farmer SF, Shahani U, Maas P, Weir AI, et al. Synchronization between motor cortex and spinal motoneuronal pool during the performance of a maintained motor task in man. *J Physiol.* 1995;489 (Pt 3):917-24.
304. Omlor W, Patino L, Hepp-Reymond MC, Kristeva R. Gamma-range corticomuscular coherence during dynamic force output. *Neuroimage.* 2007;34(3):1191-8.
305. Shibasaki H, Kuroiwa Y. Electroencephalographic correlates of myoclonus. *Electroencephalogr Clin Neurophysiol.* 1975;39(5):455-63.
306. Salenius S, Avikainen S, Kaakkola S, Hari R, Brown P. Defective cortical drive to muscle in Parkinson's disease and its improvement with levodopa. *Brain.* 2002;125(Pt 3):491-500.
307. Muthuraman M, Heute U, Arning K, Anwar AR, Elble R, Deuschl G, et al. Oscillating central motor networks in pathological tremors and voluntary movements. What makes the difference? *Neuroimage.* 2012;60(2):1331-9.
308. Mima T, Toma K, Koshy B, Hallett M. Coherence between cortical and muscular activities after subcortical stroke. *Stroke.* 2001;32(11):2597-601.
309. Ziemann U, Winter M, Reimers CD, Reimers K, Tergau F, Paulus W. Impaired motor cortex inhibition in patients with amyotrophic lateral sclerosis. Evidence from paired transcranial magnetic stimulation. *Neurology.* 1997;49(5):1292-8.
310. Vucic S, Kiernan MC. Novel threshold tracking techniques suggest that cortical hyperexcitability is an early feature of motor neuron disease. *Brain.* 2006;129(Pt 9):2436-46.
311. Chen R, Lozano AM, Ashby P. Mechanism of the silent period following transcranial magnetic stimulation. Evidence from epidural recordings. *Exp Brain Res.* 1999;128(4):539-42.

312. Werhahn KJ, Kunesch E, Noachtar S, Benecke R, Classen J. Differential effects on motorcortical inhibition induced by blockade of GABA uptake in humans. *J Physiol.* 1999;517 (Pt 2):591-7.
313. Zanette G, Tamburin S, Manganotti P, Refatti N, Forgione A, Rizzuto N. Changes in motor cortex inhibition over time in patients with amyotrophic lateral sclerosis. *J Neurol.* 2002;249(12):1723-8.
314. Vucic S, Kiernan MC. Transcranial Magnetic Stimulation for the Assessment of Neurodegenerative Disease. *Neurotherapeutics.* 2017;14(1):91-106.
315. Vucic S, Cheah BC, Yiannikas C, Kiernan MC. Cortical excitability distinguishes ALS from mimic disorders. *Clin Neurophysiol.* 2011;122(9):1860-6.
316. Wittstock M, Wolters A, Benecke R. Transcallosal inhibition in amyotrophic lateral sclerosis. *Clin Neurophysiol.* 2007;118(2):301-7.
317. Mills KR, Nithi KA. Corticomotor threshold is reduced in early sporadic amyotrophic lateral sclerosis. *Muscle Nerve.* 1997;20(9):1137-41.
318. Floyd AG, Yu QP, Piboolnurak P, Tang MX, Fang Y, Smith WA, et al. Transcranial magnetic stimulation in ALS: utility of central motor conduction tests. *Neurology.* 2009;72(6):498-504.
319. Bae JS, Simon NG, Menon P, Vucic S, Kiernan MC. The puzzling case of hyperexcitability in amyotrophic lateral sclerosis. *J Clin Neurol.* 2013;9(2):65-74.
320. Braak H, Braak E. Neuropathological staging of Alzheimer-related changes. *Acta Neuropathol.* 1991;82(4):239-59.
321. Schulte-Mattler WJ, Muller T, Zierz S. Transcranial magnetic stimulation compared with upper motor neuron signs in patients with amyotrophic lateral sclerosis. *J Neurol Sci.* 1999;170(1):51-6.
322. Brownell B, Oppenheimer DR, Hughes JT. The central nervous system in motor neurone disease. *J Neurol Neurosurg Psychiatry.* 1970;33(3):338-57.
323. Boillée S, Cleveland DW. Gene therapy for ALS delivers. *Trends Neurosci.* 2004;27(5):235-8.
324. Nayak MS, Kim YS, Goldman M, Keirstead HS, Kerr DA. Cellular therapies in motor neuron diseases. *Biochim Biophys Acta.* 2006;1762(11-12):1128-38.
325. Traynor BJ, Bruijn L, Conwit R, Beal F, O'Neill G, Fagan SC, et al. Neuroprotective agents for clinical trials in ALS: a systematic assessment. *Neurology.* 2006;67(1):20-7.
326. Brownstone RM, Lancelin C. Escape from homeostasis: spinal microcircuits and progression of amyotrophic lateral sclerosis. *J Neurophysiol.* 2018;119(5):1782-94.
327. Iyer PM, Mohr K, Broderick M, Gavin B, Burke T, Bede P, et al. Mismatch Negativity as an Indicator of Cognitive Sub-Domain Dysfunction in Amyotrophic Lateral Sclerosis. *Front Neurol.* 2017;8:395.
328. Querin G, El Mendili MM, Lenglet T, Behin A, Stojkovic T, Salachas F, et al. The spinal and cerebral profile of adult spinal-muscular atrophy: A multimodal imaging study. *Neuroimage Clin.* 2019;21:101618.
329. Boonstra TW. The potential of corticomuscular and intermuscular coherence for research on human motor control. *Front Hum Neurosci.* 2013;7:855.
330. Mima T, Hallett M. Electroencephalographic analysis of cortico-muscular coherence: reference effect, volume conduction and generator mechanism. *Clin Neurophysiol.* 1999;110(11):1892-9.
331. Gonzalez H, Olsson T, Borg K. Management of postpolio syndrome. *Lancet Neurol.* 2010;9(6):634-42.

332. The Amyotrophic Lateral Sclerosis Functional Rating Scale. Assessment of activities of daily living in patients with amyotrophic lateral sclerosis. The ALS CNTF treatment study (ACTS) phase I-II Study Group. *Arch Neurol*. 1996;53(2):141-7.
333. Cedarbaum JM, Stambler N, Malta E, Fuller C, Hilt D, Thurmond B, et al. The ALSFRS-R: a revised ALS functional rating scale that incorporates assessments of respiratory function. BDNF ALS Study Group (Phase III). *J Neurol Sci*. 1999;169(1-2):13-21.
334. Abrahams S, Newton J, Niven E, Foley J, Bak TH. Screening for cognition and behaviour changes in ALS. *Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration*. 2014;15(1-2):9-14.
335. Niven E, Newton J, Foley J, Colville S, Swingler R, Chandran S, et al. Validation of the Edinburgh Cognitive and Behavioural Amyotrophic Lateral Sclerosis Screen (ECAS): A cognitive tool for motor disorders. *Amyotroph Lateral Scler Frontotemporal Degener*. 2015;16(3-4):172-9.
336. Lee HJ, DeLisa JA, Lee HJ. *Manual of nerve conduction study and surface anatomy for needle electromyography*. Philadelphia: Lippincott Wilkins and Williams; 2005.
337. Barbero M, Merletti R, Rainoldi A. *Atlas of muscle innervation zones : understanding surface electromyography and its applications*. Milan; New York: Springer; 2012.
338. Pease WS, Lew HL, Johnson EW. *Johnson's practical electromyography*. Philadelphia: Lippincott Williams & Wilkins; 2007.
339. Brainard DH. The Psychophysics Toolbox. *Spat Vis*. 1997;10(4):433-6.
340. Oostenveld R, Fries P, Maris E, Schoffelen JM. FieldTrip: Open source software for advanced analysis of MEG, EEG, and invasive electrophysiological data. *Comput Intell Neurosci*. 2011;2011:156869.
341. Bradshaw LA, Wikswo JP. Spatial Filter Approach for Evaluation of the Surface Laplacian of the Electroencephalogram and Magnetoencephalogram. *Annals of Biomedical Engineering*. 2001;29(3):202-13.
342. McFarland DJ, McCane LM, David SV, Wolpaw JR. Spatial filter selection for EEG-based communication. *Electroencephalography and Clinical Neurophysiology*. 1997;103(3):386-94.
343. Nasserolelami B, Dukic S, Broderick M, Mohr K, Schuster C, Gavin B, et al. Characteristic Increases in EEG Connectivity Correlate With Changes of Structural MRI in Amyotrophic Lateral Sclerosis. *Cereb Cortex*. 2019;29(1):27-41.
344. Bigdely-Shamlo N, Mullen T, Kothe C, Su K, Robbins K. The PREP pipeline: standardized preprocessing for large-scale EEG analysis. *Front Neuroinform*. 9 (2015). 2015.
345. Kohe C. Fit clean channels-SCCN https://scn.ucsd.edu/wiki/Fit_clean_channels 2010 [
346. Niinimaa A, Oja H. Multivariate Median. *Encyclopedia of Statistical Sciences: Wiley StatsRef: Statistics Reference Online*; 2014.
347. Nasserolelami B, Dukic S, Buxo T, Coffey A, McMackin R, Muthuraman M, et al. Non-Parametric Rank Statistics for Spectral Power and Coherence. *bioRxiv*. 2019:818906.
348. Van Veen BD, Van Drongelen W, Yuchtman M, Suzuki A. Localization of brain electrical activity via linearly constrained minimum variance spatial filtering. *IEEE Transactions on biomedical engineering*. 1997;44(9):867-80.
349. Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, et al. Automated Anatomical Labeling of Activations in SPM Using a

- Macroscopic Anatomical Parcellation of the MNI MRI Single-Subject Brain. *NeuroImage*. 2002;15(1):273-89.
350. Fonov VS, Evans AC, McKinstry RC, Almlí CR, Collins DL. Unbiased nonlinear average age-appropriate brain templates from birth to adulthood. *Neuroimage*. 2009;47:S102.
 351. Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, et al. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage*. 2002;15(1):273-89.
 352. Stouffer SA, Suchman EA, Devinney LC, Star SA, Williams Jr RM. *The American soldier: Adjustment during army life. (Studies in social psychology in World War II), Vol. 1.* Oxford, England: Princeton Univ. Press; 1949. xii, 599-xii, p.
 353. Westfall PH. Combining P-values. *Wiley StatsRef: Statistics Reference Online*; 2014.
 354. Halliday DM, Rosenberg JR, Amjad AM, Breeze P, Conway BA, Farmer SF. A framework for the analysis of mixed time series/point process data--theory and application to the study of physiological tremor, single motor unit discharges and electromyograms. *Prog Biophys Mol Biol*. 1995;64(2-3):237-78.
 355. Benjamini Y, Krieger AM, Yekutieli D. Adaptive linear step-up procedures that control the false discovery rate. *Biometrika*. 2006;93(3):491-507.
 356. Efron B, Tibshirani R, Storey JD, Tusher V. Empirical Bayes Analysis of a Microarray Experiment. *Journal of the American Statistical Association*. 2001;96(456):1151-60.
 357. Nasserouleslami B. An Implementation of Empirical Bayesian Inference and Non-Null Bootstrapping for Threshold Selection and Power Estimation in Multiple and Single Statistical Testing. *bioRxiv*. 2019:342964.
 358. van Es MA, Hardiman O, Chio A, Al-Chalabi A, Pasterkamp RJ, Veldink JH, et al. Amyotrophic lateral sclerosis. *Lancet*. 2017;390(10107):2084-98.
 359. Pender N, Pinto-Grau M, Hardiman O. Cognitive and behavioural impairment in amyotrophic lateral sclerosis. *Curr Opin Neurol*. 2020;33(5):649-54.
 360. Mathis S, Goizet C, Soulages A, Vallat JM, Masson GL. Genetics of amyotrophic lateral sclerosis: A review. *J Neurol Sci*. 2019;399:217-26.
 361. Agosta F, Valsasina P, Absinta M, Riva N, Sala S, Prella A, et al. Sensorimotor functional connectivity changes in amyotrophic lateral sclerosis. *Cereb Cortex*. 2011;21(10):2291-8.
 362. Siegel M, Donner TH, Engel AK. Spectral fingerprints of large-scale neuronal interactions. *Nat Rev Neurosci*. 2012;13(2):121-34.
 363. Laufs H. Endogenous brain oscillations and related networks detected by surface EEG-combined fMRI. *Hum Brain Mapp*. 2008;29(7):762-9.
 364. Ludolph A, Drory V, Hardiman O, Nakano I, Ravits J, Robberecht W, et al. A revision of the El Escorial criteria - 2015. *Amyotroph Lateral Scler Frontotemporal Degener*. 2015;16(5-6):291-2.
 365. Kimura F, Fujimura C, Ishida S, Nakajima H, Furutama D, Uehara H, et al. Progression rate of ALSFRS-R at time of diagnosis predicts survival time in ALS. *Neurology*. 2006;66(2):265-7.
 366. de Carvalho M, Scotto M, Lopes A, Swash M. Clinical and neurophysiological evaluation of progression in amyotrophic lateral sclerosis. *Muscle Nerve*. 2003;28(5):630-3.

