



Trinity College Dublin
Coláiste na Tríonóide, Baile Átha Cliath
The University of Dublin

Novel Non-Invasive Investigation of Spinal Cord Electrical Activity in Health and Neurodegeneration

A Thesis Submitted to School of Medicine, Trinity College Dublin,
the University of Dublin, in partial fulfilment of the requirements for the
degree of Doctor of Philosophy, 2025

By

Prabhav Mehra

B.E. (Hons.), MSc

Supervised by:

Assistant Prof. Bahman Nasserolelami

Declaration, online access, and general data protection regulation

I declare that this thesis has not been submitted as an exercise for a degree at this or any other

university and it is entirely my own work.

I agree to deposit this thesis in the University's open access institutional repository or allow the Library to do so on my behalf, subject to Irish Copyright Legislation and Trinity College Library conditions of use and acknowledgement.

I consent to the examiner retaining a copy of the thesis beyond the examining period, should they so wish (EU GDPR May 2018).

- Prabhav Mehra,
September 2024

Summary

The spinal cord (SC) extends from the lower end of the medulla oblongata to the lumbar region and is divided into five segments: cervical, thoracic, lumbar, sacral, and coccygeal. The spinal cord, as part of the central nervous system, consists of grey and white matter. The spinal grey matter is surrounded by white matter, unlike the brain, where the cortical grey matter makes up the outer layers. The spinal cord is a crucial component of the sensory-motor system and has direct connections with the brain and the periphery, indicating its integral role in sensory-motor communication. The SC has been reported to be affected in neurodegenerative conditions like multiple sclerosis, with the extent of spinal neural degeneration exhibiting a strong correlation with both present and future levels of disability. Despite playing a crucial role in sensory-motor communication, the SC has been largely ignored by studies investigating the neurophysiology/pathophysiology of motor control in health and neurodegeneration.

Here, we aim to develop a novel technique for non-invasive recording of high-density (HD) neuroelectric signals from the spinal cord, electrospinalography (ESG), to investigate spinal activity in health and disease. This study features three broad objectives; 1) to design a standardized electrode placement system to record HD-ESG, 2) to investigate spinal neural oscillatory characteristics and spinal functional connectivity with the cortex during motor control in healthy individuals, 3) to investigate the evoked high-frequency oscillations in health and neurodegeneration (Multiple Sclerosis, MS).

First, a standardized electrode placement system (SC10X/U) was proposed for recording HD-ESG signals to investigate the spinal neuroelectric activity at the cervical and upper thoracic vertebral levels. This addresses the technical gaps; despite several recent studies utilizing high-density electrode montages to record spinal activity in response to peripheral stimulation, no electrode placement system exists to facilitate standardized recording across individuals and research groups. The proposed SC10X/U system can incorporate up to 76 electrodes, with their locations standardized w.r.t sixteen distinct surface anatomical markers. The system divided the total medial-lateral distance in equal proportions of 10%, and the total medial-sagittal distance was divided in proportion to the inter-vertebral spinous distances. The electrode space was symmetrically divided on either side of the spinal cord, and a labelling nomenclature featuring two uppercase letters followed by a number was proposed for identifying the unique 76 locations.

Second, HD-ESG was recorded in conjunction with HD-EEG to investigate spinal neural activity characteristics and cortico-spinal functional connectivity during voluntary isometric motor task. To our knowledge, this is the first study to non-invasively evaluate the spatio-spectral characteristics of spinal neural activity and its coherence with brain activity. Frequency-dependent neural oscillations and their neurophysiology have been widely reported in the cortex. Here, we demonstrated that spinal neurophysiology also exhibits frequency-dependent neural activity. Spinal signals in the beta-band demonstrated significant ipsi-antrolateralized ($p < 0.05$) activity that remained activate for the sustained part of the contraction. Furthermore, ipsi-antrolateral spinal electrodes indicated significant coherence ($p < 0.05$) with the contra-lateral motor cortex, indicating its involvement in descending cortico-spinal control. Unlike the beta-band, no significant ipsi-lateralization was observed for low-gamma spinal activity during the sustained period of contraction and bi-lateral activity was observed in the high-gamma band. In the low-gamma band, dorsal midline electrodes demonstrated increased spinal activity. A significant coherence ($p < 0.05$) between the spinal dorsal-midline region and central-midline EEG electrode was also observed in the low-gamma band. Additionally, ipsi-antrolateral electrodes demonstrated a steady increase in low-gamma band activity over time during the sustained phase. These findings indicate the inherent role of frequency-dependent neural oscillations in both the SC and the cortex during isometric motor control.

Third, ipsilaterally localised evoked high-frequency oscillations (HFOs) in the 400-600 Hz wide-frequency band were observed in response to the median nerve stimulation for both the control and MS participant groups. The onset latency did not show any significant difference between the two groups. The evoked HFOs at the ipsilateral electrode position, C6 vertebral level, lasted for 7.2 ± 1.5 ms and 6.7 ± 0.75 ms for the MS and control group respectively. The duration of the HFOs activity did not show significant differences between the two groups. A significant difference in the peak amplitude of the HFOs envelope was observed between the two groups for the ipsilateral electrode at the C6 vertebral level ($p = 0.021$), with people with MS demonstrating significantly lower amplitude than controls. Interestingly, the MS group had decreased spectral power in the 400-600 Hz band for the duration of the evoked-HFOs, indicating a potential disruption in the synchronization of the evoked high-frequency oscillation.

In conclusion, the proposed HD-ESG system has the potential to uncover the role of spinal neurophysiology in sensory-motor communication by non-invasive recording of the spinal

neuro-electric activity during external stimulation and/or during rest/tasks (resting-state, voluntary motor task). Here, we demonstrated that spinal neuroelectric activity/responses can be utilized to evaluate the frequency-band dependent oscillatory characteristics and cortico-spinal functional connectivity. In future, the aim is to extend the current analysis and investigate the connectivity between brain, spinal cord, and periphery during motor control. Furthermore, we aim to assess the utility of spinal neuro-oscillatory characteristics in neurodegeneration as a potential marker.

Acknowledgement

First and foremost, I would like to express my deepest gratitude to my supervisor, Dr. Bahman Nasseroleislami whose kindness and constant encouragement significantly helped me through my PhD journey. Academically, his scientific aptitude and guidance provided me the support to progress during my PhD project. I would like to show my sincere appreciation to Ms Helene Arnold, Dr. Saroj Bista, and Dr. Marjorie Metzger for their immense help with recruitment, data collection, scientific feedback, and friendly support. Furthermore, I would like to give a huge shoutout to the Neuromotor group and extended AUoN, especially Dr. Lara McManus, Dr. Gabriel Costa, Dr. Emmet Costello, Ms. Eileen Rose Giglia, Mr. Colm Peelo, Mr. Matthew Mitchell, Ms. Serena Plaitano, Ms. Bianca D'Alpaos, and Ms Adelais Farnell Sharp, with whom it was a pleasure to work with. I would like to thank my colleagues for all the insane office banter that paradoxically kept me sane.

I would like to thank all the collaborators, Prof. Orla Hardiman, Prof. Muthuraman Muthuraman, Prof. Richard Carson, Prof. Peter Bede, and Prof. Madeline Lowery who provided valuable feedback during my PhD and propelled the research to newer heights. I would also like to extend my appreciation to Dr. Hugh Kearney for his clinical expertise and support in recruitment.

I extend my appreciation to my friends for their support, shared memories, and emmet apt. movie/singing nights.

I also wish to express my love and appreciation to my fiancée, who constantly supported me, understood my stressors when I could not, and encouraged me to keep going through the tough times.

Finally, I would like to thank and share my love to my parents and family. They have always supported me throughout my life, and it was no different during my PhD. They showered constant encouragement and stood behind me like a rock so that I could progress and achieve my dreams. I will always be indebted for all the love they have shown me and all the morals they instilled in me since childhood. I would like to make a special mention to my extended family that I rekindled with in Dublin, they made me feel at home, far far away from home.

Table of Content

Declaration, online access, and general data protection regulation.....	2
Summary.....	3
Acknowledgement.....	6
Table of Content	7
List of Figures.....	11
List of Tables	15
Lists of Abbreviations.....	15
Outputs.....	19
1. Oral Presentation	19
2. Poster Presentation.....	19
I. Introduction and Background	20
1. Gross-Anatomy of Spinal Cord	20
2. Cross-Sectional Anatomy of Spinal Cord.....	22
2.1. Ascending Pathways	25
2.2. Descending Pathways.....	26
3. Imaging of Spinal Cord	27
3.1. Spinal Functional Magnetic Resonance Imaging.....	27
3.2. Non-Invasive Functional Neurophysiology	30
4. Spinal Cord in Neurodegeneration	32
5. Assessments for investigating SC (dys)Function and Sensory-Motor Tract Integrity	34
5.1. Nerve Conduction Studies.....	34
5.2. Neurophysiology Index	35
5.3. MUNE and MUNIX.....	36
5.4. Transcranial Magnetic Stimulation (TMS)	37
5.5. Beta-band Coherence	39
II. Aims and Objectives.....	40
1. Aims.....	40
2. Objectives and Rationale	40
2.1. Standardization and Feasibility of Recording HD-Electrospinography.....	40
2.2. Investigate Spinal Neural Activity and Cortico-Spinal Connectivity during Voluntary Task	41

2.3. Compare spinal neuro-electric activity recorded from people with neurodegenerative conditions.....	42
III. General Materials and Methods	43
1. Electrophysiological Signal.....	43
1.1. Brief Description of ESG Electrode Montage	44
1.2. Acquisition System	46
2. Experiment Setup	48
2.1. Interfacing System	49
2.2. Voluntary Contraction System	50
3. Experimental Procedure	51
3.1. Ethical Approval and Informed Consent.....	51
3.2. Experimental Paradigm.....	51
4. Signal Pre-Processing.....	57
4.1. Stimulation Artifact Removal	57
4.2. ESG Pre-Processing	57
4.3. EEG Pre-Processing.....	58
4.4. EMG Pre-Processing.....	58
5. Data Analysis.....	59
5.1. Median Nerve Stimulation.....	59
5.2. Pincer-Grip Voluntary Motor Contraction	62
6. Statistical Analysis	64
6.1. Parametric Statistics.....	64
6.2. Non-Parametric Statistics.....	65
6.3. Multiple Comparisons.....	65
IV. Result: SC10X/U electrode placement system - description and demonstration....	66
1. Introduction	66
2. The SC10-X/U Electrode System	69
2.1. The Design Criteria and Principles of ESG electrode placement:	70
2.2. The Design and Characteristics of the SC10-X/U Electrode Placement System	72
2.3. SC10-X/U Electrode Labels Nomenclature	74
2.4. Implementation of SC10-X/U using an Elastic Electrode Patch	76
3. Demonstration in Experiments: Methodology	76
3.1. Participant and Ethics	76
3.2. Participant Exclusion Criteria	76

3.3. Experimental Design	77
3.4. Data Analysis.....	80
4. Demonstration of HD-Electrospinography: Results	81
4.1. Average Evoked Potential	81
4.2. Topographic Map of Evoked Spinal Potentials.....	82
5. Discussion.....	83
6. Conclusion	85
V. Result: Spinal neural oscillatory characteristics and cortico-spinal coherence during motor control	85
1. Introduction	85
2. Methods	87
2.1. Participant and Ethics.....	87
2.2. Participant Exclusion Criteria	88
2.3. Experimental Paradigm	88
2.4. Experiment Setup	89
2.5. Data Acquisition.....	90
2.6. Signal Processing	90
2.7. Data Analysis.....	92
3. Results	96
3.1. Evoked Potentials.....	96
3.2. Spinal spectral power during isometric muscle activity.....	97
3.3. Event-Related Spectral Perturbations during Voluntary Motor Task	99
3.4. Cortico-Spinal coherence during isometric muscle activity.....	100
4. Discussion.....	102
VI. Result: Characteristics of evoked high-frequency oscillations in health and neurodegeneration (MS)	105
1. Introduction	105
2. Methods	106
2.1. Participants and Ethics	106
2.2. Exclusion Criteria.....	107
2.3. Experiment Setup	107
2.4. Data Acquisition.....	108
2.5. Signal Processing	108
2.6. Data Analysis.....	110
2.7. Statistical Analysis	111