367. Danna-Dos Santos A, Poston B, Jesunathadas M, Bobich LR, Hamm TM, Santello M. Influence of fatigue on hand muscle coordination and EMG-EMG coherence during three-digit grasping. *J Neurophysiol.* 2010;104(6):3576-87.
368. Witham CL, Riddle CN, Baker MR, Baker SN. Contributions of descending and ascending pathways to corticomuscular coherence in humans. *J Physiol.* 2011;589(Pt 15):3789-800.
369. Bradshaw LA, Wikswo JP, Jr. Spatial filter approach for evaluation of the surface Laplacian of the electroencephalogram and magnetoencephalogram. *Ann Biomed Eng.* 2001;29(3):202-13.
370. McFarland DJ, McCane LM, David SV, Wolpaw JR. Spatial filter selection for EEG-based communication. *Electroencephalogr Clin Neurophysiol.* 1997;103(3):386-94.
371. Dukic S, Iyer PM, Mohr K, Hardiman O, Lalor EC, Nasserolelami B. Estimation of coherence using the median is robust against EEG artefacts. *Conf Proc IEEE Eng Med Biol Soc.* 2017;2017:3949-52.
372. Mehrkanoon S, Breakspear M, Boonstra TW. The reorganization of corticomuscular coherence during a transition between sensorimotor states. *Neuroimage.* 2014;100:692-702.
373. Bae JS, Menon P, Mioshi E, Kiernan MC, Vucic S. Cortical hyperexcitability and the split-hand plus phenomenon: pathophysiological insights in ALS. *Amyotroph Lateral Scler Frontotemporal Degener.* 2014;15(3-4):250-6.
374. Dadon-Nachum M, Melamed E, Offen D. The "dying-back" phenomenon of motor neurons in ALS. *J Mol Neurosci.* 2011;43(3):470-7.
375. Bede P, Omer T, Finegan E, Chipika RH, Iyer PM, Doherty MA, et al. Connectivity-based characterisation of subcortical grey matter pathology in frontotemporal dementia and ALS: a multimodal neuroimaging study. *Brain Imaging Behav.* 2018;12(6):1696-707.
376. Hardiman O, Al-Chalabi A, Chio A, Corr EM, Logroscino G, Robberecht W, et al. Amyotrophic lateral sclerosis. *Nat Rev Dis Primers.* 2017;3:17085.
377. Zhang W, Zhang L, Liang B, Schroeder D, Zhang ZW, Cox GA, et al. Hyperactive somatostatin interneurons contribute to excitotoxicity in neurodegenerative disorders. *Nat Neurosci.* 2016;19(4):557-9.
378. Brownstone RM, Chopek JW. Reticulospinal Systems for Tuning Motor Commands. *Front Neural Circuits.* 2018;12:30.
379. Benatar M. Neuromuscular disease: evidence and analysis in clinical neurology: Springer Science & Business Media; 2007.
380. Geraedts VJ, Marinus J, Gouw AA, Mosch A, Stam CJ, van Hilten JJ, et al. Quantitative EEG reflects non-dopaminergic disease severity in Parkinson's disease. *Clin Neurophysiol.* 2018;129(8):1748-55.
381. Nasserolelami B, Broderick M, Mohr K, Dukic S, Gavin B, Coffey A, et al., editors. Impairment of cortico-cortical and cortico-muscular communication in amyotrophic lateral sclerosis. *Ir J Med Sci*; 2017: SPRINGER LONDON LTD 236 GRAYS INN RD, 6TH FLOOR, LONDON WC1X 8HL, ENGLAND.
382. Khademi F, Royter V, Gharabaghi A. Distinct Beta-band Oscillatory Circuits Underlie Corticospinal Gain Modulation. *Cereb Cortex.* 2018;28(4):1502-15.
383. Khanna P, Carmenta JM. Neural oscillations: beta band activity across motor networks. *Curr Opin Neurobiol.* 2015;32:60-7.
384. Chen G, Zhang Y, Li X, Zhao X, Ye Q, Lin Y, et al. Distinct Inhibitory Circuits Orchestrate Cortical beta and gamma Band Oscillations. *Neuron.* 2017;96(6):1403-18.e6.

385. Van Veen BD, van Drongelen W, Yuchtman M, Suzuki A. Localization of brain electrical activity via linearly constrained minimum variance spatial filtering. *IEEE Trans Biomed Eng.* 1997;44(9):867-80.
386. Roopun AK, Middleton SJ, Cunningham MO, LeBeau FE, Bibbig A, Whittington MA, et al. A beta2-frequency (20-30 Hz) oscillation in nonsynaptic networks of somatosensory cortex. *Proc Natl Acad Sci U S A.* 2006;103(42):15646-50.
387. Nihei K, McKee AC, Kowall NW. Patterns of neuronal degeneration in the motor cortex of amyotrophic lateral sclerosis patients. *Acta Neuropathol.* 1993;86(1):55-64.
388. Baker SN. Oscillatory interactions between sensorimotor cortex and the periphery. *Curr Opin Neurobiol.* 2007;17(6):649-55.
389. Hamada M, Hanajima R, Terao Y, Sato F, Okano T, Yuasa K, et al. Median nerve somatosensory evoked potentials and their high-frequency oscillations in amyotrophic lateral sclerosis. *Clin Neurophysiol.* 2007;118(4):877-86.
390. Shimizu T, Bokuda K, Kimura H, Kamiyama T, Nakayama Y, Kawata A, et al. Sensory cortex hyperexcitability predicts short survival in amyotrophic lateral sclerosis. *Neurology.* 2018;90(18):e1578-e87.
391. Saleh M, Reimer J, Penn R, Ojakangas CL, Hatsopoulos NG. Fast and slow oscillations in human primary motor cortex predict oncoming behaviorally relevant cues. *Neuron.* 2010;65(4):461-71.
392. Kopell N, Ermentrout GB, Whittington MA, Traub RD. Gamma rhythms and beta rhythms have different synchronization properties. *Proc Natl Acad Sci U S A.* 2000;97(4):1867-72.
393. Kew JJ, Brooks DJ, Passingham RE, Rothwell JC, Frackowiak RS, Leigh PN. Cortical function in progressive lower motor neuron disorders and amyotrophic lateral sclerosis: a comparative PET study. *Neurology.* 1994;44(6):1101-10.
394. Proudfoot M, van Ede F, Quinn A, Colclough GL, Wu J, Talbot K, et al. Impaired corticomuscular and interhemispheric cortical beta oscillation coupling in amyotrophic lateral sclerosis. *Clin Neurophysiol.* 2018;129(7):1479-89.
395. Penfield W, Boldrey E. Somatic motor and sensory representation in the cerebral cortex of man as studied by electrical stimulation. *Brain.* 1937;60(4):389-443.
396. Coffey A, Bista S, Fasano A, Buxo T, Mitchell M, Giglia ER, et al. Altered supraspinal motor networks in survivors of poliomyelitis: A cortico-muscular coherence study. *Clin Neurophysiol.* 2020;132(1):106-13.
397. Halstead LS, Rossi CD. Post-polio syndrome: clinical experience with 132 consecutive outpatients. *Birth Defects Orig Artic Ser.* 1987;23(4):13-26.
398. Trojan DA, Cashman NR. Post-poliomyelitis syndrome. *Muscle Nerve.* 2005;31(1):6-19.
399. Borg K, Borg J, Edström L, Grimby L. Effects of excessive use of remaining muscle fibers in prior polio and LV lesion. *Muscle Nerve.* 1988;11(12):1219-30.
400. Grimby G, Hedberg M, Henning GB. Changes in muscle morphology, strength and enzymes in a 4-5-year follow-up of subjects with poliomyelitis sequelae. *Scand J Rehabil Med.* 1994;26(3):121-30.
401. Larsson L, Li X, Tollbäck A, Grimby L. Contractile properties in single muscle fibres from chronically overused motor units in relation to motoneuron firing properties in prior polio patients. *J Neurol Sci.* 1995;132(2):182-92.
402. Borg K, Henriksson J. Prior poliomyelitis-reduced capillary supply and metabolic enzyme content in hypertrophic slow-twitch (type I) muscle fibres. *J Neurol Neurosurg Psychiatry.* 1991;54(3):236-40.

403. Luciano CA, Sivakumar K, Spector SA, Dalakas MC. Reinnervation in clinically unaffected muscles of patients with prior paralytic poliomyelitis. Correlation between macroelectromyography and histology. *Ann N Y Acad Sci.* 1995;753:394-6.
404. Murray D, Hardiman O, Meldrum D. Assessment of subjective and motor fatigue in Polio survivors, attending a Postpolio clinic, comparison with healthy controls and an exploration of clinical correlates. *Physiother Theory Pract.* 2014;30(4):229-35.
405. Murray D, Meldrum D, Moloney R, Campion A, Horgan F, Hardiman O. The effects of a home-based arm ergometry exercise programme on physical fitness, fatigue and activity in polio survivors: protocol for a randomised controlled trial. *BMC Neurol.* 2012;12:157.
406. Allen GM, Gandevia SC, Neering IR, Hickie I, Jones R, Middleton J. Muscle performance, voluntary activation and perceived effort in normal subjects and patients with prior poliomyelitis. *Brain.* 1994;117 (Pt 4):661-70.
407. Gandevia SC, Allen GM, Middleton J. Post-polio syndrome: assessments, pathophysiology and progression. *Disabil Rehabil.* 2000;22(1-2):38-42.
408. Lupu VD, Danielian L, Johnsen JA, Vasconcelos OM, Prokhorenko OA, Jabbari B, et al. Physiology of the motor cortex in polio survivors. *Muscle Nerve.* 2008;37(2):177-82.
409. Bodian D. Pathogenesis of poliomyelitis. *Am J Public Health Nations Health.* 1952;42(11):1388-402.
410. Bruno RL, Cohen JM, Galski T, Frick NM. The neuroanatomy of post-polio fatigue. *Arch Phys Med Rehabil.* 1994;75(5):498-504.
411. Andersen N-SB, Larsen SM, Nissen SK, Jørgensen SE, Mardahl M, Christiansen M, et al. Host Genetics, Innate Immune Responses, and Cellular Death Pathways in Poliomyelitis Patients. *Front Microbiol.* 2019;10(1495).
412. Girard S, Couderc T, Destombes J, Thiesson D, Delpeyroux F, Blondel B. Poliovirus induces apoptosis in the mouse central nervous system. *J Virol.* 1999;73(7):6066-72.
413. Belov GA, Romanova LI, Tolskaya EA, Kolesnikova MS, Lazebnik YA, Agol VI. The major apoptotic pathway activated and suppressed by poliovirus. *J Virol.* 2003;77(1):45-56.
414. Huttenlocher PR, Dabholkar AS. Regional differences in synaptogenesis in human cerebral cortex. *J Comp Neurol.* 1997;387(2):167-78.
415. Pascual-Leone A, Torres F. Plasticity of the sensorimotor cortex representation of the reading finger in Braille readers. *Brain.* 1993;116 (Pt 1):39-52.
416. Eyre JA, Miller S, Clowry GJ, Conway EA, Watts C. Functional corticospinal projections are established prenatally in the human foetus permitting involvement in the development of spinal motor centres. *Brain.* 2000;123 (Pt 1):51-64.
417. James LM, Halliday DM, Stephens JA, Farmer SF. On the development of human corticospinal oscillations: age-related changes in EEG-EMG coherence and cumulant. *Eur J Neurosci.* 2008;27(12):3369-79.
418. Kamp D, Krause V, Butz M, Schnitzler A, Pollok B. Changes of cortico-muscular coherence: an early marker of healthy aging? *Age (Dordr).* 2013;35(1):49-58.
419. Semmler JG, Kornatz KW, Enoka RM. Motor-unit coherence during isometric contractions is greater in a hand muscle of older adults. *J Neurophysiol.* 2003;90(2):1346-9.
420. Graziadio S, Basu A, Tomasevic L, Zappasodi F, Tecchio F, Eyre JA. Developmental tuning and decay in senescence of oscillations linking the corticospinal system. *J Neurosci.* 2010;30(10):3663-74.