3.	Results	111
3.1.	Evoked HFOs Temporal Characteristics	111
3.2.	Evoked HFOs Spectral Characteristics	113
4.	Discussion	116
VII.	Discussion	118
1.	Summary of the results	118
1.1.	Standardized Electrode Placement System	118
1.2.	Spinal Activity in response to median nerve stimulation	119
1.3.	Neural Oscillations in Spinal Cord	121
2.	Impact and Advantages of Investigating Spinal Cord using HD-ESG	122
2.1.	Assessment of spinal (dys)function	122
2.2.	Uncovering oscillatory characteristics of SC and a step forward in understanding sensorimotor control	124
3.	Limitations	125
3.1.	Participant Recruitment and Small Sample Size	125
3.2.	Non-Accessibility for highly impaired participants	125
4.	Future Work	126
4.1.	Data Collection	126
4.2.	Spinal Neural Oscillations in pwMS	126
4.3.	Investigating Spinal Activity during Motor Planning and Post-Motor Period	126
4.4.	Connectivity Measures with Brain and Muscle	127
VIII.	Bibliography	127
IX.	Appendix	159
1.	Appendix- Chapter IV	159
2.	Appendix- Chapter V	160
3.	ESG power spectrums	161
4.	Consent Forms	162
4.1.	Patient Consent Form	162
4.2.	Control Consent Form	165
5.	Edinburgh Handedness Inventory	168

List of Figures

Figure 1: This figure showcases the anterior and posterior view of the peripheral cutaneous innervations. The colored map highlights the dermatome pattern and the non-colored map highlights the peripheral nerve innervations. The The dermatomal mapping is based on study by Keegan and Garrett, 1948 and the figure is adapted from Clinical anatomy of the spine, spinal cord, and ANS, Cramer et al., 2014.....	21
Figure 2. This figure illustrates the cross-sectional view of the spinal cord. The top figure showcases the white and grey matter at the C7 spinal level, highlighting the division of the grey matter in Laminae I-IX and area X. The bottom figure showcases the ascending tracts on the left and the descending tracts on the right. The top figure is taken from Fundamental Neuroscience for Basic and Clinical Applications, Haines et al., 2018 and the bottom figure is taken from the Clinical anatomy of the spine, spinal cord, and ANS, Cramer et al., 2014.	23
Figure 3: This figure summarizes the functions and characteristics of each laminae observed in the spinal grey matter. The figure is adopted from Essential Clinical Anatomy of the Nervous System, Rea, 2015.....	24
Figure 4: Schematic illustration of the spinal activity observed in cervical and upper thoracic segments using fMRI technique. A. Cross-sectional representation of spinal fMRI activity observed at lower cervical level during both sensory stimulation and motor task paradigm. Cross-sectional representation highlights the lateralization and dorso-ventral spinal activity during the respective paradigms. B. Rostro-Caudal representation of spinal fMRI activity observed during both sensory stimulation and motor task paradigm. Rostro-caudal representation highlights the location of the observed activity over the spinal/ventral levels during the respective paradigms. The figure is taken from Landelle et al., 2021. ..	29
Figure 5: Illustration of the spatial current density observed in response to the mixed nerve stimulation. Taken from Akaza et al., 2020.	31
Figure 6: Designed SC10X/U electrode placement system and the developed electrode patch. A. The SC10X/U electrode placement system and its labels are shown. B. The developed electrode patch. The full description of the system is explained in the section IV.2.....	44
Figure 7: The schematic of the experiment setup showcasing the connections between the instruments and their respective locations.....	48
Figure 8: Schematic of the Interfacing system. The figure shows the connections for interfacing the triggers between the PC with the Biosemi trigger box and the DS7A trigger BNC port.....	49
Figure 9: The developed force sensing unit and pair of force sensors are shown. Left, force sensing units. Centre, force sensing unit and pair of force sensors. Right, the force sensor is placed in a structure during the pinch-grip task.....	50
Figure 10: A. The setup with the physio-chair and 10.1-inch screen that was used during the experiment. B. A participant sitting in an inclined position on the physio-chair. The	

participant's chest was supported by the chest rest while their head rested on the headrest and hands rested on the handrest respectively.....52

Figure 11: Illustration of the median nerve stimulation paradigm: The stimulation was applied at the wrist of the participant to illicit thumb movement. The paradigm had a total of 35 sessions (blocks) separated by a 10-second rest period. 54

Figure 12: The photo of the force sensor system and the 10.1-inch screen used during the task, from the perspective of the participant (photo taken through headrest). 55

Figure 13: Spinal Cord model for the cervical and thoracic regions designed using AutoCAD. The left figure shows lordotic and kyphotic curvatures of the spinal cord. The right figure represents inter-vertebral spinous distances. 69

Figure 14: Anatomical References selected as surface landmarks are shown on the human model, skeleton model, and the SC10-X/U Electrode Placement System. The anatomical points encompassing spinal space are labeled from a. to p. and are shown over the surface for the human model (left), skeleton model (right), and electrode system (centre). 71

Figure 15: Electrode placement system (SC10-X/U) representing spinal space for high-density surface electrospinography (HD-ESG). The SC10-X/U system for the 76-electrode locations is shown along with anatomical reference points and spatial features, which are utilized for 2D space division.....72

Figure 16: Design description of the SC10-X/U electrode placement system with electrode locations and labels (right). The left figure showcases the reference boundary, inner and outer perimeter contour. The right figure showcases all 76-electrode locations and labels. The labels are defined as, ML: Midline, PM: Posterior Midline, PL: Posterior Line, LP: Lateral Posterior, LL: Lateral Line, AL: Anterior Line.....74

Figure 17: A. The SC10-X/U electrode placement system acronym. B. The SC10-X/U electrode location labelling nomenclature.....75

Figure 18: In-house developed electrode patch based upon the SC10-X/U electrode system.
.a. The 64-electrode channels used for the recording and its corresponding electrode space.
.b. The electrode patch (up to 76 electrode channels) placed over the surface of the cervical and upper thoracic spinal cord space of a participant. 79

Figure 19: Grand-mean of the evoked potentials across all participants at ML6 electrode. Grey lines represent individual participant evoked potentials, and thick black line correspond to the grand mean evoked potential across all participants. The duration of significant evoked response is highlighted by red lines. The figure indicates significant N13 and P9 evoked potentials. 6a. ML6 response in reference to AL2 electrode (left). 6b. surface Laplacian re-referenced ML6 response (right).....81

Figure 20: Grand-mean signals plotted for SC10-X/U channels at Cv6 compatible with ring electrode placement system at Cv6. (Surface Laplacian re-referenced) 82

Figure 21: Topographic Map of Evoked Spinal Potentials. Centre Figure: Topographic Map of the grand-mean spinal evoked response over the SC10-X/U sensor space, time-window (12ms-14ms). Left figure: corresponds to the spinal activity recorded from channels ML2-ML7 (midline, posterior) for the time frame between 0.001s before stimulation and 0.025s

after stimulation. Right figure corresponds to the spinal activity recorded from lateral-anterior channels for the time frame between 0.001s before stimulation and 0.025s after stimulation. 83

Figure 22: A. The setup with the physio-chair and 10.1-inch screen that was used during the experiment. B. A participant sitting in an inclined position on the physio-chair. The participant's chest was supported by the chest rest while their head rested on the headrest and hands rested on the handrest respectively. C. The photo demonstrates the hand position during the voluntary motor task. The photo also showcases the force sensor system and the 10.1-inch screen used during the task, from the perspective of the participant (photo taken through headrest). 89

Figure 23: The HD-EEG and HD-ESG electrodes positioned over the participant's head, neck, and upper back. The photos highlight the electrode locations used for recording the HD-EEG and HD-ESG signals during the experiment paradigms. 90

Figure 24: A. Grand-mean of the evoked potentials across all participants at ML6 and AL2 electrode positions. Grey lines represent individual participant evoked potentials, and thick black line correspond to the grand mean evoked potential across all participants. The statistically significant evoked response sample points are illustrated as hollow red circles. B. Topographic Map of Evoked Spinal Potentials: Centre Figure: Topographic Map of the grand-mean spinal evoked response over the SC10-X/U sensor space, time-window (12ms-15ms). Left figure: corresponds to the spinal activity recorded from channels ML3-ML7 (midline, posterior) for the time frame between 0.001s before stimulation and 0.025s after stimulation. Right figure corresponds to the spinal activity recorded from lateral-anterior channels for the time frame between 0.001s before stimulation and 0.025s after stimulation. This figure demonstrate dorsal epicentre of activity at channel locations ML5-ML6. 96

Figure 25: This figure shows the topographic map of the grand-averaged normalized band-specific PSD in beta, low-gamma, and high-gamma bands for the cervical electrode locations. The bottom plot demonstrates the box plot of the spectral activity for the frequencies in each frequency band and three ESG regions marked in the topography map: Midline, Ipsi-anterolateral, and Contra-anterolateral. Beta band-specific PSD demonstrated highly ipsilateralized activity and showcased significantly increased spectral activity in ipsi-anterolateral region as compared to midline and contra-anterolateral. Low-gamma band-specific PSD demonstrated significant increased activity in midline as compared to contra-anterolateral region. Bi-lateral activity was observed for high-gamma band-specific PSD and no-significant differences were observed between the three regions. 98

Figure 26: The group-average band-specific ERSPs (beta, low-gamma, high-gamma band) for ipsi-anterolateral (red), midline (blue), and contra-lateral (black) spinal regions at the lower cervical cord are shown in the figure. Smoothened (averaged) over 0.75s time-window. The figure showcases the gERSPs in the pre-motor, execution, sustained, termination, and post-motor period of the motor task. gERSP in beta band showcased pre-dominant ipsi-anterolateral activity during the sustained period of the isometric contraction. In low-gamma band, dorsal midline region showcased increased gERSP for the duration of the sustained period, whereas ipsi-anterolateral region indicated a monotonic increase in gERSP during the sustained period. Increased gERSPs was observed for all three regions for the execution and termination period of the isometric contraction phase. 100

Figure 27: Corticospinal Coherence (CSC) between three spinal regions (ipsi-anterolateral, dorsal-midline, contra-anterolateral) and three EEG electrodes (C3, Cz, C4). The red-star indicates significant CSC at the corresponding frequency. The shaded area relates to the significance level, dark shade represents significance >0.1 and light shade represents significance > 0.05 & <0.1 , while the unshaded region represents significant CSC values (<0.05). 101

Figure 28: The figure illustrates the topographical map of the group averaged enveloped-HFO activity for controls (A.) and pwMs (B.). The figure demonstrates a highly localized activity over ipsilateral electrodes of the lower cervical levels for both groups. 112

Figure 29: The figure illustrates the temporal-spectral activity of controls and pwMs for event-locked (top) and non-phase locked (bottom) spinal signals at the C6 vertebral level. The activity at contra-anterolateral electrode (AL1), contralateral electrode (LL7), dorsal-midline electrode (ML6), ipsi-anterolateral electrode (AL1), and ipsilateral electrode (LL7) is shown in the figure. A. Event-Locked Temporal-spectral activity: An increase in spectral activity was observed in the 400 Hz – 600 Hz frequency band between 8 ms-14 ms post-stimulation for both groups. The increased activity was most prominent at the ipsilateral electrode position for both pwMS and controls. B. Non-phase locked temporal-spectral activity: A decrease in spectral activity was observed in the 1000 Hz – 1200 Hz frequency band for both groups, though the latency of the observed response was delayed in pwMS. 113

Figure 30: This figure showcases the significant differences between pwMS and control. A. Showcases the significant differences between control and pwMS at peak HFO amplitude (signal envelope). The differences are shown as T-values and a positive difference indicate higher signal envelope amplitude in control i.e. pwMS have a lower signal envelope amplitude. Significant differences were observed in 5 electrode regions labelled a.,b.,c.,d. B. Showcases significant differences between control and pwMS in the time-frequency domain. The differences are shown as T-values and a positive difference indicate higher spectral power in controls. Significant differences were observed in the similar 4 electrode regions (a.,b.,c.,d.). C. The C. plot showcases the difference between pwMS and controls averaged for the respective regions. The region a and b demonstrated significant differences in 400-600 Hz frequency while the regions c and demonstrated significant differences in 600-800Hz. 115