421. Oldfield RC. The assessment and analysis of handedness: The Edinburgh inventory. *Neuropsychologia*. 1971;9(1):97-113.
422. Oliveri M, Brighina F, La Bua V, Buffa D, Aloisio A, Fierro B. Reorganization of cortical motor area in prior polio patients. *Clin Neurophysiol*. 1999;110(5):806-12.
423. Jaiser SR, Baker MR, Baker SN. Intermuscular Coherence in Normal Adults: Variability and Changes with Age. *PLoS One*. 2016;11(2):e0149029-e.
424. Fuhr P, Cohen LG, Dang N, Findley TW, Haghighi S, Oro J, et al. Physiological analysis of motor reorganization following lower limb amputation. *Electroencephalogr Clin Neurophysiol*. 1992;85(1):53-60.
425. Cohen LG, Bandinelli S, Findley TW, Hallett M. Motor reorganization after upper limb amputation in man. A study with focal magnetic stimulation. *Brain*. 1991;114 (Pt 1B):615-27.
426. Galea MP, Darian-Smith I. Postnatal maturation of the direct corticospinal projections in the macaque monkey. *Cereb Cortex*. 1995;5(6):518-40.
427. Williams P, Jiang YQ, Martin JH. Motor system plasticity after unilateral injury in the developing brain. *Dev Med Child Neurol*. 2017;59(12):1224-9.
428. Jernigan TL, Baaré WF, Stiles J, Madsen KS. Postnatal brain development: structural imaging of dynamic neurodevelopmental processes. *Prog Brain Res*. 2011;189:77-92.
429. Barkovich AJ. Concepts of myelin and myelination in neuroradiology. *AJNR Am J Neuroradiol*. 2000;21(6):1099-109.
430. Barkovich AJ. Magnetic resonance techniques in the assessment of myelin and myelination. *J Inherit Metab Dis*. 2005;28(3):311-43.
431. Khanna A, Pascual-Leone A, Michel CM, Farzan F. Microstates in resting-state EEG: Current status and future directions. *Neurosci Biobehav Rev*. 2015;49:105-13.
432. Ingber L, Nunez PL. Neocortical dynamics at multiple scales: EEG standing waves, statistical mechanics, and physical analogs. *Math Biosci*. 2011;229(2):160-73.
433. Olejarczyk E, Marzetti L, Pizzella V, Zappasodi F. Comparison of connectivity analyses for resting state EEG data. *Journal of Neural Engineering*. 2017;14(3):036017.
434. Teramoto H, Morita A, Ninomiya S, Akimoto T, Shiota H, Kamei S. Relation between Resting State Front-Parietal EEG Coherence and Executive Function in Parkinson's Disease. *Biomed Res Int*. 2016;2016:2845754.
435. Pollok B, Krause V, Martsch W, Wach C, Schnitzler A, Südmeyer M. Motor-cortical oscillations in early stages of Parkinson's disease. *J Physiol*. 2012;590(13):3203-12.
436. Nardone R, Sebastianelli L, Versace V, Saltuari L, Lochner P, Frey V, et al. Usefulness of EEG Techniques in Distinguishing Frontotemporal Dementia from Alzheimer's Disease and Other Dementias. *Dis Markers*. 2018;2018:6581490.
437. Garn H, Coronel C, Waser M, Caravias G, Ransmayr G. Differential diagnosis between patients with probable Alzheimer's disease, Parkinson's disease dementia, or dementia with Lewy bodies and frontotemporal dementia, behavioral variant, using quantitative electroencephalographic features. *J Neural Transm (Vienna)*. 2017;124(5):569-81.
438. Kelly SP, Lalor EC, Reilly RB, Foxe JJ. Increases in alpha oscillatory power reflect an active retinotopic mechanism for distracter suppression during sustained visuospatial attention. *J Neurophysiol*. 2006;95(6):3844-51.
439. Başar E, Başar-Eroglu C, Karakaş S, Schürmann M. Gamma, alpha, delta, and theta oscillations govern cognitive processes. *Int J Psychophysiol*. 2001;39(2-3):241-8.
440. Fries P, Nikolić D, Singer W. The gamma cycle. *Trends Neurosci*. 2007;30(7):309-16.

441. MATLAB and Computer Vision System Toolbox. [Internet]. The MathWorks, Inc.
442. Mohr KS, Nasserroleslami B, Iyer PM, Hardiman O, Lalor EC. EyeBallGUI: A Tool for Visual Inspection and Binary Marking of Multi-channel Bio-signals. *bioRxiv*. 2017:129437.
443. Cassim F, Monaca C, Szurhaj W, Bourriez JL, Defebvre L, Derambure P, et al. Does post-movement beta synchronization reflect an idling motor cortex? *Neuroreport*. 2001;12(17):3859-63.
444. Kosaka T, Kuroha Y, Tada M, Hasegawa A, Tani T, Matsubara N, et al. A fatal neuromuscular disease in an adult patient after poliomyelitis in early childhood: consideration of the pathology of post-polio syndrome. *Neuropathology*. 2013;33(1):93-101.
445. Prior TW, Leach ME, Finanger E. Spinal Muscular Atrophy. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Mirzaa G, et al., editors. *GeneReviews*(®). Seattle (WA): University of Washington, Seattle Copyright © 1993-2021, University of Washington, Seattle. *GeneReviews* is a registered trademark of the University of Washington, Seattle. All rights reserved.; 1993.
446. Groen EJN, Perenthaler E, Courtney NL, Jordan CY, Shorrock HK, van der Hoorn D, et al. Temporal and tissue-specific variability of SMN protein levels in mouse models of spinal muscular atrophy. *Hum Mol Genet*. 2018;27(16):2851-62.
447. Lefebvre S, Sarret C. Pathogenesis and therapeutic targets in spinal muscular atrophy (SMA). *Arch Pediatr*. 2020;27(7s):7s3-7s8.
448. Ramos DM, d'Ydewalle C, Gabbeta V, Dakka A, Klein SK, Norris DA, et al. Age-dependent SMN expression in disease-relevant tissue and implications for SMA treatment. *J Clin Invest*. 2019;129(11):4817-31.
449. Burlet P, Huber C, Bertrand S, Ludosky MA, Zwaenepoel I, Clermont O, et al. The distribution of SMN protein complex in human fetal tissues and its alteration in spinal muscular atrophy. *Hum Mol Genet*. 1998;7(12):1927-33.
450. Braun S, Croizat B, Lagrange MC, Warter JM, Poindron P. Constitutive muscular abnormalities in culture in spinal muscular atrophy. *Lancet*. 1995;345(8951):694-5.
451. Boyer JG, Deguise MO, Murray LM, Yazdani A, De Repentigny Y, Boudreau-Larivière C, et al. Myogenic program dysregulation is contributory to disease pathogenesis in spinal muscular atrophy. *Hum Mol Genet*. 2014;23(16):4249-59.
452. Kim JK, Jha NN, Feng Z, Faleiro MR, Chiriboga CA, Wei-Lapierre L, et al. Muscle-specific SMN reduction reveals motor neuron-independent disease in spinal muscular atrophy models. *J Clin Invest*. 2020;130(3):1271-87.
453. Bede P, Hardiman O. Lessons of ALS imaging: Pitfalls and future directions - A critical review. *Neuroimage Clin*. 2014;4:436-43.
454. Neuwirth C, Braun N, Claeys KG, Bucelli R, Fournier C, Bromberg M, et al. Implementing Motor Unit Number Index (MUNIX) in a large clinical trial: Real world experience from 27 centres. *Clin Neurophysiol*. 2018;129(8):1756-62.
455. Ramdas S, Servais L. New treatments in spinal muscular atrophy: an overview of currently available data. *Expert Opin Pharmacother*. 2020;21(3):307-15.
456. Annoussamy M, Seferian AM, Daron A, Péréon Y, Cances C, Vuillerot C, et al. Natural history of Type 2 and 3 spinal muscular atrophy: 2-year NatHis-SMA study. *Ann Clin Transl Neurol*. 2021;8(2):359-73.
457. Chang Y. Reorganization and plastic changes of the human brain associated with skill learning and expertise. *Front Hum Neurosci*. 2014;8:35.

458. Maguire EA, Gadian DG, Johnsrude IS, Good CD, Ashburner J, Frackowiak RSJ, et al. Navigation-related structural change in the hippocampi of taxi drivers. *Proceedings of the National Academy of Sciences*. 2000;97(8):4398.
459. Artzi M, Shiran SI, Weinstein M, Myers V, Tarrasch R, Schertz M, et al. Cortical Reorganization following Injury Early in Life. *Neural Plast*. 2016;2016:8615872.
460. von Gontard A, Zerres K, Backes M, Laufersweiler-Plass C, Wendland C, Melchers P, et al. Intelligence and cognitive function in children and adolescents with spinal muscular atrophy. *Neuromuscul Disord*. 2002;12(2):130-6.
461. Keifer OP, Jr., Hurt RC, Gutman DA, Keilholz SD, Gourley SL, Ressler KJ. Voxel-based morphometry predicts shifts in dendritic spine density and morphology with auditory fear conditioning. *Nat Commun*. 2015;6:7582.
462. Finegan E, Chipika RH, Shing SLH, Hardiman O, Bede P. Primary lateral sclerosis: a distinct entity or part of the ALS spectrum? *Amyotroph Lateral Scler Frontotemporal Degener*. 2019;20(3-4):133-45.
463. Fournier CN, Murphy A, Loci L, Mitsumoto H, Lomen-Hoerth C, Kisanuki Y, et al. Primary Lateral Sclerosis and Early Upper Motor Neuron Disease: Characteristics of a Cross-Sectional Population. *J Clin Neuromuscul Dis*. 2016;17(3):99-105.
464. Geevasinga N, Menon P, Sue CM, Kumar KR, Ng K, Yiannikas C, et al. Cortical excitability changes distinguish the motor neuron disease phenotypes from hereditary spastic paraplegia. *Eur J Neurol*. 2015;22(5):826-31, e57-8.
465. Weber M, Stewart H, Hirota N, Eisen A. Corticomotoneuronal connections in primary lateral sclerosis (PLS). *Amyotroph Lateral Scler Other Motor Neuron Disord*. 2002;3(4):190-8.
466. Moeller F, Muthuraman M, Stephani U, Deuschl G, Raethjen J, Siniatchkin M. Representation and propagation of epileptic activity in absences and generalized photoparoxysmal responses. *Hum Brain Mapp*. 2013;34(8):1896-909.
467. Rayner S. Cognitive styles and learning styles. *International Encyclopedia of Social & Behavioral Sciences UK: Elsevier Science Ltd*. 2001.
468. Kim DW. IC. EEG Spectral Analysis. . CH. I, editor. Singapore: Springer; (2018)
469. Chokroverty S. *Sleep Disorders Medicine* 2009.
470. Blain-Moraes S, Mashour GA, Lee H, Huggins JE, Lee U. Altered cortical communication in amyotrophic lateral sclerosis. *Neurosci Lett*. 2013;543:172-6.
471. Danna-Dos Santos A, Poston B, Jesunathadas M, Bobich LR, Hamm TM, Santello M. Influence of fatigue on hand muscle coordination and EMG-EMG coherence during three-digit grasping. *J Neurophysiol*. 2010;104(6):3576-87.
472. Witham CL, Riddle CN, Baker MR, Baker SN. Contributions of descending and ascending pathways to corticomuscular coherence in humans. *The Journal of physiology*. 2011;589(Pt 15):3789-800.
473. Finegan E, Li Hi Shing S, Chipika RH, Doherty MA, Hengeveld JC, Vajda A, et al. Widespread subcortical grey matter degeneration in primary lateral sclerosis: a multimodal imaging study with genetic profiling. *Neuroimage Clin*. 2019;24:102089.
474. Bede P, Chipika RH, Finegan E, Li Hi Shing S, Doherty MA, Hengeveld JC, et al. Brainstem pathology in amyotrophic lateral sclerosis and primary lateral sclerosis: A longitudinal neuroimaging study. *Neuroimage Clin*. 2019;24:102054.
475. Agosta F, Canu E, Inuggi A, Chiò A, Riva N, Silani V, et al. Resting state functional connectivity alterations in primary lateral sclerosis. *Neurobiol Aging*. 2014;35(4):916-25.