Figure 31: The previous iterations of the electrode patch development. The electrode patch went through multiple iterations before the final version shown in the section IV.2.4 was realized. 1st Iteration: In the first iteration the electrode locations were marked over the skin of the participant, then flat-type electrodes were placed at the marked site and were firmly stuck to the back using adhesive tape. In the second iteration, the electrode patch was carved out of a breathable cloth. The electrode locations were cut-out in the cloth as per the designed electrode placement system. The flattype electrodes were held in the cut-out position using adhesive tape. Before placing the electrode patch on the participant adhesive pads were put on the flat-type electrodes, after which the electrode patch was placed on the participants. The electrodes which weren't held tightly was reattached for better connection. In the third iteration of the electrode patch was developed using breathable netted nylon sheet and pin-type electrodes. The pin-electrode holders were embedded in the netted fibre

as per the designed electrode placement location. The nylon electrode patch was placed over the skin of the participant and tape was used to attach it to the surface..... 159

Figure 32: The group-average unbanded ERSPs for ipsi-anterolateral (AL2), midline (ML6), and contra-lateral (AL1) ESG electrodes at the lower cervical cord are shown in the figure. The figure showcases the gERSPs in the pre-motor (-2s to 0s), execution (0s to 0.75s), sustained (0.75s to 4.75s), termination (4.75s to 5.5s), and post-motor period of the motor task (5s – 7s)..... 160

Figure 33: Average Power spectrum of ESG signal at ML6 electrode across 10 healthy particiapnts is plotted alongside the average EEG power spectrum at C3 electrode location and average bi-EMG power spectrum (ABP) during sustained contraction. The plotted power spectrum is normalized w.r.t. the total power, i.e. area under the curve for all three spectra equals 1. The figure shows differences between ESG, EEG, and EMG spectral power distribution..... 161

Figure 34: The power spectra of ESG (n=10) at the lower cervical level (left) and upper cervical level (right) during the eyes-open (red, EO) and eyes-closed (blue, EC) resting state paradigm. A significant difference in spectral power between EO and EC paradigm was observed in the delta frequency band for both cervical levels. However, only the upper cervical level demonstrated a significant difference in spectral power in the alpha frequency-band between EO and EC..... 161

List of Tables

Table 1: Demographic Details of the people with MS who were recruited for the study, ... 107

Lists of Abbreviations

ADM	Abductor Digiti Minimi
AFC	Activation-Flow Coupling
ALS	Amyotrophic lateral sclerosis
AMT	Active Motor Threshold
APB	Abductor Pollicis Brevis
BOLD	Blood Oxygen Level-Dependent
CCA	Canonical Component Analysis
CMAP	Compound Muscle Action Potential

CMC	Corticomuscular Coherence
CMCT	Central Motor Conduction Time
CSA	Cross-Sectional Area
CSC	Cortico-Spinal Coherence
cSP	Cortical Silent Period
CST	Cortico-Spinal Cord
DAQ	Data Acquisition system
EC	Eyes Closed
ECG	Electrocardiography
EEG	Electroencephalography
EMG	Electromyogram
EO	Eyes-Open
ERSP	Event-Related Spectral Perturbations
ESG	Electrospinography
FA	Fractional Anisotropy
FDI	First Dorsal Interosseous
FDR	False Discovery Rate
fMRI	Functional Magnetic Resonance Imaging
fNIRS	Functional Near-Infrared Spectroscopy
FPB	Flexor Pollicis Brevis
HD	High-Density
HFO	High-Frequency Oscillation
ICA	Independent Component Analysis
LMN	Lower Motor Neuron

LPA	Left Pre-auricular Points
MEG	Magnetoencephalography
MEP	Motor Evoked Potential
MND	Motor Neuron Disease
MPS	Multi-point stimulation
MSG	Magnetospinography
MS	Multiple Sclerosis
MUNE	Motor Unit Number Estimation
MUNIX	Motor Unit Number Index
MVC	Maximum Voluntary Contraction
NI	Neurophysiology Index
OPM	Optically Pumped Magnetometers
PCA	Principal Component Analysis
PMA	Progressive Muscular Atrophy
PSD	Power Spectral Density
pwMS	People with Multiple Sclerosis
RMT	Resting Motor Threshold
RPA	Right Pre-auricular Points
RRMS	Relapse-Remitting Multiple Sclerosis
RS	Resting State
SC	Spinal Cord
SEP	Somatosensory Evoked Potential
SHI	Split-Hand Index
SIP	Surface electromyography Interference Pattern

SMA	Spinal Muscular Atrophy
SQUID	Superconducting Quantum Interference Device
TF	Time-Frequency
TMS	Transcranial Magnetic Stimulation
TRCA	Task-Related Component Analysis
UMN	Upper Motor Neuron

Outputs

1. Oral Presentation

Mehra P., Bista S., Marjorie M., ..., Nasserroleslami B. Novel Non-invasive Assessment of Brain-Body Connection in health and neurodegeneration. **40 min presentation** – *Visiting researcher, University of Birmingham, 2023.*

2. Poster Presentation

Mehra P., Bista S., Marjorie M., ..., Nasserroleslami B. Non-invasively-recorded spinal cord responses to median nerve stimulation demonstrate stronger high-frequency oscillations in anterior versus posterior electrode locations, *33rd Neural Control of Movement, 2024.*

Mehra P., Bista S., Marjorie M., ..., Nasserroleslami B. Evaluating the characteristics of evoked spinal cord high-frequency wave potentials using posterior and anterior surface electrodes: Preliminary Study, *Neurophysiological Bases of Human Movement, 2023.*

Mehra P., Bista S., Marjorie M., ..., Nasserroleslami B. Investigating the characteristics of High-Frequency Oscillations as a potential biomarker for sensorimotor dysfunction: Preliminary Study, *34th International Motor Neuron Disease Association (MND) symposium 2023.*

Mehra P., Bista S., Marjorie M., ..., Nasserroleslami B. Evaluating the characteristics of High-Frequency Oscillations as a potential biomarker for sensorimotor dysfunction in ALS: Preliminary Study, *European Network to Cure ALS (ENCALS) 2023.*

I. Introduction and Background

This chapter includes an introduction to the spinal cord and presents the background (literature review) for the work presented in this thesis. The first two sections (section 1 and 2) of this chapter focuses on the introduction to the anatomy of the spinal cord. The last three (section 3, 4, and 5) sections are focused on presenting a review of the previous scientific literature as the background for the work presented in this thesis.

1. Gross-Anatomy of Spinal Cord

The spinal cord is a long cylindrical structure that makes up the lower part of the central nervous system. It extends from the lower end of the medulla oblongata to the lumbar region. The spinal cord is enclosed in the vertebral column and consists of grey and white matter. The vertebral column along with cerebrospinal fluid and meninges (group of members) provide an external protective structure around the spinal cord. The average length of the spinal cord in adults is 42 - 45 cm (Cramer et al., 2014; Landelle et al., 2021) and it weighs approximately 30-35 grams (Cramer et al., 2014). In adults, the spinal cord's length lags behind the vertebral column's length with the spinal cord ending approximately at the level of L1 vertebrae (occupying around two-thirds of the vertebral column)(Rea, 2015).

The spinal cord is composed of 31 spinal levels and is divided into 5 segments: Cervical (8 spinal levels), Thoracic (12 spinal levels), Lumbar (5 spinal levels), Sacral (5 spinal levels), and Coccygeal (1 spinal level). Each of these 31 spinal levels has corresponding pair of spinal nerves (31 pairs)(Haines et al., 2018). The spinal nerve at each level is created by anterior and ventral roots coming together within the intervertebral foramen (Cramer et al., 2014). The dorsal root carries afferent information from the periphery to the spinal cord. The pseudo-unipolar cell bodies of the fibers that carry afferent information are located outside of the spinal cord in an oval structure called the dorsal root ganglion. The pseudo-unipolar neuron has a peripheral process that gathers sensory information from the periphery and a central process that carries the sensory information into the spinal cord via the dorsal root that divides into multiple dorsal rootlets before entering the dorsal spinal cord. The ventral root, on the other hand, contains efferent fibers that carry motor information to the periphery. The efferent fibers exit from the anterior spinal cord as ventral

rootlets, which then combine to form the ventral root. The spinal nerves are thereby mixed nerves that carry both sensory and motor information.

The first seven spinal nerves exit above their corresponding vertebrae whereas all other spinal nerves exit below their corresponding vertebrae. This lets the 8 spinal nerves exit from 7 vertebral columns at the cervical segment leading to one additional spinal nerve as compared to the number of cervical vertebrae. Additionally, two enlargements can be found along the spinal cord: the first enlargement is seen at the cervical segment (C3-T2), and the second enlargement is seen at the lumbar segment (L1-S3). These enlargements help to incorporate more afferent fibers, efferent fibers, and cell bodies of efferent neurons needed for communicating and controlling the upper and lower extremities respectively. The locations of spinal enlargements are associated with the respective extremities, as more structures are required for carrying sensory and motor signals to and from the limbs. The mapping of the dermatome and cutaneous peripheral nerve distribution is shown in Figure 1.

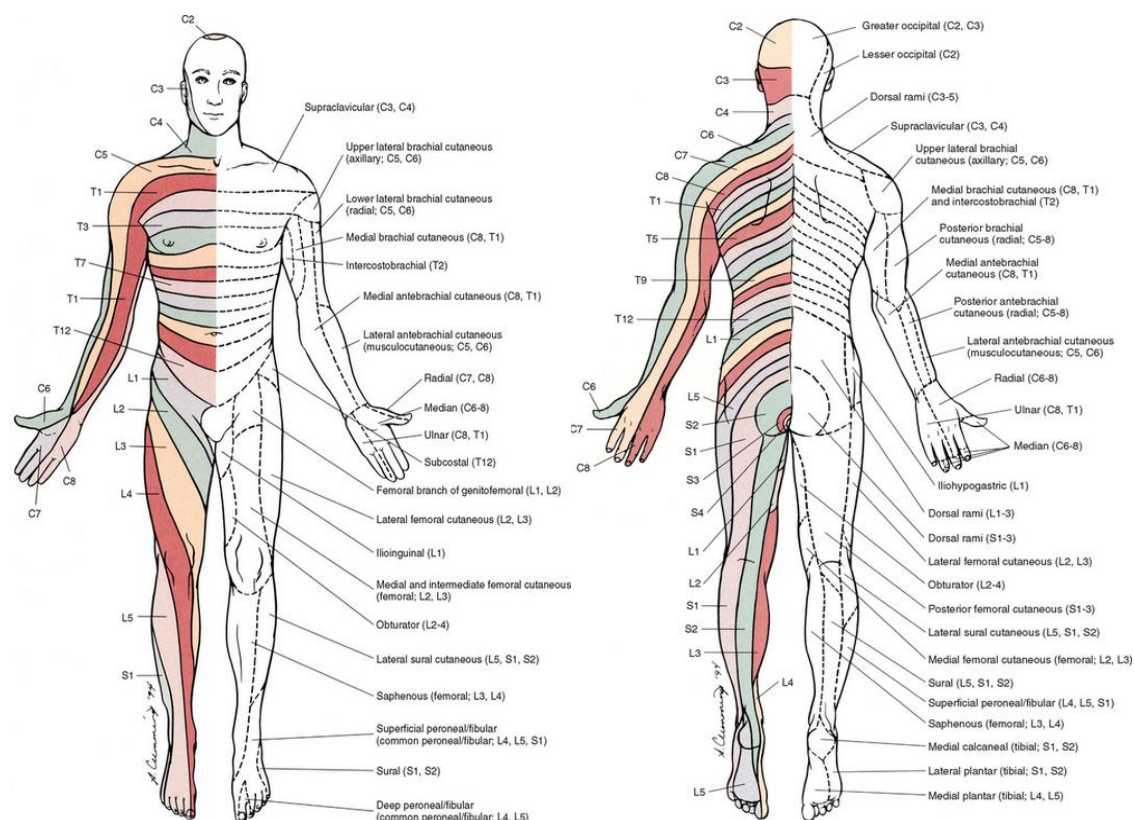


Figure 1: This figure showcases the anterior and posterior view of the peripheral cutaneous innervations. The colored map highlights the dermatome pattern and the non-colored map highlights the peripheral nerve innervations. The dermatomal mapping is based on study by Keegan and Garrett, 1948 and the figure is adapted from *Clinical anatomy of the spine, spinal cord, and ANS*, Cramer et al., 2014.

2. Cross-Sectional Anatomy of Spinal Cord

The spinal cord has a characteristic cross-sectional neuroanatomy throughout its levels, although the composition of grey and white matter may differ at different spinal levels. In the cross-sectional view, the grey matter is located in the center and is surrounded by white matter. This is in contrast to the brain, where the white matter is surrounded by the grey matter. The spinal cord segments (cervical, thoracic, lumbar) display distinct features that distinguish them from each other. The cervical segment displays high white matter as compared to other segments. The thoracic region displays a presence of lateral horn that is absent in other segments. The lumbar segment displays increased grey matter.

The grey matter in the spinal cord has a characteristic H-shape otherwise known as the butterfly shape as shown in Figure 2.A., the figure is adopted from Haines et al., 2018. A medial-sagittal division of the spinal cord results in identical halves, featuring identical grey and white matter organization. The grey matter is broadly divided into posterior horn and anterior horn and an intermediate area between the two horns (Cramer et al., 2014; Haines et al., 2018). The posterior or dorsal horns are broadly responsible for sensory information processing while the anterior or ventral horns for motor function. The grey matter in each half is further segmented into 10 areas arranged over the dorso-ventral axis, namely Lamina I-XI and area X. The categorization of the spinal cord in these 10 areas was proposed by Rexed (Rexed, 1954, 1952) based on the neuronal soma size. The macro function and characteristics of each lamina are listed in Figure 3, the figure is adopted from Rea, 2015.

The white matter in the spinal cord can be largely divided into three large sections (Haines et al., 2018), posterior funiculus, lateral funiculus, and anterior funiculus, as shown in Figure 2.A, the figure is adopted from Haines et al., 2018. The posterior funiculus is defined between the posterior median septum and the medial edge of the dorsal horn. The lateral funiculus is defined between posterolateral and anterolateral sulci. Finally, the anterior funiculus is defined between the anterolateral sulcus and the ventral median fissure. The lateral funiculus and anterior funiculus contain both ascending and descending tracts whereas the posterior funiculus only contains the ascending tracts.

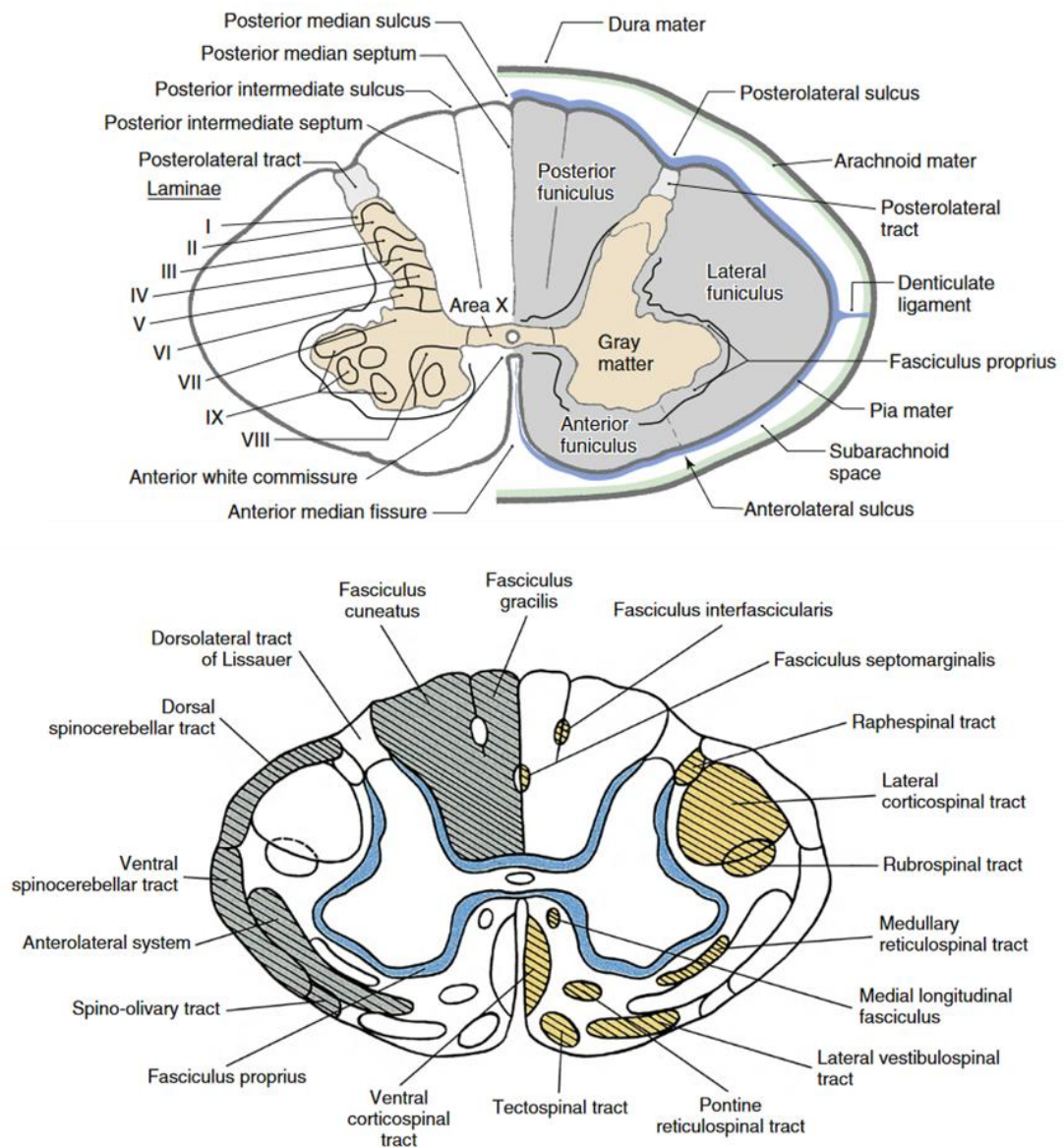


Figure 2. This figure illustrates the cross-sectional view of the spinal cord. The top figure showcases the white and grey matter at the C7 spinal level, highlighting the division of the grey matter in Laminae I-IX and area X. The bottom figure showcases the ascending tracts on the left and the descending tracts on the right. The top figure is taken from *Fundamental Neuroscience for Basic and Clinical Applications*, Haines et al., 2018 and the bottom figure is taken from the *Clinical anatomy of the spine, spinal cord, and ANS*, Cramer et al., 2014.

Lamina	Characteristics	Input	Output	Function
I	Small neurons and large marginal cells. Fusiform, multipolar, flattened and pyramidal cells. Small dendrites	Fine myelinated and unmyelinated dorsal root fibers	Contralateral spinothalamic tract	Noxious and thermal sensation
II	Stalked and islet cells. Interneurons	Unmyelinated primary afferent input. Very little input from large myelinated primary afferents. Descending dorsolateral fasciculus fibers	Some neurons projecting from the spinal cord (projection neurons), some passing to different laminae and some with axons confined to a lamina in the region of the dendritic tree of that cell, e.g. intralaminar interneurons, local interneurons and Golgi Type II cells	Modulate the activity of nociceptive and temperature afferent fibers
III	Slightly larger cells than lamina II, but with a neurophil similar to that of lamina II. Variable cell size	Hair follicles. Pacinian corpuscles. Rapidly and slowly adapted fibers. A β fibers. Dendrites of cells from laminae III-VI	Propriospinal and interneurons	Light tactile stimulation and strong pressure
IV	Variety of cell types	Collaterals and from large primary afferent fibers. Input also arises from the substantia gelatinosa (lamina II)	Projection to the ipsilateral and contralateral spinothalamic tracts. Spinocervical, spinocerebellar and spinohypothalamic tracts. Nucleus accumbens and the lateral parabrachial area and reticular nuclei	Pain, temperature and crude touch. Light mechanical stimulation
V	Thick nerve fibers. Comprised of a lateral and medial zone. Triangular, star shaped and spindle cells. Difficult to distinguish lamina V from lamina VI.	Large primary afferent collaterals. Monosynaptic information from A β , A δ and C fibers. Descending fibers from the corticospinal and rubrospinal tracts	Brainstem and thalamus via ipsilateral and contralateral spinothalamic tracts. Cerebellum, dorsal column nuclei, reticular formation, rostromedial medulla. Local projections (more typical of the lateral area)	Multireceptive. Nociception and mechanoreception
VI	Broad layer. Developed most in the cervical and lumbar enlargements. Variety of cell shapes here	Joint and muscle spindle afferents. Rubrospinal and corticospinal pathways (in its lateral portion)	Spinocerebellar pathway	Integration of somatic motor processes. Reflex pathways via interneurons
VII	Renshaw cells. Triangular, fusiform and multipolar cell types found here	Tendon and muscle afferents. Laminae II-VI. Sympathetic preganglionic neurons constituting the intermediolateral cell column in the thoracolumbar (T1-L2/3) and the parasympathetic neurons located in the lateral aspect of the sacral cord (S2-4), i.e. visceral afferent fibers	Innervates postganglionic cells in the sympathetic or parasympathetic ganglia. Inhibitory interneurons which synapse on the alpha motor neurons	Autonomic nervous system. Inhibitory interneurons which synapse on the alpha motor neurons
VIII	Shape varies across the spinal cord	Vestibulospinal and reticulospinal fibers. Descending motor tracts from the cerebral cortex and the brainstem	Motor neurons innervating the intrafusal fibers	Modulate motor activity
IX	Large and smaller motor neurons. Size and shape varies across the spinal cord	Descending motor tracts from the cerebral cortex and the brainstem	Motor neurons	Extrafusal skeletal muscle fibers. Intrafusal muscle fibers. Nociceptive
X	Decussation of axons. Small to medium sized cells. Triangular, star shaped and spindle cells	Small myelinated and unmyelinated fibers. Convergence of somatic and visceral primary afferent input	Lateral parabrachial nucleus, amygdala and the nucleus of the solitary tract, medullary and pontine reticular formation	Nociceptive and mechanoreceptive information

Figure 3: This figure summarizes the functions and characteristics of each laminae observed in the spinal grey matter. The figure is adopted from *Essential Clinical Anatomy of the Nervous System*, Rea, 2015.

2.1. Ascending Pathways

Ascending pathways contain the ascending tracts that carry information from the periphery through the spinal cord to the brain. Here we have briefly described three primary ascending tracts: dorsal column, spinocerebellar tract, and spinothalamic tract (part of the anterolateral tract system) are shown in Figure 2B.

- **Posterior Column / Dorsal Column** (Cramer et al., 2014; Haines et al., 2018; Kandel et al., 2021; Rea, 2015): The dorsal/posterior column primarily carries peripheral information related to fine touch, discriminative touch (two-point discrimination), pressure, proprioception, and vibration. It is composed of heavily myelinated fibres with fast conduction velocity (Haines et al., 2018). The dorsal column displays a somatotopic arrangement, with medial fibres corresponding to the caudal periphery. The dorsal column is comprised of two parts, cuneate fasciculus and gracile fasciculus. Cuneate fasciculus carries information from the periphery superior to the vertebral level T6 while the gracile fasciculus carries information from the periphery inferior to T6. Dorsal Column is part of the dorsal column-medial lemniscus pathway that carries information from the periphery to the primary somatosensory cortex. These pathways feature three cell bodies that synapse and are named as orders. The first-order cell is found at the ipsilateral dorsal root ganglion that receives the sensory information from the periphery and ascends along the dorsal column that terminates at the second-order neuron. The second-order neuronal cell body is found either at the cuneate nucleate for fibres that travel along the cuneate fasciculus or at the gracile nucleus for fibres that travel along the gracile fasciculus. The third-order neuron is found at the contralateral ventral posterolateral thalamic nucleus, this information is then carried from the thalamic nucleus to the primary somatosensory cortex via the thalamocortical tract. In the dorsal column – medial lemniscus pathway, the dorsal column travels along the ipsilateral while the medial lemniscus travels contralaterally. The pathway crosses from the ipsilateral to the contralateral side at the medulla oblongata before medial lemniscus. Interestingly, the dorsal column fibre does not feature any synaptic order at the spinal cord but has indirect projections that transmit information to the spinal cord for reflex activity among other functionalities (Cramer et al., 2014; Haines et al., 2018).