476. Meoded A, Morrisette AE, Katipally R, Schanz O, Gotts SJ, Floeter MK. Cerebro-cerebellar connectivity is increased in primary lateral sclerosis. *Neuroimage Clin.* 2015;7:288-96.
477. Santhosh J, Bhatia M, Sahu S, Anand S. Decreased electroencephalogram alpha band [8-13 Hz] power in amyotrophic lateral sclerosis patients: a study of alpha activity in an awake relaxed state. *Neurol India.* 2005;53(1):99-101.
478. Mai R, Facchetti D, Micheli A, Poloni M. Quantitative electroencephalography in amyotrophic lateral sclerosis. *Electroencephalogr Clin Neurophysiol.* 1998;106(4):383-6.
479. Merker B. Cortical gamma oscillations: the functional key is activation, not cognition. *Neurosci Biobehav Rev.* 2013;37(3):401-17.
480. Fries P, Reynolds JH, Rorie AE, Desimone R. Modulation of oscillatory neuronal synchronization by selective visual attention. *Science.* 2001;291(5508):1560-3.
481. Herrmann CS, Munk MHJ, Engel AK. Cognitive functions of gamma-band activity: memory match and utilization. *Trends Cogn Sci.* 2004;8(8):347-55.
482. Singer W. Consciousness and the binding problem. *Ann N Y Acad Sci.* 2001;929:123-46.
483. Bai O, Vorbach S, Hallett M, Floeter MK. Movement-related cortical potentials in primary lateral sclerosis. *Ann Neurol.* 2006;59(4):682-90.
484. de Vries BS, Spreij LA, Rustemeijer LMM, Bakker LA, Veldink JH, van den Berg LH, et al. A neuropsychological and behavioral study of PLS. Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration. 2019;20(5-6):376-84.
485. Herrmann CS, Strüber D, Helfrich RF, Engel AK. EEG oscillations: From correlation to causality. *Int J Psychophysiol.* 2016;103:12-21.
486. Agarwal S, Highton-Williamson E, Caga J, Matamala JM, Dharmadasa T, Howells J, et al. Primary lateral sclerosis and the amyotrophic lateral sclerosis-frontotemporal dementia spectrum. *J Neurol.* 2018;265(8):1819-28.
487. Piquard A, Le Forestier N, Baudoin-Madec V, Delgadillo D, Salachas F, Pradat PF, et al. Neuropsychological changes in patients with primary lateral sclerosis. *Amyotroph Lateral Scler.* 2006;7(3):150-60.
488. Canu E, Agosta F, Galantucci S, Chiò A, Riva N, Silani V, et al. Extramotor damage is associated with cognition in primary lateral sclerosis patients. *PLoS One.* 2013;8(12):e82017.
489. Brier MR, Thomas JB, Ances BM. Network dysfunction in Alzheimer's disease: refining the disconnection hypothesis. *Brain Connect.* 2014;4(5):299-311.
490. Sisková Z, Mahad DJ, Pudney C, Campbell G, Cadogan M, Asuni A, et al. Morphological and functional abnormalities in mitochondria associated with synaptic degeneration in prion disease. *Am J Pathol.* 2010;177(3):1411-21.
491. Ince PG, Evans J, Knopp M, Forster G, Hamdalla HH, Wharton SB, et al. Corticospinal tract degeneration in the progressive muscular atrophy variant of ALS. *Neurology.* 2003;60(8):1252-8.
492. Eisen A, Weber M. The motor cortex and amyotrophic lateral sclerosis. *Muscle Nerve.* 2001;24(4):564-73.
493. Braak H, Brettschneider J, Ludolph AC, Lee VM, Trojanowski JQ, Del Tredici K. Amyotrophic lateral sclerosis--a model of corticofugal axonal spread. *Nat Rev Neurol.* 2013;9(12):708-14.
494. Uchida A, Sasaguri H, Kimura N, Tajiri M, Ohkubo T, Ono F, et al. Non-human primate model of amyotrophic lateral sclerosis with cytoplasmic mislocalization of TDP-43. *Brain.* 2012;135(Pt 3):833-46.

495. Kater SB, Rehder V. The sensory-motor role of growth cone filopodia. *Curr Opin Neurobiol.* 1995;5(1):68-74.
496. Rosenfeld J, Swash M. What's in a name? Lumping or splitting ALS, PLS, PMA, and the other motor neuron diseases. *Neurology.* 2006;66(5):624-5.
497. Sanes JN, Suner S, Donoghue JP. Dynamic organization of primary motor cortex output to target muscles in adult rats. I. Long-term patterns of reorganization following motor or mixed peripheral nerve lesions. *Exp Brain Res.* 1990;79(3):479-91.
498. van Nuenen BF, Helmich RC, Buenen N, van de Warrenburg BP, Bloem BR, Toni I. Compensatory activity in the extrastriate body area of Parkinson's disease patients. *J Neurosci.* 2012;32(28):9546-53.
499. Bobkova N, Vorobyov V. The brain compensatory mechanisms and Alzheimer's disease progression: a new protective strategy. *Neural Regen Res.* 2015;10(5):696-7.
500. Cech DJ, Martin ST. Chapter 3 - Motor Development. In: Cech DJ, Martin ST, editors. *Functional Movement Development Across the Life Span (Third Edition)*. Saint Louis: W.B. Saunders; 2012. p. 45-67.
501. Edelman GM. *Neural Darwinism: The theory of neuronal group selection*: Basic books; 1987.
502. Calvin WH. *Neural Darwinism. The Theory of Neuronal Group Selection*. Gerald M. Edelman. Basic Books, New York, 1987. xxii, 371 pp., illus. \$29.95. *Science.* 1988;240(4860):1802.
503. Vucic S, Nicholson GA, Kiernan MC. Cortical hyperexcitability may precede the onset of familial amyotrophic lateral sclerosis. *Brain.* 2008;131(6):1540-50.
504. Menke RAL, Proudfoot M, Wu J, Andersen PM, Talbot K, Benatar M, et al. Increased functional connectivity common to symptomatic amyotrophic lateral sclerosis and those at genetic risk. *Journal of Neurology, Neurosurgery & Psychiatry.* 2016;87(6):580.
505. Horwitz B, Rowe JB. Functional biomarkers for neurodegenerative disorders based on the network paradigm. *Prog Neurobiol.* 2011;95(4):505-9.
506. Eisen A, Kiernan M, Mitsumoto H, Swash M. Amyotrophic lateral sclerosis: a long preclinical period? *Journal of Neurology, Neurosurgery & Psychiatry.* 2014;85(11):1232.
507. Krause V, Wach C, Südmeyer M, Ferrea S, Schnitzler A, Pollok B. Cortico-muscular coupling and motor performance are modulated by 20 Hz transcranial alternating current stimulation (tACS) in Parkinson's disease. *Front Hum Neurosci.* 2013;7:928.
508. Park H, Kim JS, Paek SH, Jeon BS, Lee JY, Chung CK. Cortico-muscular coherence increases with tremor improvement after deep brain stimulation in Parkinson's disease. *Neuroreport.* 2009;20(16):1444-9.
509. Lou X, Xiao S, Qi Y, Hu X, Wang Y, Zheng X. Corticomuscular coherence analysis on hand movement distinction for active rehabilitation. *Comput Math Methods Med.* 2013;2013:908591.
510. Schmidt ER, Pasterkamp RJ, van den Berg LH. Axon guidance proteins: novel therapeutic targets for ALS? *Prog Neurobiol.* 2009;88(4):286-301.

11. Appendices

11.1. Appendix 4.1

Ethics approval reviewed under GDPR guidelines

REC Reference: 2019-05 List 17 (01)

Previous REC Reference: 2015-01 Chairman's Action (1)

(Please quote reference on all correspondence)

EudraCT Number: N/A

Date of Valid Submission to REC: 26.03.2019

Date of Ethical Review: 08.05.2019

R&I application Number: N/A

Dear Ms Coffey,

Thank you for your correspondence in which you submitted an amendment for the above named study.

The Chairman has reviewed the documentation you submitted and approved this amendment. The following documents were reviewed:

- Non-clinical Amendment Request Form, dated 22.03.2019
- PIL & CF
- Protocol
- DPIA
- EMG Electrodes booklet
- Medical history form

Applicants must submit an annual report for ongoing projects and an end of project report upon completion of the study. It is the responsibility of the researcher/research team to ensure all aspects of the study are executed in compliance with the General Data Protection regulation (GDPR), Health Research Regulations and the Data Protection Act 2018. Additionally, please note for documents submitted for GDPR purposes that the REC and the Chair are not confirming that you're documents are GDPR compliant, they are approving the document from an ethical perspective.

Yours sincerely,



REC Officer – Dr Sadhbh O'Neill - SJH/TUH Research Ethics Committee

11.2. Appendix 4.2

Patient consent form for CMC- based study participants

**SJH / AMNCH RESEARCH ETHICS COMMITTEE.
PATIENT CONSENT FORM**

Study title: "Impairments of Neuro-muscular Communication in Motor-Neuron Disease: A Bio-Marker for Early and Personalised Diagnosis"

I have read and understood the Information Leaflet about this research project. The information has been fully explained to me and I have been able to ask questions, all of which have been answered to my satisfaction.	Yes ☐	No ☐
I understand that I don't have to take part in this study and that I can opt out at any time. I understand that I don't have to give a reason for opting out and I understand that opting out won't affect my future medical care.	Yes ☐	No ☐
I am aware of the potential risks, benefits and alternatives of this research study.	Yes ☐	No ☐
I give permission for researchers with delegated authority from Professor Hardiman and her research team to look at my medical records to get information. I have been assured that information about me will be kept private and confidential.	Yes ☐	No ☐
I agree to take part in EEG and EMG recording sessions, every 4 months (up to 3 sessions).	Yes ☐	No ☐
I agree to take part in the MUNIX arm of this study, every 4 months (up to 3 sessions)	Yes ☐	No ☐
I have been given a copy of the Information Leaflets and this completed consent form for my records. I understand that a copy is maintained in my medical records and a copy will be sent to the principal investigator.	Yes ☐	No ☐
I consent to take part in this research study having been fully informed of the risks, benefits and alternatives.	Yes ☐	No ☐
I give informed explicit consent to have my data processed as part of this research study.	Yes ☐	No ☐
I consent to be contacted by researchers as part of this research study.	Yes ☐	No ☐

<u>FUTURE CONTACT</u> [please choose one or more as you see fit]		
OPTION 1: I consent to be re-contacted by researchers about possible future MND research related to the current study for which I may be eligible.	Yes ☐	No ☐
OPTION 2: I consent to be re-contacted by researchers about possible future MND research unrelated to the current study for which I may be eligible.	Yes ☐	No ☐

STORAGE AND FUTURE USE OF INFORMATION		
RETENTION OF RESEARCH MATERIAL IN THE FUTURE [please choose one or more as you see fit]		
OPTION 1: I give permission for material/data to be stored for <u>possible future research related</u> to the current study <u>only if consent is obtained</u> at the time of the future research but only if the research is approved by a Research Ethics Committee.	Yes ☐	No ☐
OPTION 2: I give permission for material/data to be stored for <u>possible future research related</u> to the current study <u>without further consent being required</u> but only if the research is approved by a Research Ethics Committee.	Yes ☐	No ☐
OPTION 3: I give permission for material/data to be stored for <u>possible future research unrelated</u> to the current study <u>only if consent is obtained</u> at the time of the future research but only if the research is approved by a Research Ethics Committee.	Yes ☐	No ☐
OPTION 4: I give permission for material/data to be stored for <u>possible future research unrelated</u> to the current study <u>without further consent</u> being required but only if the research is approved by a Research Ethics Committee (As detailed in "Consent to Future Uses" in the information leaflet).	Yes ☐	No ☐
OPTION 5: I agree that some future research projects may be carried out by researchers working for <u>commercial/pharmaceutical companies</u> .	Yes ☐	No ☐
OPTION 6: I understand <u>I will not be entitled to a share of any profits</u> that may arise from the future use of my material/data or products derived from it.	Yes ☐	No ☐

PARTICIPANT'S NAME: _____

PARTICIPANT'S SIGNATURE: _____

DATE: _____

Date on which the participant was first furnished with this form: _____

Where the participant is incapable of comprehending the nature, significance and scope of the consent required, the form must be signed by a person competent to give consent to his or her participation in the research study (other than a person who applied to undertake or conduct the study). If the subject is a minor (under 18 years old) the signature of parent or guardian must be obtained:-

NAME OF CONSENTOR, PARENT or GUARDIAN: _____

SIGNATURE: _____

RELATION TO PARTICIPANT: _____

Where the participant is capable of comprehending the nature, significance and scope of the consent required, but is physically unable to sign written consent, signatures of two witnesses present when consent was given by the participant to a registered medical practitioner treating him or her for the illness.