- **Spinothalamic Tract** (Cramer et al., 2014; Haines et al., 2018; Kandel et al., 2021; Rea, 2015): The spinothalamic tract primarily carries peripheral information related to pain, temperature, crude touch, and pressure through direct and indirect paths of the anterolateral system (Haines et al., 2018). There are two spinothalamic tracts: lateral spinothalamic tract and anterior (ventral) spinothalamic tract. These tracts feature three cell bodies that synapse and are named as orders. The first-order cell is found at the ipsilateral dorsal root ganglion that receives the sensory information from the periphery. The second-order neuronal cell body is found at the dorsal spinal cord and ascends along the contralateral spinothalamic tract by crossing via ventral white commissure, terminating at the third-order neuron. The third-order neuron is found at the contralateral ventral thalamic nucleus, this information is then carried through the internal capsule to the cortex. These tracts also feature projections to reticular formation along the pathway. The anterolateral system also features a somatotopic arrangement, where the information from the caudal periphery is more posterolateral and the successive rostral information orderly arranges itself sequentially along the posterolateral–anteromedial axis.
- **Spinocerebellar Tract** (Cramer et al., 2014; Haines et al., 2018; Kandel et al., 2021; Rea, 2015): The spinocerebellar tract carries peripheral information that corresponds to unconscious proprioception and limited cutaneous signals (Cramer et al., 2014; Haines et al., 2018), allowing the cerebellum to coordinate movements and posture. The spinocerebellar tract comprises of multiple tracts including dorsal spinocerebellar tract, anterior spinocerebellar tract, cuneocerebellar tract, and rostral cerebellar tract.

2.2. Descending Pathways

Descending pathways contain the descending tracts that carry information from the brain to the periphery. One type of such tract called the cortico-spinal tract carries information from the cortex to the periphery through the spinal cord and is most affected in neuromotor conditions like stroke, multiple sclerosis, motor neuron diseases, etc. Here we have briefly described the corticospinal.

- **Corticospinal Tract (CST)**: The corticospinal tract is the largest descending tract in humans and is part of the pyramidal tract (Cramer et al., 2014). The corticospinal

tract originates from layer V of the sensory-motor cortex with ~60% originating from the motor cortex and remaining from the sensory cortex (Haines et al., 2018). The axons in CST are comprised of varying diameters, 90% of the diameter is less than 5µm and the larger axons ranging from 11 – 22 µm make up less than 2% of the axons (Cramer et al., 2014). The larger axons are myelinated while the remaining axons are either lightly myelinated or unmyelinated (Haines et al., 2018). The CST fibres originating from the cortex pass through the corona radiata and coverage before passing from the internal capsule. The CST fibres then pass through the caudal medulla and divide into lateral and anterior CST. The lateral CST makes up for the majority of the fibres (85%), these fibres cross over to the opposite side and this crossing-over is termed pyramidal decussation. The remaining CST fibres descend uncrossed making up anterior CST. *Lateral CST*: Descend in the lateral funiculus and are topographically organized where axons terminating at lower caudal spinal levels are organized more laterally. This pattern leads to the adjacent more lateral fascicles shifting medially as the medially located fibres terminate in the more rostral spinal level. Approximately 55% of the lateral CST fibres leave the tract and terminate at the ventral horn and intermediate area of the cervical spinal segment providing fine motor control to the muscles in the upper extremities (Cramer et al., 2014). *Anterior CST*: Descend uncrossed in the ventral white funiculus and terminate at the ventral horn of the spinal cord on the other side. These axons cross at the spinal cord level.

3. Imaging of Spinal Cord

3.1. Spinal Functional Magnetic Resonance Imaging

Functional Magnetic Resonance Imaging (fMRI) is an indirect non-invasive imaging technique that is used to investigate task-related or resting-state functional neural correlates (Kim et al., 2010). The imaging technique exploits the contrast of BOLD (blood oxygen level dependent) physiological signals to record regional functional changes. This relies on the changes in the closed coupling between metabolism and blood flow in the regional central nervous system (Detre and Floyd, 2001), referred to as activation-flow coupling (AFC), acting as a surrogate marker or neural correlate. The fMRI signals utilize BOLD contrast or BOLD signal change in the form of a percentage change during task-related

activity, typically ranging from 0.5% to 5%. The region's functional activity is reflected in increased BOLD contrast due to the change of deoxygenated blood, which has paramagnetic properties, compared to non-magnetic oxygenated blood (Detre and Floyd, 2001). The interactions between blood flow, blood oxygenation/deoxygenation, and metabolism occur during both neuronal excitation and inhibition (Nudo and Masterton, 1986), which are observed during fMRI using pulse sequences.

The fMRI technique has been used to investigate the neural correlates in spinal grey matter, the first such study being reported in 1996 by Yoshizawa et al. (Yoshizawa et al., 1996). Despite being approximately 30 years old, spinal fMRI has received little attention. This can be associated with spinal fMRI suffering from some major challenges (Nawal Kinany et al., 2023). Firstly, the spinal cord is a long structure, which makes it challenging to cover its entire rostro-caudal length posing a limitation for fMRI machines. Secondly, the spinal cord is small in diameter which requires very high spatial resolution for an adequate signal-to-noise ratio (Bede et al., 2012; Nawal Kinany et al., 2023). Thirdly, cumbersome anatomical location, the spinal cord is surrounded by bones, tissues, and fluids leading to magnetic inhomogeneities distorting the magnetic signals (Finsterbusch, 2014; Nawal Kinany et al., 2023). Finally, physiological noise due to motion, breathing, and cardiac activity due to mobile vertebral structure and its vicinities with visceral organs (Nawal Kinany et al., 2023; Piché et al., 2009). The advancements in high-field fMRI (improved spatial resolution), advanced pulse sequences (Yacoub and Wald, 2018), and improved processing/segmentation techniques (De Leener et al., 2017, 2016) in the past decades have mitigated some of the aforementioned limitations. Furthermore, recent development of a standardized template for the spinal cord (De Leener et al., 2018) has further enabled group-level inferences which were few and far between before. These advances have led to an increase in both task-dependent and resting-state fMRI studies in the last decade. The spinal fMRI studies have been comprehensively summarized and analyzed in a recent review by Landelle et al. (Landelle et al., 2021). The spinal fMRI predominantly studies the cervical region compared to the lumbar/lower-thoracic region due to the rostro-caudal length limitation in carrying out lower limb studies.

The review by Landelle et al. (Landelle et al., 2021) highlighted the reported literature on lateral, dorso-ventral, and rostro-caudal representation of spinal activity during sensory stimulation, motor task, and resting-state fMRI as shown in Figure 4. The sensory stimulation studies report significant lateralization of activity showing predominant

activation of ipsilateral side (Ghazni et al., 2010; Lawrence et al., 2008). The motor-task-related fMRI studies show more heterogeneity between reported lateralization, reported results included bi-lateral activation (Bouwman et al., 2008), predominant ipsilateral activation (Maieron et al., 2007), and significant lateralization (Weber et al., 2016). The lateralization in motor-task-based spinal activity showed increased variability between literature, the lateralization varying with the chosen motor task, and the analysis method. The dorso-ventral localization for both sensory stimulation (dorsal dominant) and motor-task-based (ventral dominant) experiments showcased widespread activation across dorso-ventral horns (Backes et al., 2001; Kinany et al., 2019; Stroman and Ryner, 2001; Weber et al., 2020). This demonstrates less specificity in the dorsoventral plane at a specific cervical level. The rostro-caudal presentation of upper-limb sensory stimulation and motor-task-based studies are predominately localized in the C5-T1 cervical level region (Lawrence et al., 2008; Madi et al., 2001). The presentation of the activity at cervical levels in response to different sensory stimulation and distinct motor-task is shown in Figure 4 (adopted from Landelle et al., 2021).

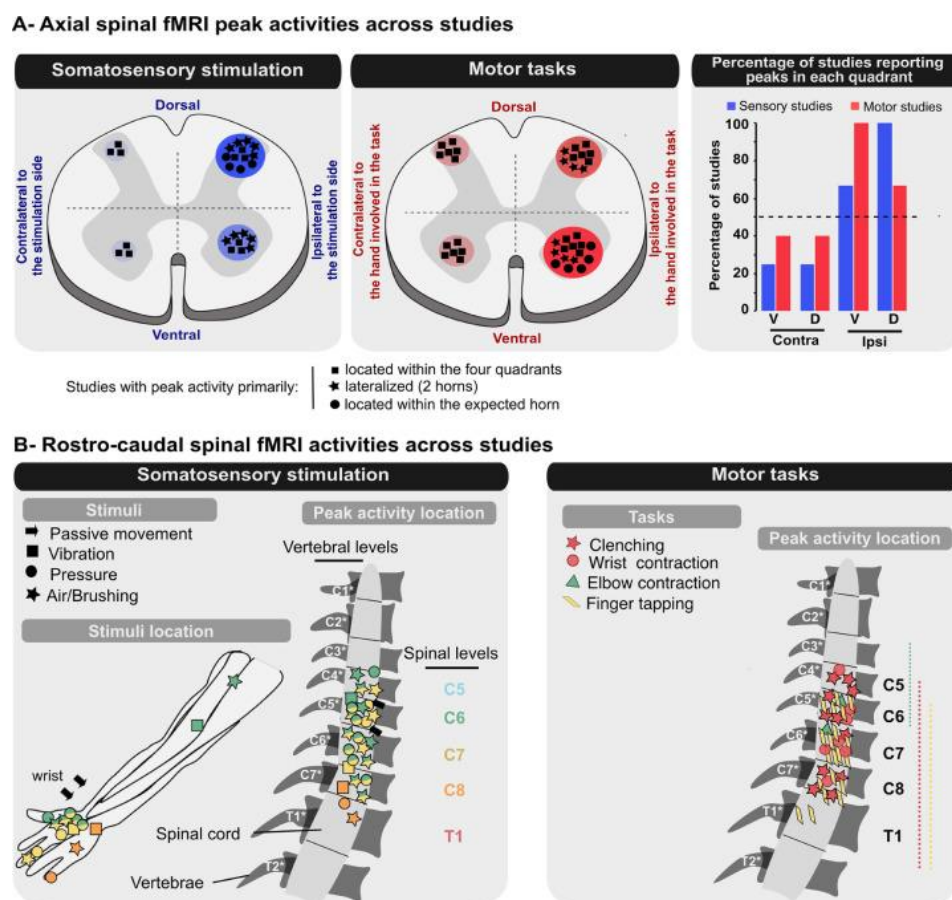


Figure 4: Schematic illustration of the spinal activity observed in cervical and upper thoracic segments using fMRI technique. A. Cross-sectional representation of spinal fMRI activity observed at lower cervical level

during both sensory stimulation and motor task paradigm. Cross-sectional representation highlights the lateralization and dorso-ventral spinal activity during the respective paradigms. B. Rostro-Caudal representation of spinal fMRI activity observed during both sensory stimulation and motor task paradigm. Rostro-caudal representation highlights the location of the observed activity over the spinal/ventral levels during the respective paradigms. The figure is taken from Landelle et al., 2021.

3.2. Non-Invasive Functional Neurophysiology

Non-invasive neurophysiology studies utilize neuro-electric or neuro-magnetic techniques to record spinal electric activity. In the early last decade, Adachi et. al. (Adachi et al., 2013) developed a high-resolution superconducting quantum interference device (SQUID) based magnetic flux sensor to record functional spinal activity non-invasively, Magnetospinography (MSG). The developed system featured an array of 40 SQUID-magnetic sensors and utilized source localization techniques for high-resolution functional spinal imaging. The MSG system was used to record evoked spinal magnetic fields at cervical spinal levels in response to transcutaneous thoracic stimulation (Sumiya et al., 2017) and median nerve stimulation (Akaza et al., 2020). The activity recorded at cervical levels in response to the median nerve stimulation showed P9, N11, and N13 evoked responses. Reconstruction of spatial current density demonstrated three components as shown in Figure 5: entry action currents, traveling action currents (leading, trailing), and intracellular currents. The first component highlighted the current entering the intervertebral foramen at C5-C7 levels which was cranially directed, followed by a perpendicular inward volumetric current at lower cervical levels, and finally a caudally directed trailing axonal current. Moreover, this technique has also been used in areas beyond the cervical region. SQUID-based sensors were utilized to record the evoked magnetic field activity at the brachial plexus (Watanabe et al., 2019), the cauda equina (Ushio et al., 2019), and the elbow (Hoshino et al., 2022) in response to peripheral nerve stimulations. In rabbits (Sakaki et al., 2020), the MSG system was used to evaluate evoked magnetic fields at the lumbar region in healthy and lesioned spinal cords.

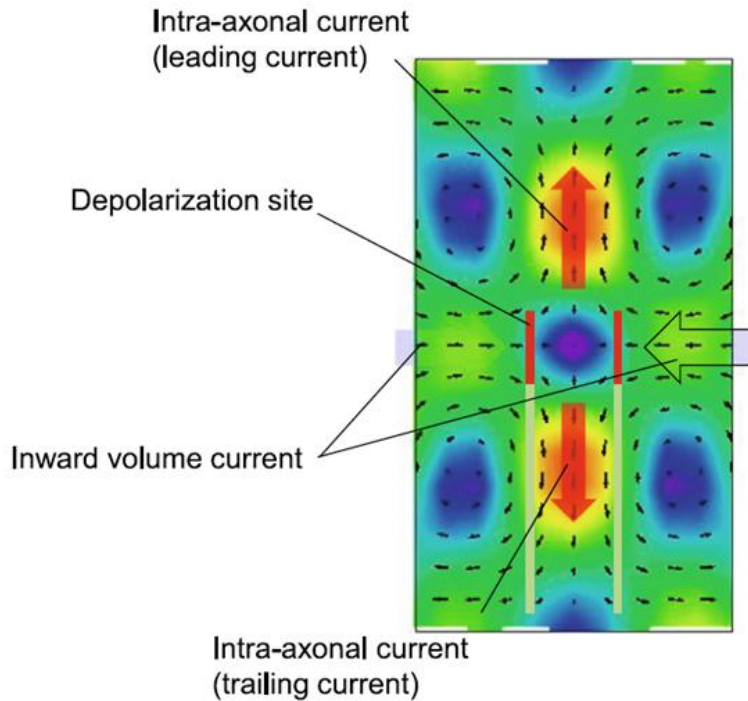


Figure 5: Illustration of the spatial current density observed in response to the mixed nerve stimulation. Taken from Akaza et al., 2020.

The SQUID-based MSG recording has highlighted the potential of high-resolution non-invasive neurophysiology techniques. The SQUID-based MSG utilizes highly specialized equipment that isn't widely accessible and also doesn't offer simultaneous recording of brain and spinal activity at present. Recently, Mardelle et. al. (Mardell et al., 2022) utilized optically pumped magnetometers (OPM) to record spinal evoked potentials using a wearable system that can be used with OPM-MEG systems. The OPM-MSG system needs a custom-built wearable cast for each individual to record the spinal evoked system (Mardell et al., 2022). The OPM-based MSG system is still in the early stages and needs further development and studies to investigate the reliability and robustness across multiple participants.

Nierula et. al. (Nierula et al., 2022) studies spinal somatosensory evoked responses using high-density multichannel non-invasive electrophysiology activity at both cervical and lumbar levels. Nierula et. al. demonstrated that high-density spinal electrophysiology recordings can be used to study spatial spinal activity and utilize spatial filtering techniques to enhance the signal-to-noise ratio of the evoked spinal activity. Earlier multichannel studies (Jeanmonod et al., 1991; Mauguière, 2003a; Restuccia and Mauguière, 1991; Schabrun et al., 2012) reported evoked spinal somatosensory activity in response to

peripheral nerve stimulation. Studies featuring median nerve stimulation reported cervical potentials including N11 and N13. The N13 potentials are suspected to reflect the compound postsynaptic potentials by group I and group II in the dorsal horns at Laminae IV and V (Desmedt and Cheron, 1983; Emerson et al., 1984; Insola et al., 2008; Restuccia and Mauguière, 1991). The N11 potential could be described by very high-frequency oscillation (HFO) with a maximum peak at approximately 11 ms latency (Prestor et al., 1997), though further research is required to characterize N11 activity. Very high-frequency oscillations (greater than 200Hz) at cervical spinal levels in response to median nerve stimulation have been recorded by earlier invasive epidural studies (Insola et al., 2008; Prestor et al., 1997) and in a recent non-invasive study by Chander et. al. (Bankim Subhash Chander et al., 2022). The origin of these very high frequency oscillations are still unknown. However, Prestor et. al. have suggested that they might be generated by presynaptic afferents of the long sensory tract (cuneate fascicle). The studies by Insola et. al. and Chander et. al. has indicated that very high-frequency oscillations might be composed of two separate bursts, first at around 500 Hz and second at 1200 Hz. They argued that the 500Hz burst might have a presynaptic origin but 1200 Hz seem to indicate postsynaptic activity from the dorsal horn. Further research is required in the field of evoked HFO activity.

4. Spinal Cord in Neurodegeneration

The spinal cord is affected in varying degrees depending on the neurodegenerative condition. In this section, we primarily focus on Multiple Sclerosis (MS) and Motor Neuron Disease (MND) like Amyotrophic lateral sclerosis (ALS), in which the spinal cord is heavily affected.

Multiple Sclerosis (MS): Multiple Sclerosis is a chronic inflammatory demyelinating neurodegenerative disease that affects the central nervous system. Spinal cord lesions have been reported in approximately 90% of MS patients during the postmortem analysis (Ikuta and Zimmerman, 1976; Nijeholt et al., 2001; Waldman et al., 2022). The spinal cord is shown to be affected early in MS (Abdel-Aziz et al., 2015; Biberacher et al., 2015) and is part of the McDonald criteria for diagnosing MS (Chen et al., 2020; Thompson et al., 2018). Multiple pathophysiological processes present themselves in the spinal cord similar to the brain, these include demyelination, axonal loss, inflammation, and gliosis (Chen et al., 2020; Gass et al., 2015; McGavern et al., 2000).

Focal lesions with primary demyelination in the spinal cord are a hallmark pathophysiology of MS, the demyelination can be observed both in white matter as well as the grey matter (Kreiter et al., 2024). The lesions are predominantly found to be located in the posterior and/or lateral part of the spinal cord and are found to progress to the anterior and central part only in the later stages of progressive MS. The postmortem histopathology studies suggest that a larger portion of grey matter is demyelinated as compared to white matter in the spinal cord (Gilmore et al., 2006; Kreiter et al., 2024). The MRI scans have shown that demyelination and spinal lesions are often observed at the cervical segment of the spinal cord (Moccia et al., 2019; Waldman et al., 2024), and half of the MS patient population report spinal lesions on their first clinic visit (Arrambide et al., 2018; Kreiter et al., 2024). These lesions show a peripheral profile (subpial) i.e. they are predominantly observed on the outer most layer of the spinal cord in MRI scans (Chen et al., 2020). Along with focal lesions diffused partial demyelination can also be observed in the spinal cord, though is more common in the progressive subtype of MS.

Axonal loss pathophysiology is more closely related to the disability in MS (Andelova et al., 2019; Daams et al., 2014) and is a significant predictor of future further disability (Rocca et al., 2023; Valsasina et al., 2022). The rate of atrophy is also reported to be much higher in the spinal cord as compared to the brain (Casserly et al., 2018; Chen et al., 2020), with the cervical segment being the most prominent location for spinal atrophy (Bonacchi et al., 2020). The rate of atrophy in MS is reported to be around 1.78% in spinal cord as compared to 0.5 - 1% observed in the brain (Casserly et al., 2018; Chen et al., 2020). Furthermore, reduced neurite density was observed in the cervical spinal cord which strongly correlated with physical disability (Collorone et al., 2020). The role of cervical spinal cord is further exacerbated where 1% rate of spinal cord volume loss was associated with a 28% increased risk of developing disability in the coming year (Moccia et al., 2019). The cross-sectional area (CSA) of the spinal cord has shown to correlate with the MS disability (Chen et al., 2020). CSA has also been used as an indirect measure of axonal loss, though the correlation between CSA and axonal loss seems limited (Petrova et al., 2018).

Motor Neuron Disease (MND): MND refers to a set of neurological conditions in which motor neurons progressively degenerate leading to severe disability, these conditions affect both brain and spinal cord. The MND disease family is comprised of conditions like primary lateral sclerosis (PLS) in which upper motor neurons (UMNs) are dominantly affected, Progressive/Spinal Muscular Atrophy (PMA/SMA) in which lower motor neurons

(LMNs) are dominantly affected, and Amyotrophic Lateral Sclerosis (ALS) in which both LMNs and UMNs are affected (Rosenfeld and Swash, 2006). Here we will briefly discuss the involvement of the spinal cord in ALS.

Spinal cord atrophy, corticospinal degeneration, axonal and cell body loss have been reported as the hallmark pathophysiological phenomenon in the ALS population (Rossi et al., 2013). In the spinal cord, ALS has been associated to present prominent anterior horn pathology (Paquin et al., 2018; Valsasina et al., 2007) though the involvement of the dorsal and lateral spinal cord has also been observed (Cohen-Adad et al., 2013). CSA has been utilized as a measure of spinal atrophy and have significantly correlated with disease severity and survival in ALS (Branco et al., 2014; Mendili et al., 2014). Recent advancements in spinal MRI have resulted in advanced neuroimaging measures to characterize how ALS affects the spinal cord (Bede et al., 2012; El Mendili et al., 2019). Decreased fractional anisotropy (FA) using diffusion tensor MRI techniques has been reported in the cervical spinal cord indicating reduced tract integrity and CST degeneration (Agosta et al., 2009; Nair et al., 2010; Valsasina et al., 2007). This was more pronounced in the distal cervical segments as compared to the rostral (Nair et al., 2010). FA has been shown to correlate with the clinical measures (Cohen-Adad et al., 2013; Valsasina et al., 2007), though contrary observations have also been reported (Agosta et al., 2009).

5. Assessments for investigating SC (dys)Function and Sensory-Motor Tract Integrity

5.1. Nerve Conduction Studies

a. Compound Muscle Action Potential (CMAP)

CMAP is recorded in response to peripheral nerve stimulation. CMAP is recorded from the desired muscle and represents the summation of muscle fibre action potentials generated in response to nerve stimulation in near synchrony (Dengler et al., 2020). CMAP has been utilized in clinical diagnosis to examine the dysfunction of lower motor neurons (LMN) and can indicate combined effects of reinnervation, denervation, muscle atrophy, and the number of innervated fibres (de Carvalho et al., 2005; Shin-Yi Lin et al., 2024).

Maximal CMAP amplitude has shown diagnostic utility in characterizing LMN dysfunction in both SMA (Axente et al., 2022; Lewelt et al., 2010) and ALS (Daube, 1985; Kent-Braun

et al., 1998). The CMAP amplitude correlates with ALS disability and muscle function decline (Nandedkar et al., 2015). Split-Hand Index (SHI) is a measure developed to assess the muscle atrophy in MND conditions where increased muscle weakness and atrophy are observed in FDI and/or APB as compared to ADM (Hannaford et al., 2021). Reduced SHI is observed in patients with ALS (Hannaford et al., 2021; Menon et al., 2013) and a systemic review showcases that SHI shows potential diagnostic potential (Hu et al., 2021).

$$SHI = (FDI_{CMAP_{Amp}} * APB_{CMAP_{Amp}}) / ADM_{CMAP_{Amp}}$$

b. F-wave

F wave results from the summated action potentials of a single or small number of motor units activated from antidromic activation of the anterior horn in the spinal cord (Jerath and Kimura, 2019). The action potential generated by stimulation first travels antidromically to the spinal cord and then travels orthodromically from the spinal cord to the muscle, resulting in F-wave latency (Jerath and Kimura, 2019). This longer F-wave latency during distal stimulation creates a distinct separation between CMAP (M-wave) and the F-wave. F-waves have been shown to exhibit higher variability in latencies and properties due to the activation of different motor units. Repeater F-waves are defined as the F-waves which exhibit the same properties and latency (Chroni et al., 2017). They are proposed to be generated from the backfiring of the same individual motor neurons (Chroni et al., 2017; Oguz Akarsu et al., 2020). F-wave properties are linked with UMN dysfunction (Bischoff et al., 1992; Fierro et al., 1990). While the repeater F-waves are linked with LMN dysfunction (Oguz Akarsu et al., 2020). Relation of F-wave frequency with UMN still seems to be questionable, de Carvalho et. al. found no correlation between UMN dysfunction and F-wave frequency (Carvalho et al., 2002). There seems to be less consensus regarding the application of F-wave in clinical practice and more research is needed to further understand F-wave as a viable assessment tool in neurodegenerative conditions.

5.2. Neurophysiology Index

De Carvalho and Swash (de Carvalho and Swash, 2000) proposed neurophysiology index (NI) as a clinical assessment method that combined indices from nerve conduction methodologies as given by the equation below.

$$NI = CMAP_{amp} * F - wave_{frequency} / CMAP_{onset-latency}$$

NI has demonstrated sensitivity to LMN loss and muscle weakness in patients with ALS (de Carvalho et al., 2003; de Carvalho and Swash, 2010). NI also seems to correlate with the survival of patients with ALS as reported in a recent study (Cao et al., 2019). The studies indicate that NI could be a valuable potential surrogate marker for diagnosis and prognosis in MND conditions.

5.3. MUNE and MUNIX

Motor Unit Number Estimation (MUNE) was first proposed by McComas et. al. in 1971 (McComas et al., 1971) to electrophysiological quantify the number of active and functional motor units in the targeted muscle. MUNE provides a quantitative measure to assess the denervation and loss of motor LMNs, which can be utilized in the prognosis and diagnosis of MND.