NAME OF FIRST WITNESS: _____ SIGNATURE: _____
NAME OF SECOND WITNESS: _____ SIGNATURE: _____

Statement of responsibility of the Principal investigator's or his/her nominated experimenter's: I have taken the time to explain the nature, purpose, procedures, benefits, and risks of this research study to the above patient in a way they could understand. I have offered to answer any questions about any aspect of the study that concerns them and fully answered such questions. I believe that the participant understands my explanation and has freely given informed consent.

Experimenter's Name: _____

Experimenter's signature: _____

Date: _____

The 3 copies needed: 1 original for the participant's medical records in hospital, 1 copy for the participant, 1 copy for the investigator's records.

Page 3

11.3. Appendix 4.3

Questionnaire for clinical exam and ALSFRS-R

"MotorMarker - Patient Recruitment (Inclusion/Exclusion) Questionnaire"

Patient EEG/EMG code:

Date: dd / mm / year

Inclusion Criteria:

Diagnosis: ☐ ALS

☐ PLS

☐ Pollio

El Escorial:

____ Month Follow-up

☐ Possible

☐ Probable

☐ Definite

Age: ____ ☐ between 18-65 years of age.

Medical Hx:

Exclusion Criteria:

Diagnosed with:

Transient Ischemic Attack (TIA)

☐ No ☐ Yes

Multiple Sclerosis (MS)

☐ No ☐ Yes

Stroke

☐ No ☐ Yes

Epilepsy

☐ No ☐ Yes

seizure disorder

☐ No ☐ Yes

brain tumours

☐ No ☐ Yes

structural brain diseases

☐ No ☐ Yes

other neuro-degenerative diseases

☐ No ☐ Yes

known terminal conditions (e.g. human immunodeficiency virus)

☐ No ☐ Yes

psychiatric diseases

☐ No ☐ Yes

History of:

regular substance abuse

☐ No ☐ Yes

alcohol dependence syndrome,

☐ No ☐ Yes

neurological abnormality or disease

☐ No ☐ Yes

neuromuscular diseases or dysfunction

☐ No ☐ Yes

peripheral movement restrictions (e.g. neuropathy)

☐ No ☐ Yes

musculoskeletal abnormalities and disorders in right upper limb

☐ No ☐ Yes

serious injuries in their wrist and digits

☐ No ☐ Yes

any other conditions that could affect neuro-motor performance

☐ No ☐ Yes

Note to the Participant:

- No Caffeine (e.g. tea or coffee) in the day of Experiment or
- No sedatives in the past 7 days of the experiment day) for.

Recruited ☐ No ☐ Yes

MotorMarker - Disease Evaluation Questionnaire

Patient EEG/EMG code: _____

Date: dd / mm / yyyy

Edinburgh Handedness Inventory (EHI) Score (original 10+2 questions, Oldfield 1971): _____

ALSFRS-R Score and Motor Sub-scores:

Total: _____

4. Handwriting: _____

5. Cutting food and handling utensils: _____

6. Dressing and hygiene: _____

7. Turning in bed and adjusting bed clothes: _____

8. Walking: _____

9. Climbing stairs: _____

MotorMarker Study- Clinical Examination Sheet

Patient EEG code:

Date: dd / mm / yyyy

Reflexes



assessment	Absent	Decreased	Normal	Increased	Not Done
Biceps-left					
Biceps-right					
Triceps-left					
triceps-right					
Brachioradials-left					
Brachioradials-right					

Ashworth score

0	No increase in tone
1	Slight increase in tone (catch or minimal resistance)
2	Marked increase in tone through most ROM, but affected parts easily moved
3	Considerable increase in muscle tone
4	Affected part(s) rigid in flexion or extension
6	Not done

Right Elbow _____	Right Wrist _____
Left Elbow _____	Left Wrist _____

Power

assessment	No contraction	Flicker of movement	Active movement without gravity	Active movement against gravity	Active movement against resistance	Normal power	Not done
Deltoid-Left							
Deltoid-Right							
Triceps-Left							
Triceps-Right							
Biceps-Left							
Biceps-Right							
Wrist flexor-Left							

assessment	No contraction	Flicker of movement	Active movement without gravity	Active movement against gravity	Active movement against resistance	Normal power	Not done
Wrist extensor-Left							
Wrist extensor-Right							
Finger Flexor-Left							
Finger Flexor-Right							
Finger extensor-Right							
Finger extensor-Left							
FDI-Left							
FDI-Right							
APB-Left							
APB-Right							
ADM-Left							
ADM-Right							

Signs

Wasting- Thenar eminence	Yes	No
Wasting- Hypothenar eminence	Yes	No
Fasciculations (upper limbs)	Yes	No
Clonus	Yes	No
Babinski sign	Yes	No
Hoffmans sign	Yes	No

MotorMarker Study- ALSFRS-R

Patient EEG/EMG code:

Date: dd / mm / ~~yyyy~~

1. Speech	
4	Normal speech processes
3	Detectable speech disturbance
2	Intelligible with repeating
1	Speech combined with ~~nonverbal~~ communication
0	Loss of useful speech
2. Salivation	
4	Normal
3	Slight but definite excess of saliva in mouth; may have nighttime drooling
2	Moderately excessive saliva; may have minimal drooling
1	Marked excess of saliva with some drooling
0	Marked drooling; requires constant tissue or handkerchief
3. Swallowing	
4	Normal eating habits
3	Early eating problems — occasional choking
2	Dietary consistency changes
1	Needs supplemental tube feeding
0	NPO (exclusively parenteral or enteral feeding)
4. Handwriting	
4	Normal
3	Slow or sloppy: all words are legible
2	Not all words are legible
1	Able to grip pen but unable to write
0	Unable to grip pen
5a. Cutting food and handling utensils (patients without gastrostomy)?	
4	Normal
3	Somewhat slow and clumsy, but no help needed
2	Can cut most foods, although clumsy and slow; some help needed
1	Food must be cut by someone, but can still feed slowly
0	Needs to be fed
5b. Cutting food and handling utensils (alternate scale for patients with gastrostomy)?	
4	Normal
3	Clumsy but able to perform all manipulations independently
2	Some help needed with closures and fasteners
1	Provides minimal assistance to caregiver
0	Unable to perform any aspect of task
6. Dressing and hygiene	
4	Normal function

3	Independent and complete self-care with effort or decreased efficiency
2	Intermittent assistance or substitute methods
1	Needs attendant for self-care
0	Total dependence
7. Turning in bed and adjusting bed clothes	
4	Normal
3	Somewhat slow and clumsy, but no help needed
2	Can turn alone or adjust sheets, but with great difficulty
1	Can initiate, but not turn or adjust sheets alone
0	Helpless
8. Walking	
4	Normal
3	Early ambulation difficulties
2	Walks with assistance
1	~~Nonambulatory~~ functional movement
0	No purposeful leg movement
9. Climbing stairs	
4	Normal
3	Slow
2	Mild unsteadiness or fatigue
1	Needs assistance
0	Cannot do
10. Dyspnea	
4	None
3	Occurs when walking
2	Occurs with one or more of the following: eating, bathing, dressing (ADL)
1	Occurs at rest, difficulty breathing when either sitting or lying
0	Significant difficulty, considering using mechanical respiratory support
11. Orthopnea	
4	None
3	Some difficulty sleeping at night due to shortness of breath, does not routinely use more than two pillows
2	Needs extra pillows in order to sleep (more than two)
1	Can only sleep sitting up
0	Unable to sleep
12. Respiratory insufficiency	
4	None
3	Intermittent use of BiPAP
2	Continuous use of BiPAP during the night
1	Continuous use of BiPAP during the night and day
0	Invasive mechanical ventilation by intubation or tracheostomy

MotorMarker Study – Edinburgh Handedness Inventory
 Patient EEG/EMG code: _____ Date: dd / mm / yyyy

Activity	Right	Left	Either
Writing			
Drawing			
Throwing			
Scissors			
Toothbrush			
Knife(without fork)			
Spoon			
Broom (upper hand)			
Striking match(match)			
Opening box/lid			
Which foot prefer to kick with?			
Which eye used when only using one?			

Difference	Cumulative Total	Result

11.4. Appendix 6.1

Coffey et al., 2021



Altered supraspinal motor networks in survivors of poliomyelitis: A cortico-muscular coherence study

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HIGHLIGHTS

- Abnormal coherence patterns provide neurophysiologic evidence of supraspinal change in those affected by poliomyelitis.
- Cortico-Muscular Coherence changes in Polio patients reflect functional re-organisation of the central-peripheral network.
- Cortico-Muscular Coherence is a potential biomarker of altered Motor network function in Polio, SMA and other related conditions.

ABSTRACT

Objective: Poliomyelitis results in changes to the anterior horn cell. The full extent of cortical network changes in the motor physiology of polio survivors has not been established. Our aim was to investigate how focal degeneration of the lower motor neurons (LMN) in infancy/childhood affects motor network connectivity in adult survivors of polio.

Methods: Surface electroencephalography (EEG) and electromyography (EMG) were recorded during an isometric pincer grip task in 25 patients and 11 healthy controls. Spectral signal analysis of cortico-muscular (EEG-EMG) coherence (CMC) was used to identify the cortical regions that are functionally synchronous and connected to the periphery during the pincer grip task.

Results: A pattern of CMC was noted in polio survivors that was not present in healthy individuals. Significant CMC in low gamma frequency bands (30–47 Hz) was observed in frontal and parietal regions.

Conclusion: These findings imply a differential engagement of cortical networks in polio survivors that extends beyond the motor cortex and suggest a disease-related functional reorganisation of the cortical motor network.

Abbreviations: ADM, abductor digiti minimi; APB, abductor pollicis brevis; APL, abductor pollicis longus; CMC, corticomuscular coherence; DFT, discrete Fourier transform; EDC, extensor digitorum communis; EEG, electroencephalography; EMG, electromyography; EPB, extensor pollicis brevis; FDI, first dorsal interosseous; FDR, false discovery rate; FDS, flexor digitorum superficialis; FPB, flexor pollicis brevis; FDMB, flexor digiti minimi brevis; LMN, lower motor neuron; MRC, medical research council; MVC, maximum voluntary contraction; PPS, post polio syndrome; SMA, spinal muscular atrophy.

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Significance: This research has implications for other similar LMN conditions, including spinal muscular atrophy (SMA). CMC has potential in future clinical trials as a biomarker of altered function in motor networks in post-polio syndrome, SMA, and other related conditions.

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1. Introduction

15–20 million people across the world experience the late sequelae of poliomyelitis (Mehndiratta et al., 2014). There is evolving evidence that central pathways for movement may be affected in polio survivors, either directly as a result of the original illness, or as a compensatory process following the loss of motor neurons (Allen et al., 1994; Lupu et al., 2008; Bodian, 1949, 1952). Moreover, post-mortem studies have demonstrated supraspinal involvement, as evidenced by gliosis of white matter tracts (Bruno et al., 1994), punctate lesions of grey matter within motor midbrain areas (particularly the reticular formation) (Bruno et al., 1994; Bodian, 1949, 1952) and lesions of the motor cortex (Bodian, 1949, 1952).

Despite the presence of pathologic evidence, there have been few detailed neurophysiological studies to investigate the nature and extent of alteration in network dynamics in the supraspinal components of the motor system.

Cortico-muscular coherence (Boonstra, 2013; Conway et al., 1995) is an emerging neurophysiological approach that can be applied to examine the integrated physiology of cortical, corticospinal and neuromuscular systems in neurological conditions (Proudfoot et al., 2018).

Cortico-muscular coherence provides a measure of coupling between cortical oscillations and motor unit firing patterns along the corticospinal tract (Mima and Hallett, 1999; Boonstra, 2013). The presence of coherence can be explained by an adequate number of motor neurons receiving temporally synchronised transmissions of synaptic input from cortical projections, coupled with afferent feedback from muscle to spinal and cortical networks (Conway et al., 1995). CMC frequency bands and their physiological correlates have been described in literature in healthy subjects (see Table 1), as such we aim to build on these findings and explore CMC changes in lower motor neuron conditions.

We have used cortico-muscular coherence to investigate the presence of alterations in the motor networks in survivors of poliomyelitis. We show that patients who suffered polio in childhood exhibit distinct changes in cortical connectivity that extend beyond the primary motor cortex.

2. Methods

2.1. Ethics

The study was approved by the Tallaght University Hospital/St. James's Hospital Joint Research Ethics Committee – Dublin [REC Refer-

ence: 2019-05 List 17 (01)] and performed in accordance with the Declaration of Helsinki. All patients provided informed written consent to the procedures before undergoing assessment.

2.2. Patient cohort

Patients were prospectively recruited in this cross-sectional study between June 2017–November 2019 through the national clinic for polio survivors at Beaumont Hospital. All patients had a verified diagnosis of poliomyelitis in childhood, and all had supportive clinical and electromyographic findings. Healthy controls, age-matched to patients, were recruited from database of healthy controls interested in taking part in research.

Subjects with a history of major head trauma or other neurological conditions that could affect cognition, alcohol dependence syndrome, current use of neuroleptic medications or high-dose psychoactive medication were excluded. Those with diabetes mellitus, a history of cerebrovascular disease, and those with neuropathy from other causes were also excluded.

2.3. Clinical assessment

On the day of EEG recording all patients underwent an extensive clinical assessment. Disease duration from symptom onset and site of disease onset were recorded. Muscle strength was assessed using the Medical Research Council (MRC) score in 9 bilateral (i.e. 18) upper limb muscles, including deltoid, triceps, biceps, wrist flexors and extensors, fingers flexors and extensors, and abductors of the index fingers and thumbs. The degree of clinical lower motor neuron (LMN) involvement in the upper limbs was graded by a LMN score (de Carvalho et al., 2003) from these MRC scores, which ranged from 90 (absent LMN signs) to 0 (severe LMN signs).

Handedness was assessed in all participants with the Edinburgh Handedness Inventory (Oldfield et al., 1971).

2.4. Experimental paradigm

Assessment was conducted in the same manner for the patients and control groups. All participants were asked to minimise their eye movements and to relax during the experiment. Subjects sat in a comfortable chair that supported them in a stable posture, in front of a 23" computer monitor (distance from eyes: ~1 m), with upper arm elevated at approximately 40° from shoulder and elbow at 90° resting on a pillow over a desk.

Participants attempted maximum voluntary contraction (MVC) of the pincer grip between the thumb and the index finger of their right hand (Fig. 1) guided by visual cues. Each MVC exertion was requested for 5 s, with 30 s delays between trials. MVC was taken as the average peak force of the three trials which were within 10% of each other. Note MVC trials were used for the purpose of calibration of force and visual interface and not for physiological signal recordings.

Participants attempted 30 voluntary isometric pincer grips between the thumb and the index finger of their right upper limb, according to visual cues. The onset and offset of the exertion were signalled to the subject by visual cues. Each exertion was requested at 10%MVC for 5 s, with 10 s delays between trials for rest. Partic-

Table 1
Frequency bands and their physiological correlates described in literature in healthy controls.

Band	Frequency	Physiological Correlates
Alpha Band	8–12 Hz	Physiological tremor (McAuley and Marsden, 2000)
Beta Band	15–30 Hz	Sustained muscle contraction (Conway et al., 1995)
Gamma Band	30–60 Hz	Dynamic force output (Omlor et al., 2007)

participant were instructed to use their preferred pace for increasing and decreasing the grip force and to avoid making abrupt changes in force. The force level exerted by the participant was deemed to be acceptable if the error was less than 10% of the range. The aim was to capture the cortico-muscular coherence, only during the exertion of these low-levels of steady-state force at 10% MVC. A visuomotor force-control isometric task was used, rather than position-control grip task at lower force levels, with a view to maintaining a low level of baseline CMC. We verified this by comparing the beta-band CMC between C3-FPB in a pilot position control task (significant classic CMC present, $p = 0.0102$) and our main visuomotor task (non-significant CMC, $p = 0.9429$), were a significant difference ($p = 0.0068$, corrected for multiple comparisons) was observed. This was asserted by preliminary analysis of CMC in our control group, and according to previous findings on CMC (Liu et al., 2019). It was anticipated that this would permit discrimination across patient groups. The time window during steady-state force was used for analysis.

2.4.1. Force

Grip force was recorded using 2 flat resistive force sensors (FlexiForce A201 Sensor, Tekscan, Inc., Boston, MA, USA) with their circular sensing area ($d = 9.7$ mm) attached to the 2 bases of a wooden hexagon (Fig. 1). The resistance was converted to analogue voltage using a small circuit board (Tekscan, Inc., Boston, MA, USA) and was recorded and digitised using a Data Acquisition Card (PCIe-6321, National Instruments, Austin, TX, USA) at 2000 Hz in real time, and subsequently visualised and presented to the participant.

2.4.2. Visual interface

The visual stimuli and the visual feedback of the grip force was programmed in MATLAB® (Mathworks, Inc., Natick, MA, USA) using Psychophysics Toolbox (Brainard, 1997) at a screen refresh rate of 60 Hz. The typical delay between the visualisation loop and the recording loop was 1–3 ms.

2.5. Data acquisition

EEG data were recorded in a special purpose laboratory, electromagnetically shielded as a Faraday cage, using 128-channel scalp electrode cap, filtered over the range 0–400 Hz and digitized at 2048 Hz using the BioSemi® ActiveTwo system (BioSemi B.V., Amsterdam, Netherlands). Each participant was fitted with an appropriately sized EEG cap.

Surface EMG was recorded (Fig. 1) from 8 muscles in the right upper arm: FDI (first dorsal interosseous); EDC (Extensor Digitorum Communis); FDS (Flexor Digitorum Superficialis); APL (Abductor Pollicis Longus) and EPB (Extensor Pollicis Brevis); FPB (Flexor Pollicis Brevis); APB (Abductor Pollicis Brevis); ADM (Abductor

Digiti Minimi); FDMB (Flexor Digiti Minimi Brevis). These 8 muscles were chosen for recording surface EMG and were not the same as the 9 muscle pairs chosen for strength-based clinical assessment. Bipolar channels were used according to the provided recommendation by SENIAM (Hermens et al., 2000; Merletti and Hermens, 2000). Surface EMG recordings were conducted using flat active sintered Ag-AgCl electrodes (BioSemi B.V., Amsterdam, The Netherlands), which provided a circular recording area ($d = 3$ mm) in a 17×10 mm support surface area. An interelectrode distance of 1 cm (up to a maximum of 2 cm) was used for bipolar recording. The placement of EMG electrodes with respect to the muscle locations followed previously reported recommendations (Lee et al., 2005; Barbero et al., 2012; Pease et al., 2007). The sampling frequency and the filter settings for the EMG channels were the same as the EEG channels.

2.6. Data analysis

EEG/EMG data analysis was performed by an engineer who was blind to clinical assessments. Five EEG channels (Cz, Pz, C4, Fz, C3) and 3 EMG channels (APB, FDI, FPB) were chosen prior to the analysis of cortico-muscular coherence (CMC). This selection was based on the biomechanical involvement of the muscles in the pincer grip task (Danna-Dos Santos et al., 2010), and the suitability of the EEG and EMG channels for assessing CMC (Witham et al., 2011). A time window/epoch duration of 4 s (starting 1 s after the visual cue) was chosen for analysis; data epochs where the target force was not correctly achieved were excluded. The raw EEG data was (re-)referenced using (large) surface Laplacian spatial filter (Bradshaw and Wikswo, 2001; McFarland et al., 1997) which is a spatial filter for removing spurious signal components in EEG channels, and EMG data (signal amplitude) were normalized with respect to the Force value during 100 % MVC. EEG/EMG data were filtered between 1 and 100 Hz using a dual-pass 4th order Butterworth bandpass filter. This was followed by a 50 Hz Discrete Fourier Transform (DFT) filter in the FieldTrip toolbox to remove power line noise (Supplementary Fig. S2). An automatic artefact detection and rejection was performed using FieldTrip toolbox (Oostenveld et al., 2011) to remove eye-blinks, muscle, and electrode jump artefacts from EEG signals. The auto-spectrum of each EEG/EMG signal, and cross-spectrum between all combinations of EEG-EMG signals (frequency resolution 1 Hz, bandwidth 2–100 Hz) was calculated using FieldTrip toolbox (Hanning taper and frequency smoothing at 1 Hz). The auto- and cross-spectra at each frequency (2–100 Hz) was converted into 8 band values-delta (2–4 Hz), theta (5–7 Hz), lower alpha (8–10 Hz), higher alpha (11–13 Hz), lower beta (14–20 Hz), higher beta (21–30 Hz), lower gamma (31–47 Hz), and higher gamma (53–97 Hz).



Fig. 1. Pincer grip isometric task used to assess cortico-muscular coherence (CMC) (left) and the recording of surface EMG during the experiment (right).

excluding the 48–52 Hz range to avoid mains power noise. The formation of band-specific values was carried out by taking Spatial Median (a variation of the median operator for complex-valued spectra, chosen and preferred over the algebraic averaging to provide robustness against outlier values) (Niinimaa and Oja, 2014; Nasserolelami et al., 2019) of the spectra at corresponding frequencies. The spectral coherence (cortico-muscular coherence in this study) was obtained by normalizing the cross-spectrum by the respective auto-spectra (Supplementary Fig. S2) (Nasserolelami et al., 2019).

The selection of the parameters and methods for signal processing (e.g. band-specific analysis and the use of non-parametric methods) was based on our previous EEG studies (Nasserolelami et al., 2017; Dukic et al., 2019) that provided robust estimations not sensitive to outliers or observations in individual subjects (Dukic et al., 2017).

2.7. Statistics

Participant-level statistics were calculated using one-sample non-parametric rank statistics for spectral coherence (Nasserolelami et al., 2019). This method gave individual p-values for spectral cortico-muscular coherence in each frequency band for both patients and control groups (Fig. 2). Stouffer's method (Stouffer et al., 1949; Westfall, 2014) was used to combine individual p values to derive group average p value. This procedure is equivalent to the pooled coherence analysis (Halliday et al., 1995).

Correction for multiple comparisons was performed using the adaptive false discovery rate at $q = 0.05$ (Benjamini et al., 2006), which was applied by correcting the p-values in the coherence spectra. Negative logarithm of p-values, i.e. $-\log_{10}(p)$, was used to visualize cortico-muscular coherence. The band-specific values of coherence, expressed in $-\log_{10}(p)$, was used to represent the values for all of the frequencies in that frequency band.

3. Results

3.1. Patients' clinical profile

A total of 25 patients affected by Poliomyelitis were successfully recruited from a cohort Polio clinic based in Beaumont hospital, Dublin (see Tables 2 and 3). One patient was subsequently excluded from analysis as recording of the motor task was carried out using his left hand, due to inadequate strength in right hand. The analysed patient group included 17 female and 7 male patients (mean age of 67.04 ± 6.8 (Standard deviation), 22 right hand dominant). From this group, 8 patients suffered poliomyelitis in first 24 months of life with 16 contracting polio after 24 months of age. Muscle weakness, graded by the LMN score, was 85.2 ± 6.6 points. A total of 11 healthy controls were successfully recruited (mean age of 61.09 ± 14.8 standard deviation).

3.2. Abnormal cortico-muscular coherence in the PPS patient group

Cortico-muscular coherence (CMC), during steady low force isometric pincer grip (Fig. 3), was calculated between intrinsic hand muscles (using bipolar EMG) and surface EEG electrodes over scalp. In this context, we identified patterns of CMC in the PPS group that differed from those of the controls (see Table 1). Specifically, the CMC in the patient group did not show the typical beta-band CMC over contralateral motor area (as intended by the task selection), and the CMC across the 5 tested EEG electrodes, 3 muscles, and the frequency bands were scattered and inconsistent (Supplementary Fig. S1). These null findings were in accordance with expectations from our pilot study in controls, as well as the anticipation of typical beta-band cortico-muscular coherence primarily over primary motor cortex, C3 electrode (seen in low force sustained muscle contraction (Salenius et al., 1997; Mima and Hallett, 1999; Conway et al., 1995).