Fundamentally, MUNE calculation features obtaining a CMAP and dividing the CMAP with average single motor unit potential to quantify MUNE. Multiple methodologies have been proposed to calculate MUNE reliably. The incremental MUNE method was proposed by McComas et. al. to estimate the motor unit number by incrementally increasing the stimulation intensity and calculating the average single unit. This method suffers from multiple drawbacks including MU alteration (Brown and Milner-Brown, 1976). Multi-point stimulation (MPS) (Doherty and Brown, 1993) method was proposed to address some of the drawbacks faced by the incremental-MUNE method. Later, Adaptive-MPS method was proposed with combined incremental and MPS estimation methodology (Wang and Delwaide, 1995). In Adaptive-MPS, the nerve is stimulated at multiple sites (minimum three sites recommended), and at each site, the incremental stimulation method is used to calculate the average MUNE across the sites. Other advanced methodologies include statistical motor unit estimation, CMAP scan, etc. A recent method MScanFit (Bostock, 2016) has gained popularity due to its reliability, sensitivity, and faster estimation along with openly available software (Gunes et al., 2021; Jacobsen et al., 2017).

Motor Unit Number Index (MUNIX) was proposed by Nandedkar et. al. as a mathematical index to evaluate the functional LMNs innervating the targeted muscle (Nandedkar et al., 2010, 2004). MUNIX is mathematically calculated based on CMAPs and surface electromyography interference pattern (SIP) (Fatehi et al., 2018) resulting in an index for the motor unit number rather than estimating the count of active motor units. MUNIX requires three steps; first obtaining a supramaximal CMAP, the second step is recording

SIPs at distinct force levels ranging from slight to maximum, and finally calculating MUNIX based upon the power regression of the collected measures (Shin-Yi Lin et al., 2024). MUNIX is a faster and better tolerated neurophysiological index to assess LMN functionality (de Carvalho et al., 2018; Verber et al., 2019). MUNIX has shown reproducibility (Ahn et al., 2010; Escorcio-Bezerra et al., 2016; Fathi et al., 2016; Nandedkar et al., 2011) and sensitivity (de Carvalho and Swash, 2010; Fatehi et al., 2018; Neuwirth et al., 2017, 2010) to functional loss in MND across multiple studies. MUNIX has also shown a significant correlation with MUNE techniques (Benmouna et al., 2018; Boeckstein et al., 2012).

5.4. Transcranial Magnetic Stimulation (TMS)

TMS was first proposed by Baker et. al. (Barker et al., 1985) as an safer and less painful alternative to the high-voltage transcranial electrical stimulation. TMS is a non-invasive brain stimulation technique that utilizes a very-short lasting (150 – 300 μ s) and rapidly changing magnetic field that induces electric current at the cortex (Zewdie and Kirton, 2016). TMS when delivered to motor cortex can elicit a motor response in the desired periphery. TMS assess the UMN functional integrity by non-invasively eliciting multiple descending volleys in the corticospinal tract (Zewdie and Kirton, 2016). TMS descending volleys contain both direct excitation (D-wave) of corticospinal tract and indirect tract (I-waves) activation through cortical intraneuronal firing. TMS based motor-assessments are applied either as a single-pulse TMS (sTMS) or as paired pulse TMS (pTMS). In sTMS, a single pulse is used to elicit a motor evoked potential at the desired muscle. sTMS is largely used to study the corticospinal excitability and motor descending pathways. In pTMS, a stimulation pulses is applied as a conditioning pulse to the cortex prior to the second pulse called the test pulse. The paired-pulse technique is largely used to assess the cortical inhibition/facilitation in response to the conditioning pulse. Here, we discuss the assessment parameters evaluated using sTMS technique that is primary associated with corticospinal pathways.

sTMS techniques has been used to propose multiple assessment parameters that have been associated with descending pathways. In these assessments the sTMS technique is accompanied by recording surface-Electromyography (sEMG) signals from the muscle of interest to measure the evoked potential. The assessment parameters are briefly described below:

Cortical Motor Threshold: The cortical motor threshold is defined as the minimum intensity to elicit reproducible evoked response amplitude for at least 50% of the consecutive 10-20 trials. Two cortical motor thresholds are widely used for assessment: resting motor threshold (RMT) and active motor threshold (AMT). The RMT is defined as the intensity where reproducible evoked responses of amplitude at least 50 μ V are elicited by consecutive single pulses. The AMT is defined as the intensity where reproducible evoked responses of amplitude at least 50 μ V are elicited by consecutive single pulses.

Motor-Evoked Potential (MEP): Motor-evoked potential is observed in the sEMG signal in response to the transcranial magnetic stimulation. The MEP is resultant of the sum of the motor unit activated by the descending tracts due to the stimulation pulse. The peak-to-peak amplitude of the evoked potential is considered as the MEP and the onset of the evoked potential is recorded as the MEP latency.

Central Motor Conduction Time (CMCT): CMCT is the estimate of the latency between the stimulation pulse and the activation of lower motor neurons. CMCT is calculated by subtracting the peripheral conduction time from the onset latency of evoked potential due to TMS. The peripheral conduction time can be calculated by either delivering a magnetic stimulation over the spinal cord to record the latency of the subsequently evoked potential or by delivering distal nerve stimulation to record the conduction time of the antidromic volley (F-wave).

Cortical Silent Period (cSP): A suppression of EMG activity is observed in response to a TMS pulse applied during voluntary background contraction. The duration of this suppressed EMG activity is referred to as the cSP.

These sTMS parameters have shown varying degree of sensitive as markers for UMN dysfunction in neurological conditions (de Carvalho and Swash, 2010). CMCT has shown some promising results as a viable assessment, it has shown prolonged latency in ALS (Berardelli et al., 1987; Huynh et al., 2016; Inghilleri and Iacovelli, 2011) and MS (Alusi et al., 2021; Di Sapio et al., 2014; Ingram et al., 1988) groups as compared to the healthy population. CMCT has also shown a significant correlation with clinical scores in MS (Jones et al., 1991; Kale et al., 2009; Sue et al., 1997) .

5.5. Beta-band Coherence

Beta oscillations are observed to be a prominent feature of voluntary motor contraction (Khanna and Carmena, 2015). Beta oscillations in the motor cortex are most evident during steady contractions (isometric) and suppressed during movement execution (Baker, 2007; Chakraborty et al., 2023; Tzagarakis et al., 2010). A prominent coupling of the beta oscillation between the brain and periphery has been observed during isometric contraction (Chakarov et al., 2009; Kilner et al., 1999). This coupling can be calculated using coherence analysis. The brain-muscle (cortico-muscular) and inter-muscle coherence are proposed to be able to indirectly assess the functionality of the corticospinal tract and UMN integrity (Farmer et al., 1993; Fisher et al., 2012).

A distributed coherence has been observed in multiple neurological conditions including stroke, Parkinson, MNDs, etc (Bao et al., 2021; Fisher et al., 2012; Little and Brown, 2014; Mima et al., 2001). In our group, Bista et. al. have previously shown that altered cortico-muscular coherence was observed in primary lateral sclerosis, a UMN disorder (Bista et al., 2023). The study also indicated that the disruption in beta-band coherence was significantly correlated with clinical scores. Previously, Fisher et. al. utilized inter-muscular coherence and observed significantly decreased beta-band coherence in participants with primary lateral sclerosis (Fisher et al., 2012). The same study also reported that no such disruptions were observed in progressive muscle atrophy, an LMN disorder. Similarly, Issa et. al. found 3.8 times decreased beta-band inter-muscular coherence in participants with ALS as compared to healthy participants (Issa et al., 2017) while Proudfoot et al. reported significantly decreased cortical-muscular coherence in ALS (Proudfoot et al., 2018).

Proposed Role of Beta Band: It has been suggested that lower frequency neural oscillations like beta oscillations might exhibit dynamic mechanisms that enable the beta rhythms to synchronize over longer distances and conduction delays (Kopell et al., 2000). Beta band activation is proposed to signify a signature of promoting the existing active contraction while inhibiting other movements (Gilbertson et al., 2005; Swann et al., 2015), suggestive of an ‘active akinetic’ process (Brown and Williams, 2005; Engel and Fries, 2010). The ‘active akinetic’ role of beta band activation was proposed to replace the previously proposed hypothesis suggesting that beta oscillations might represent the ‘idling rhythm’ of the motor system (Pfurtscheller et al., 1996).

II. Aims and Objectives

1. Aims

The overarching aim of my PhD was to develop Electrospinography (ESG) as a novel non-invasive technique to record evoked and task-related spinal electrical activity. The developed ESG technique should feature a standardized electrode location system to record high-density neuro-electrical signals from the cervical spinal cord, facilitating comparative analysis between population groups. This PhD aims to address the role of spinal (dys)function in health and disease which has been largely neglected in the field of non-invasive neurophysiology. The spinal tract evaluation plays an important role in assessing the motor function in health and neurodegenerative conditions, the developed ESG aims to provide an easy-to-use technique to investigate the spinal integrity and its functional connectivity with the cortex. More specifically, this project aims to undertake the following objectives:

- Design an electrode montage for the standardized recording of the high-density electrospinography across participants and population groups.
- Investigate neuro-electric spinal activity and its functional communication with the brain during voluntary motor activity.
- Compare the spinal neural activity between people with neurodegenerative conditions and controls.

2. Objectives and Rationale

2.1. Standardization and Feasibility of Recording HD-Electrospinography

- a. To design an electrode montage for the standardized recording of HD-ESG from the cervical spinal cord.*

Standardization of electrode locations is an important criterion for developing a foundation of a high-density electrophysiology system to facilitate reliable and reproducible recordings between individuals and population groups. Non-invasive electrophysiology techniques like EEG (Chatrian et al., 1985; H, 1958) and EMG (Hermens et al., 2000) have recommended electrode placement systems that enables standardized recording between individuals, groups, and study centres. Recently, a standardized template (De Leener et al.,

2018) was proposed for spinal fMRI for reliable and reproducible recording of spinal fMRI, further showcasing the need for standardization in non-invasive recordings. Despite a standardized system being a foundational step of non-invasive recording techniques, no such standardized placement system has been proposed for recording HD-ESG signals from the cervical spinal cord. HH Jasper (H, 1958) proposed 10/20 electrode system for EEG recording which was further advanced to 10/10 (Chatrian et al., 1985) and 10/5 (Oostenveld and Praamstra, 2001) system to accommodate high-density EEG electrode locations, our objective is to propose a complementary novel electrode placement system for HD-ESG recording.

b. To develop an easy-to-use electrode patch and demonstrate the feasibility of HD-ESG

The proposed electrode placement system for HD-ESG needs to be realized in an electrode patch as an easy-to-use system for recording the spinal signals. The interspinous distances and the anatomical characteristics vary considerably between individuals (Ernst et al., 2019, 2013), this exacerbates a need to develop an elastic electrode patch that could easily accommodate these inter-individual body characteristics to record HD-ESG comfortably during the recording sessions.

The evoked spinal responses have been reported using low-density multichannel electrodes in response to peripheral stimulation (Jeanmonod et al., 1991; Mauguière, 2003a; Restuccia and Mauguière, 1991; Schabrun et al., 2012). The responses by utilizing the HD-ESG system should record these spinal responses and corroborate the evoked response properties reported in previous studies. The HD-ESG should further demonstrate similar spatial topography at cervical vertebral level 6 as seen previously.

2.2. Investigate Spinal Neural Activity and Cortico-Spinal Connectivity during Voluntary Task

a. Evaluate spinal neuro-electric characteristics during voluntary isometric contraction in the healthy population.

Supraspinal studies have highlighted the role of frequency band-dependent neural oscillations during voluntary motor activity (Baker, 2007; Muthukumaraswamy, 2010; Pfurtscheller et al., 2003). Previous studies have reported that neural oscillations might

indicate an indirect representation of neural functions underlying the frequency-dependent oscillatory activation (Engel and Fries, 2010; Kilavik et al., 2013; Nowak et al., 2018; Ulloa, 2022). Recently, epidural spinal recording also demonstrated similar neural oscillatory characteristics as that of the cortex during the eyes-open and eyes-closed resting state paradigm (Wang et al., 2021). Furthermore, spinal fMRI studies have highlighted the complex spinal activation pattern during motor control, summarized in a review by Landelle et al., 2021. The neural oscillatory characteristics observed in the spinal cord during rest and the complex spinal activation pattern observed in fMRI lead to a hypothesis that frequency-dependent neural oscillatory characteristics could play a crucial role during motor control and sensorimotor communication that could be extracted using HD-ESG.

b. Evaluate functional connectivity between the brain and spinal cord by utilizing multimodal recording of HD-ESG and HD-EEG.