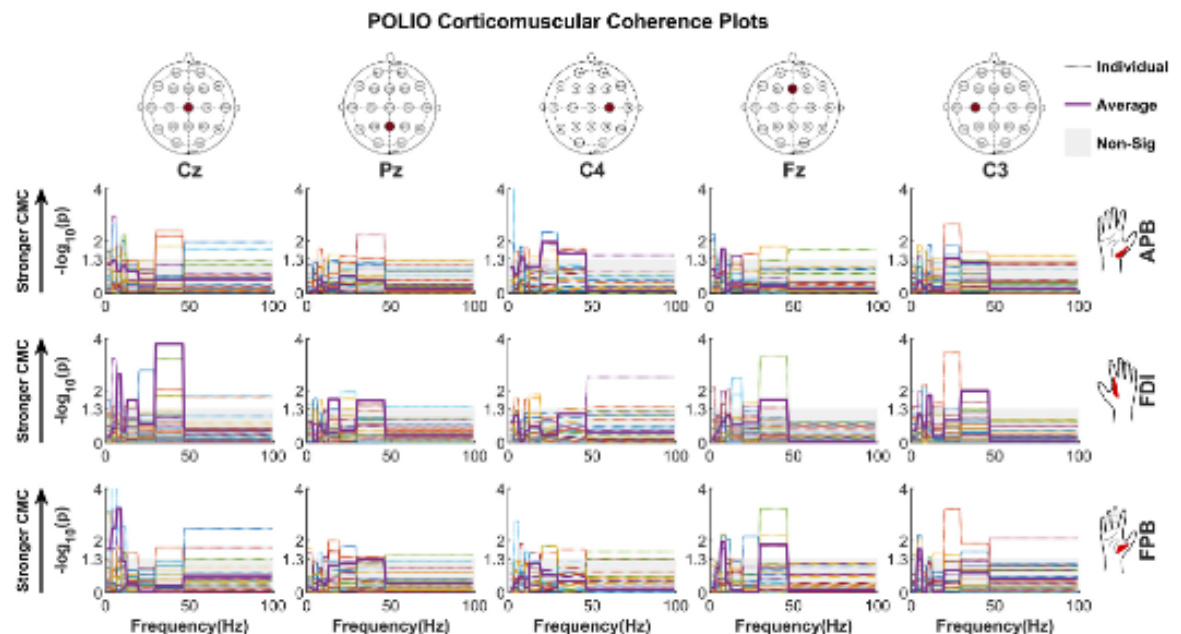


Fig. 2. The cortico-muscular coherence (CMC) spectra in the polio patient group, showing the significant values at individual and group-level. *Abductor Pollicis Brevis (APB); First dorsal interosseous (FDI); Flexor Pollicis Brevis (FPB).

Table 2
Clinical and demographic data of analysed patients.

	Patient Group	Controls
Gender (F/M)	17/7	4/7
Average age at recording (F/M)	68.5 ± 3.6/63.5 ± 10.8	64.7 ± 4.2/59 ± 18.5
Onset < 24 months	8/16	–
LMN score ^a (max 90)	85.2 ± 6.6	90

^a LMN (Lower Motor Neuron Score).**Table 3**
Clinical and demographic data of patient sub-groups.

	Infant-onset (<24 months)	Childhood-onset (≥24 months)
Number of Patients	8	16
Average age of onset (in yrs)	0.97 ± 0.5	4.12 ± 2.4
LMN score ^a	86.6 ± 4.1	84.5 ± 7.6

^a LMN (Lower Motor Neuron Score).

In the PPS group the CMC was statistically significant ($p < 0.05$, $q = 0.05$) at the group level (Fig. 3), and appeared at frequencies different from the commonly observed and expected beta (14–30 Hz) frequency bands. Instead, the significant CMC appeared consistently in the low gamma (31–47 Hz) frequency band across several EEG-EMG channels (see Fig. 2). Importantly, this abnormal low gamma-band coherence was observed not only over primary motor (C3 and C2, and C4) areas, but also over parietal and frontal areas (Fz and Pz). The abnormal CMC appeared in other (e.g. alpha) frequency bands but was less consistently across EEG-EMG channels. These CMC patterns differed from the control cohort in frequency (beta vs. low-gamma) and location (contralateral motor C3 vs Frontal Fz and Parietal Pz) (Conway et al., 1995, Mehrkanoon et al., 2014), (Figs. 2 and 3).

The pathological gamma-band CMC was a consistent finding, and was observed at both frontal and parietal electrodes and in both FDI and FPB muscles (Fig. 2 and Supplementary Fig. S1).

3.3. Polio sub-groups

The analysis of CMC in separate patient subgroups, revealed evidence of differences between those affected by polio virus in infancy (define as <24 months) and those who developed the paralysis in later childhood (≥24 months) in frontal and parietal areas (Fig. 4).

4. Discussion

This study provides robust neurophysiologic evidence of extensive supraspinal changes in those affected by poliomyelitis in childhood. The physiological correlates of frequency bands have been described in literature, demonstrating that beta band coherence appears during weak tonic contraction especially when directed towards a motor task (Kristeva-Feige et al., 2002). Gamma band coherence becomes more apparent on strong muscle contraction (Kilner et al., 1999), while Alpha band coherence has been described in resting physiological tremor (McAuley and Marsden, 2000). The presence of all of these frequency bands in unexpected cortical areas within the patient group implies the presence of a disrupted central-peripheral network.

These abnormal patterns, with increases in the gamma band, were consistent at group level in 24 patients despite major differences and heterogeneity in clinical disability (affected limb), and age of first polio diagnosis. These changes most likely reflect functional re-organisation of the central-peripheral network, possibly

as a compensatory response to continuous remodelling of the motor units (Oliveri et al., 1999).

Our observations are consistent with recent TMS studies which show enlarged motor maps in the cortical areas of Motor Evoked Potentials (MEPs) in adult patients who contracted polio prior to reaching 18 months of age (Oliveri et al., 1999). Our findings are also congruent with existing knowledge of cortical neurophysiology (Jaisner et al., 2016). The role of sensory feedback loops in regulating the motor cortex output has been well described (Fuhr et al., 1992; Cohen et al., 1991), and it is plausible that changes in the normal proprioceptive feedback due to muscle spindle dysfunction (as a consequent of both alpha and gamma motor neuron degeneration) contribute to the observed CMC changes (Oliveri et al., 1999). The observed CMC pattern may also be influenced by functional changes in projections from cortical layers to spinal cord, and transcortical and the corticofugal pathways, although multi-modal or source analysis studies will be required to further elucidate the underlying neuroanatomical and neurophysiological origins of the abnormal CMC.

The observed trend toward differences in CMC patterns between infancy and childhood onset patients are likely to reflect differences in compensatory patterns that occur as the neuroaxis matures. During development, the human cortex is closely linked to the spinal motor centre, reflecting the uniquely dominant role of the corticomotoneuronal system in human control of movement (Eyre et al., 2000; Galea and Darian-Smith, 1995; Williams et al., 2017). Early assaults to the anterior horn cell are more likely to radically alter motor circuitry, as formation and elimination of synapses occurs during infancy and childhood (Huttenlocher and Dabholkar, 1997). This “heterochronous synaptogenesis” (Graziadio et al., 2010) could differentially influence the development of a compensatory processes following anterior horn cell injury in infancy and later in life respectively.

This observation of alterations in corticomotor circuitry following poliomyelitis has implications for other conditions, notably spinal muscular atrophy (SMA), for which quantitative biomarkers of drug efficacy are urgently required. In conditions such as SMA, for example, a consistent abnormal CMC measure in (adult) SMA, could be used as a potential biomarker to track network function. Such biomarkers can identify network-level changes associated with lower motor neurons following administration of a disease modifying agent, thus providing a quantitative biomarker of efficacy in clinical trials. Indeed, recent imaging studies have suggested the presence of altered cortical connectivity in SMA patients (Querini et al., 2019), implying that the CMC changes observed may not be specific to polio patients, but rather a more generic compensatory physiologic reorganization of cortical circuitry following damage to the lower motor neuron.

This study is not without limitations, which include a small sample size which precluded detailed analysis of subgroups. This is a function of the relative rarity of polio survivors in European countries. Accordingly, while the overall group sample size provides robust statistical results, interpretation of the subsequent subcategorizations are preliminary. Source analysis of brain sources, increased sample sizes, and multivariate spectral analysis will be instrumental in further elucidating the patterned changes of motor circuitry in future studies.

Notwithstanding, our study demonstrates that CMC is a powerful tool that can evaluate the function of the motor circuits as an entire connected network. The unmasking of activity in networks upstream to the anterior horn suggests plasticity of motor circuitry especially when these disruptions occur at a younger age. Further investigation will be required to provide a more complete understanding of how modulation of cortical circuitry occurs in pure lower motor neuron disorders. However, this study provides a robust proof of concept demonstrating that interrogation of CMC

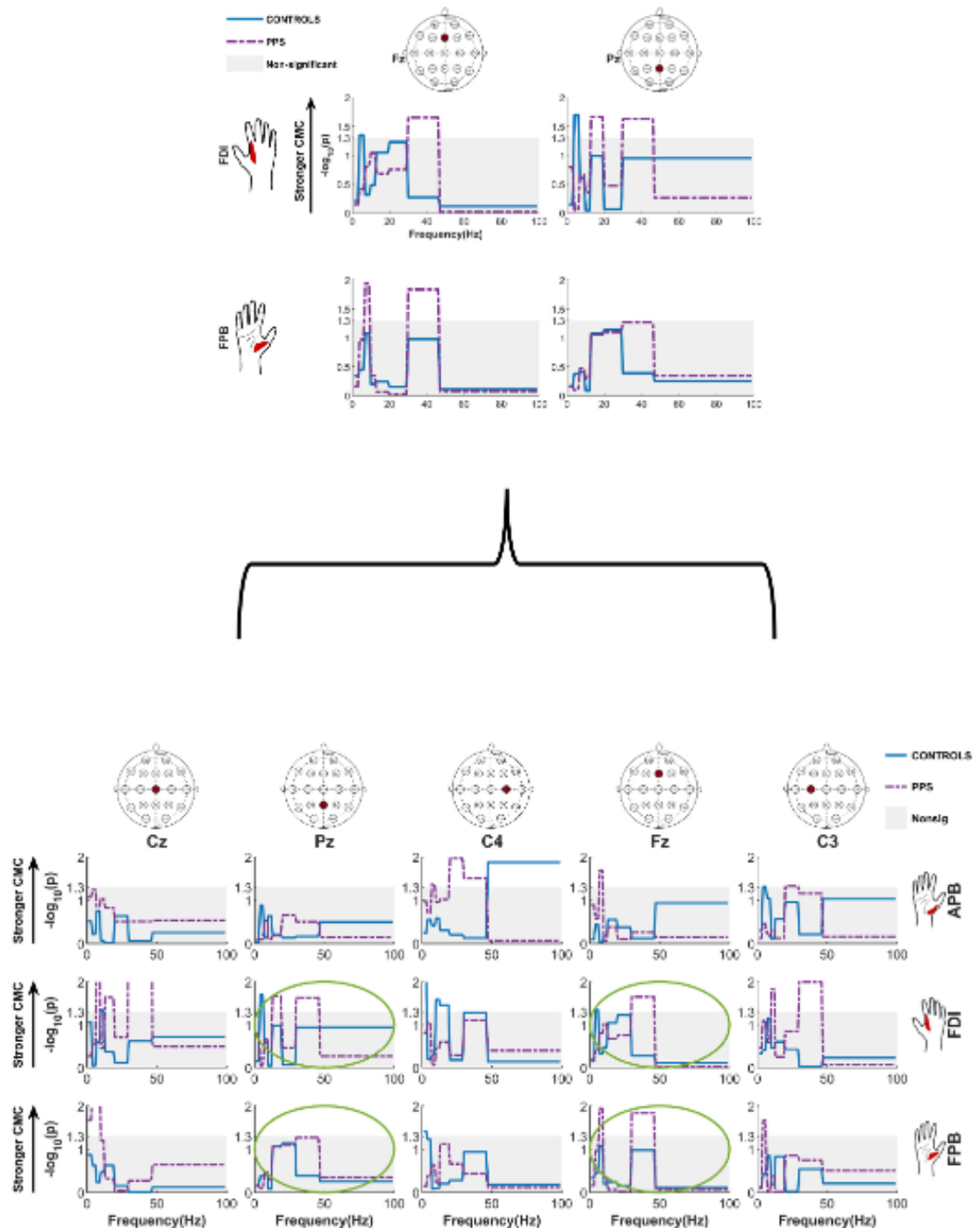


Fig. 3. Patients with Post-Polio Syndrome (PPS) show abnormal group-level Cortico-Muscular (EEG-EMG) Coherence (CMC) in non-primary motor area. The cortico-muscular coherence spectra, expressed in $-\log_{10}(p)$, show the synchrony between the EEG electrodes (over the frontal area, Fz, and parietal area, Pz) and EMG (First Dorsal Interosseous and Flexor Pollicis Brevis muscles) in different frequency bands. The lower values show less synchrony, whereas the higher value show higher EEG-EMG synchrony. The shaded area corresponds to the non-significant values at $\alpha = 0.05$ threshold for p-values (corrected for multiple comparison using false discovery rate (FDR) at $q = 0.05$). Notice the dominant abnormal coherence in PPS coherence in low gamma-band (30–47 Hz) which is present in both muscles and in both Pz and Fz electrodes. *Abductor Pollicis Brevis (APB); First dorsal interosseous (FDI); Flexor Pollicis Brevis (FPB).

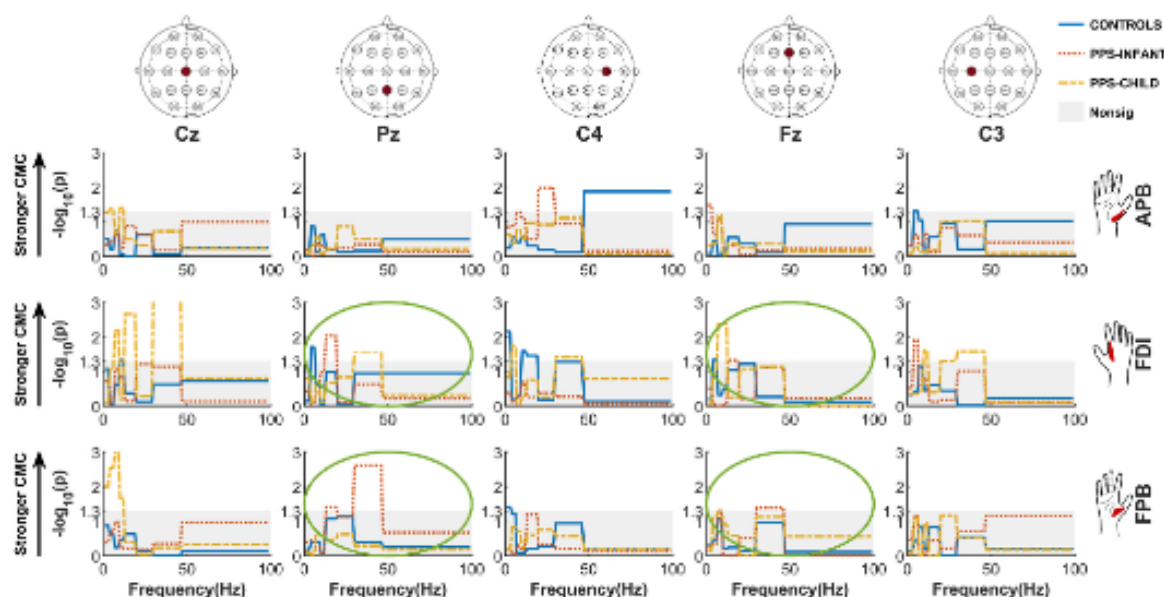


Fig. 4. Patient subgroups with onsets in infancy (<24 months) vs. childhood (>24 months) show different abnormal Cortico-Muscular (EEG-EMG) Coherence (CMC) patterns. The subgroups show subgroup-specific abnormal patterns. Notice the similarity to the group-level findings as well as differential trends in the 2 subgroups. The shaded area corresponds to the non-significant values at $\alpha = 0.05$ threshold for p-values (corrected for multiple comparison using false discovery rate (FDR) at $q = 0.05$). *Abductor Pollicis Brevis (APB); First dorsal interosseus (FDI); Flexor Pollicis Brevis (FPB).

patterns could be developed as a reliable marker of therapeutic efficacy in conditions such as PPS and SMA, where current quantitative clinical outcome measurements are limited by severe motor disability.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.clinph.2020.10.011>.

References

- Allen GM, Gandevia SC, Neering IR, Hickie I, Jones R, Middleton J. Muscle performance, voluntary activation and perceived effort in normal subjects and patients with prior poliomyelitis. *Brain* 1994;117:661–70.
- Barbero M, Merletti R, Rainoldi A. Atlas of muscle innervation zones: understanding surface electromyography and its applications. Milan (New York): Springer; 2012.
- Benjamini Y, Krieger AM, Yekutieli D. Adaptive linear step-up procedures that control the false discovery rate. *Biometrika* 2006;93:491–507.
- Bodian D. Histopathologic basis of clinical findings in poliomyelitis. *Am J Med* 1949;6:563–78.
- Bodian D. Pathogenesis of poliomyelitis. *Am J Public Health Nations Health* 1952;42:1388–402.
- Boonstra TW. The potential of corticomuscular and intermuscular coherence for research on human motor control. *Front Hum Neurosci* 2013;7:855.
- Bradshaw LA, Wikswo Jr JP. Spatial filter approach for evaluation of the surface Laplacian of the electroencephalogram and magnetoencephalogram. *Ann Biomed Eng* 2001;29:202–13.
- Brainard DH. The psychophysics toolbox. *Spat Vis* 1997;10:433–6.
- Bruno RL, Cohen JM, Galski T, Frick NM. The neuroanatomy of post-polio fatigue. *Arch Phys Med Rehabil* 1994;75:498–504.
- Cohen LG, Bandinelli S, Findley TW, Hallett M. Motor reorganization after upper limb amputation in man. A study with focal magnetic stimulation. *Brain* 1991;114:615–27.
- Conway BA, Halliday DM, Farmer SF, Shahani U, Maas P, Weir AJ, et al. Synchronization between motor cortex and spinal motoneuron pool during the performance of a maintained motor task in man. *J Physiol* 1995;489:917–24.
- Danna-Dos Santos A, Poston B, Jesunathadas M, Bobich LR, Hamm TM, Santello M. Influence of fatigue on hand muscle coordination and EMG-EMG coherence during three-digit grasping. *J Neurophysiol* 2010;104:3576–87.
- de Carvalho M, Scotto M, Lopes A, Swash M. Clinical and neurophysiological evaluation of progression in amyotrophic lateral sclerosis. *Muscle Nerve* 2003;28:630–3.
- Dukic S, Iyer PM, Mohr K, Hardiman O, Labor EC, Nasserollesami B. Estimation of coherence using the median is robust against EEG artefacts. *Conf Proc IEEE Eng Med Biol Soc* 2017;2017:3949–52.
- Dukic S, McMackin R, Buxo T, Fasano A, Chipika R, Pinto-Grau M, et al. Patterned functional network disruption in amyotrophic lateral sclerosis. *Hum Brain Mapp* 2019;40:4827–42.
- Eyre JA, Miller S, Clowry GJ, Conway EA, Watts C. Functional corticospinal projections are established prenatally in the human foetus permitting involvement in the development of spinal motor centres. *Brain* 2000;123:51–64.

- Fuhr P, Cohen LG, Dang N, Findley TW, Haghighi S, Oro J, et al. Physiological analysis of motor reorganization following lower limb amputation. *Electroencephalogr Clin Neurophysiol* 1992;85:53–60.
- Galea MP, Darian-Smith I. Postnatal maturation of the direct corticospinal projections in the macaque monkey. *Cereb Cortex* 1995;5:518–40.
- Graziadio S, Basu A, Tomasevic L, Zappasodi F, Tecchio F, Eyre JA. Developmental tuning and decay in senescence of oscillations linking the corticospinal system. *J Neurosci* 2010;30:3663–74.
- Halliday DM, Rosenberg JR, Amjad AM, Breeze P, Conway BA, Farmer SF. A framework for the analysis of mixed time series/point process data—theory and application to the study of physiological tremor, single motor unit discharges and electromyograms. *Prog Biophys Mol Biol* 1995;64:237–78.
- Hermens HJ, Freriks B, Disselhorst-Klug C, Rau G. Development of recommendations for SEMG sensors and sensor placement procedures. *J Electromyogr Kinesiol* 2000;10(5):361–74. [https://doi.org/10.1016/S1050-6411\(00\)00027-4](https://doi.org/10.1016/S1050-6411(00)00027-4)
- Huttenlocher JR, Dabholkar AS. Regional differences in synaptogenesis in human cerebral cortex. *J Comp Neurol* 1997;387:167–78.
- Jaiser SR, Baker MR, Baker SN. Intermuscular coherence in normal adults: variability and changes with age. *PLoS One* 2016;11. e0149029–e149029.
- Kilner JM, Baker SN, Salenius S, Jousmaki V, Hari R, Lemon RN. Task-dependent modulation of 15–30 Hz coherence between rectified EMGs from human hand and forearm muscles. *J Physiol* 1999;516:559–70.
- Kristeva-Feige R, Fritsch C, Timmer J, Lücking CH. Effects of attention and precision of exerted force on beta range EEG-EMG synchronization during a maintained motor contraction task. *Clin Neurophysiol* 2002;113:124–31.
- Lee HJ, Delisa JA, Lee HJ. Manual of nerve conduction study and surface anatomy for needle electromyography. Philadelphia: Lippincott Williams and Williams; 2005.
- Liu J, Sheng Y, Liu H. Corticomuscular coherence and its applications: A review. *Front Hum Neurosci* 2019;13:100.
- Lupu VD, Danielian L, Johnsen JA, Vasconcelos OM, Prokhorenko OA, Jalbary B, et al. Physiology of the motor cortex in polio survivors. *Muscle Nerve* 2008;37:177–82.
- McAuley JH, Marsden CD. Physiological and pathological tremors and rhythmic central motor control. *Brain* 2000;123:1545–67.
- McFarland DJ, McCane LM, David SV, Wolpaw JR. Spatial filter selection for EEG-based communication. *Electroencephalogr Clin Neurophysiol* 1997;103:386–94.
- Mehndiratta MM, Mehndiratta P, Pande R. Poliomyelitis: historical facts, epidemiology, and current challenges in eradication. *Neurohospitalist* 2014;4:223–9.
- Mehrkanoon S, Breakspear M, Boonstra TW. The reorganization of corticomuscular coherence during a transition between sensorimotor states. *Neuroimage* 2014;100:692–702.
- Merletti R, Hermens H. Introduction to the special issue on the SENIAM European Concerted Action. *J Electromyogr Kinesiol* 2000;10(5):283–6. [https://doi.org/10.1016/S1050-6411\(00\)00019-5](https://doi.org/10.1016/S1050-6411(00)00019-5)
- Mima T, Hallett M. Corticomuscular coherence: a review. *J Clin Neurophysiol* 1999;16:501–11.
- Nasserollesami B, Dukic S, Broderick M, Mohr K, Schuster C, Gavin B, et al. Characteristic increases in EEG connectivity correlate with changes of structural MRI in amyotrophic lateral sclerosis. *Cereb Cortex* 2017;1–15.
- Nasserollesami B, Dukic S, Buxo T, Coffey A, McMackin R, Muthuraman M, et al. Non-parametric rank statistics for spectral power and coherence. *bioRxiv* 2019.
- Niinimäa A, Oja H. Multivariate median. *Encyclopedia of statistical sciences*. Wiley StatsRef: Statistics Reference Online; 2014.
- Oldfield RC. The assessment and analysis of handedness: The Edinburgh inventory. *Neuropsychologia* 1971;9:97–113.
- Oliveri M, Brighina F, la Bua V, Buffa D, Aloisio A, Fierro B. Reorganization of cortical motor area in prior polio patients. *Clin Neurophysiol* 1999;110:806–12.
- Omlor W, Patino L, Hepp-Reymond MC, Kristeva R. Gamma-range corticomuscular coherence during dynamic force output. *Neuroimage* 2007;34:1191–8.
- Oostenveld R, Fries P, Maris E, Schoffelen JM. FieldTrip: Open source software for advanced analysis of MEG, EEG, and invasive electrophysiological data. *Comput Intell Neurosci* 2011;2011:156869.
- Pease WS, Lew HL, Johnson EW. Johnson's practical electromyography. Philadelphia: Lippincott Williams & Wilkins; 2007.
- Proudfoot M, van Ede F, Quinn A, Colclough GL, Wu J, Talbot K, et al. Impaired corticomuscular and interhemispheric cortical beta oscillation coupling in amyotrophic lateral sclerosis. *Clin Neurophysiol* 2018;129:1479–89.
- Querini G, El Mendili MM, Lenglet T, Behin A, Stojkovic T, Salachas F, et al. The spinal and cerebral profile of adult spinal-muscular atrophy: A multimodal imaging study. *Neuroimage Clin* 2019;21.
- Salenius S, Portin K, Kajola M, Salmelin R, Hari R. Cortical control of human motoneuron firing during isometric contraction. *J Neurophysiol* 1997;77:3401–5.
- Stouffer SA, Suchman EA, Devinney LC, Star SA, Williams Jr RM. The American soldier: Adjustment during army life. (Studies in social psychology in World War II) 1949;vol. 1. Oxford (England): Princeton Univ. Press; 1949.
- Westfall PH. Combining P-values. *Wiley StatsRef: Statistics Reference Online*; 2014.
- Williams P, Jiang YQ, Martin JH. Motor system plasticity after unilateral injury in the developing brain. *Dev Med Child Neurol* 2017;59:1224–9.
- Witham CL, Riddle CN, Baker MR, Baker SN. Contributions of descending and ascending pathways to corticomuscular coherence in humans. *J Physiol* 2011;589:3789–800.