Functional connectivity measures like cortico-muscular coherence analysis are utilized to assess the (dys)function of the corticospinal tract (Chakarov et al., 2009; Kilner et al., 1999). The beta band coherence has been widely reported as a feature of sensorimotor communication and UMN integrity (Farmer et al., 1993; Fisher et al., 2012). These methods have largely ignored the role of the spinal cord, despite the spinal cord being an integral part of the corticospinal tract and the spinal grey matter featuring the termination point of UMNs. The spinal interneurons have been shown to have neuro-modulatory effects during sensorimotor communication (Moreno-López et al., 2016; Stachowski and Dougherty, 2021), demonstrating the need to evaluate the functional role of the spinal cord during motor control. This leads us to hypothesize that the coherence as a functional connectivity measure between the brain (HD-EEG) and spinal cord (HD-ESG) could uncover the sensorimotor communication neurophysiology and highlight the UMN function that has been missing before, making it first of its kind analysis.

2.3. Compare spinal neuro-electric activity recorded from people with neurodegenerative conditions.

Indirect assessments including but not limited to TMS, CMAP, and MUNIX have been utilised to assess the sensorimotor pathways in people with neurodegenerative conditions. Multiple sclerosis is a neurodegenerative condition in which the spinal cord is heavily affected (Ikuta and Zimmerman, 1976; Nijeholt et al., 2001; Waldman et al., 2022). The

cervical spinal cord has been especially has been reported to the predominant location of focal lesions and axonal loss (Bonacchi et al., 2020; Waldman et al., 2024). In relapse-remitting MS approximately 60% of focal lesions are reported in the cervical spinal cord (Bot et al., 2004), furthermore, reduced neurite density was prominently observed in the cervical spinal cord of RRMS participants (Collorone et al., 2020). The posterior and lateral SC is found to be especially affected in MS, and the degree of spinal cord atrophy is significantly correlated with the current and future disability in MS (Chen et al., 2020; Collorone et al., 2020). Currently, cross-section area has been used to assess the degree of spinal cord impairment though limited correlation between CSA and axonal has been reported (Petrova et al., 2018). This highlights the crucial role of the spinal cord in MS and leads us to hypothesize that the comparison of direct neuro-electric signals from the spinal cord could indicate the dysfunctional SC neural signalling in the MS population as compared to controls. This could further provide the functional disruptions caused by MS and potentially highlight its role in current and future disability.

III. General Materials and Methods

1. Electrophysiological Signal

Multimodal Electrophysiological signals were recorded using Biosemi ActiveTwo system (BioSemi B.V, Amsterdam, Netherlands), a total of 204+8 channels of signals were simultaneously recorded using the active electrode system. The multimodal signals consisted of high-density EEG (128 channels), high-density ESG (64 channels), ECG (5 channels), EMG (7 channels), and EXG (8 channels) electrophysiological signals. The signals were recorded at a sampling frequency of 8192 Hz.

The Biosemi Activetwo system, used to record multimodal electrophysiological signals, adopts active electrodes instead of passive ones. These active electrodes contains electronic circuit that amplifies the signals at the electrode. In other words, the electrophysiological signals are amplified at the electrode itself before entering the electrode lead. On the other hand, passive electrodes send unamplified signals to the amplifier box, where the signal is then amplified and converted into digital signals (analog-to-digital conversion). The use of active amplification reduces ambient and electrical-line noise during recording. Conductive gel is used in conjunction to further limit the external noise by improving the contact

between the skin and electrode. The enhanced contact reduces the impedance between skin and electrode thus improving the signal-to-noise ratio.

1.1. Brief Description of ESG Electrode Montage

This section briefly describes the developed SC10X/U electrode placement system used for recording the cervical and upper thoracic spinal activity used for analysis in this thesis. The extensive description and development of the electrode placement system is described in Chapter IV.

A high-density electrode placement system was developed to non-invasively record the electric activity from the spinal cord. This aimed to standardize the electrode location with respect to the sources of neural activity in the spinal cord. The design was based on human anatomical landmarks suited for guiding the electrode placement locations. The principles of standardizing formerly used in EEG were extended to the spinal cord for designing a high-density ESG recording electrode placement system.

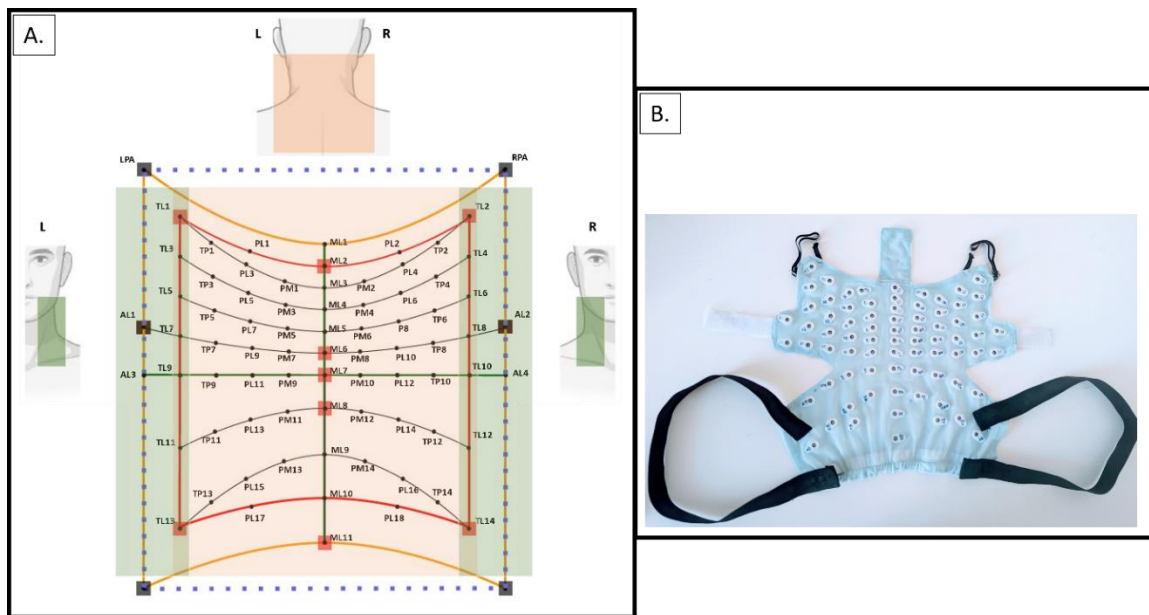


Figure 6: Designed SC10X/U electrode placement system and the developed electrode patch. A. The SC10X/U electrode placement system and its labels are shown. B. The developed electrode patch. The full description of the system is explained in the section IV.2.

Principles of ESG electrode placement:

1. Two-dimensional representation of the space: The cervical-thoracic spinal space can be envisioned as two curved cylinders joined together. The cervical curvature (lordotic) and the thoracic curvature (kyphotic) can be characterized as concave and convex cylinders,

respectively, when viewed posteriorly. The 2D representation of cervical-upper thoracic space can be characterized by the curved surface of the two cylinders in 2D plane. This is described by a rectangle with curved top and bottom sides.

2. Anatomical Landmarks: The anatomical references for representing cervical and upper thoracic spinal space include: occipital protrusion, C2 spinous process, cervical and thoracic spinous process, left and right pre-auricular points, transverse spinous process of the atlas, carotid tubercles, root of the scapula, intersection of the transverse plane through C5 spinous process and the sagittal plane passing through the inferior angle of the scapula.

3. Division of 2D space: The lateral space is divided into 10 equal spaces each accounting for 10% of the total lateral-medial distance. The medial-sagittal space along the length and direction of the spinal cord is divided in proportion to the average inter-vertebrae spinous distances contained in the electrode space. The medial sagittal space encompassing the cervical spine region is divided in proportion to the average cervical intervertebral spinous distance relative to the average thoracic intervertebral spinous distance. Similarly, the space that encompasses the thoracic region is divided in proportion to the average thoracic intervertebral spinous distance relative to the average cervical intervertebral spinous distance.

The implementation of the above principles initializes the mapping of the spinal space onto the electrode space. After the initial mapping of the spinal space, the electrode placement system was realized by expanding the proportional division to the whole electrode space resulting in the SC10X/U electrode placement system.

Non-invasive ESG electrode recording patch: A novel non-invasive electrode patch was developed for non-invasive recording of the spinal electrophysiological signals over the cervical and upper thoracic spinal space (over the neck and upper back), as shown in Figure 6. The recording patch was developed in accordance with the SC10X/U electrode placement system described above. The electrode patch covers the cervical and upper thoracic (up to T4) spinal space. The electrode patch was realized in three sizes: small, medium, and large to incorporate inter-individual spinal cord length variability. The average inter-cervical and inter-thoracic spinous distance for each electrode patch are determined based on studies by Ernst, M. J., et al. (Ernst et al., 2013).

1.2. Acquisition System

Electroencephalography: The spinal activity was recorded using a 128-channel high-density active electrode system. The EEG electrodes were positioned based on the 10/5 placement system. The pin-type electrodes were attached to the electrode holder using a highly conductive gel (signa-gel) to enhance the skin-electrode connection.

Acquisition Procedure: The participants head circumference was measured and appropriate size headcap was placed over the head. The head cap was adjusted such that the center electrode was placed at the center of the scalp. The center of the scalp was identified as the point that equally divided the distance between the occipital protrusion and inion sagittally, as well as the distance between the right and left pre-auricular points laterally. The chinstraps were fastened after the successful alignment of the cap. The conductive gel was filled in the individual electrode holders using a blunt syringes to decrease the skin-electrode impedance.

Electrospinography: The spinal activity was recorded using a 64-channel high-resolution active electrode system. The ESG electrodes were positioned based on the SC10X/U placement system. The pin-type electrodes were attached to the electrode holder using a high-viscosity conductive paste (elefix) with adhesive properties to secure the electrode positions and enhance the skin-electrode connection.

Acquisition Procedure: The anatomical landmarks listed in section IV.2.2 were marked on the participant's skin. The neck's circumference and the average cervical interspinous distance were measured to select the appropriately sized electrode patch. The electrode patch was placed over the skin of the participant and positioned accurately with respect to the marked anatomical landmarks. Velcro and elastic straps were fastened around the neck and shoulders, respectively. High-viscosity conductive paste was applied to the individual electrode holders using a blunt syringe to reduce skin-electrode impedance. The adhesive quality of the conductive paste also aided in securing the patch to the skin.

Electromyography: The muscle activity was recorded using seven flat active Ag-AgCl electrodes. The electrodes provided a circular recording area ($d=3\text{mm}$) located in a $17\times 10\text{mm}$ support surface area. Two unipolar electrodes were placed in accordance with SENIAM recommendation (at the center of the muscle belly) for each of the three muscles:

FDI (first dorsal interosseous); APB (Abductor Pollicis Brevis); FPB (Flexor Pollicis Brevis) used to acquire bi-polar surface EMG. An additional electrode was placed over the wrist bone as reference.

Acquisition Procedure: The electrode positions were marked according to SENIAM for the three muscles of interest. Adhesive rings were placed over the support area of the flat electrodes, and conductive gel was applied over the circular recording area. The flat unipolar electrodes were then placed over the marked positions and further secured using micropore medical tape.

Electrocardiography: The cardiac activity was recorded using flat active Ag-AgCl electrodes. Surface ECG was placed in accordance with the 5-electrode ECG system. The 7-lead ECG signals were extracted using the 5-electrode ECG system.

1. $Lead\ I = LA - RA$
2. $Lead\ II = LL - RA$
3. $Lead\ III = LL - LA$
4. $aVR = RA - \frac{1}{2}(LA + LL)$
5. $aVL = LA - \frac{1}{2}(RA + LL)$
6. $aVF = LL - \frac{1}{2}(RA + LA)$
7. $Lead\ V1 = V1 - \frac{1}{3}(RA + LA + LL)$

EXG: Eight external channels were connected using flat active sintered Ag-AgCl electrodes. The position of external electrodes were— (a) left and right earlobe, (b) to the left of left eye and to the right of right eye, (c) left and right clavicle, (d) below left eye, (e) erb's point (brachial plexus).

2. Experiment Setup

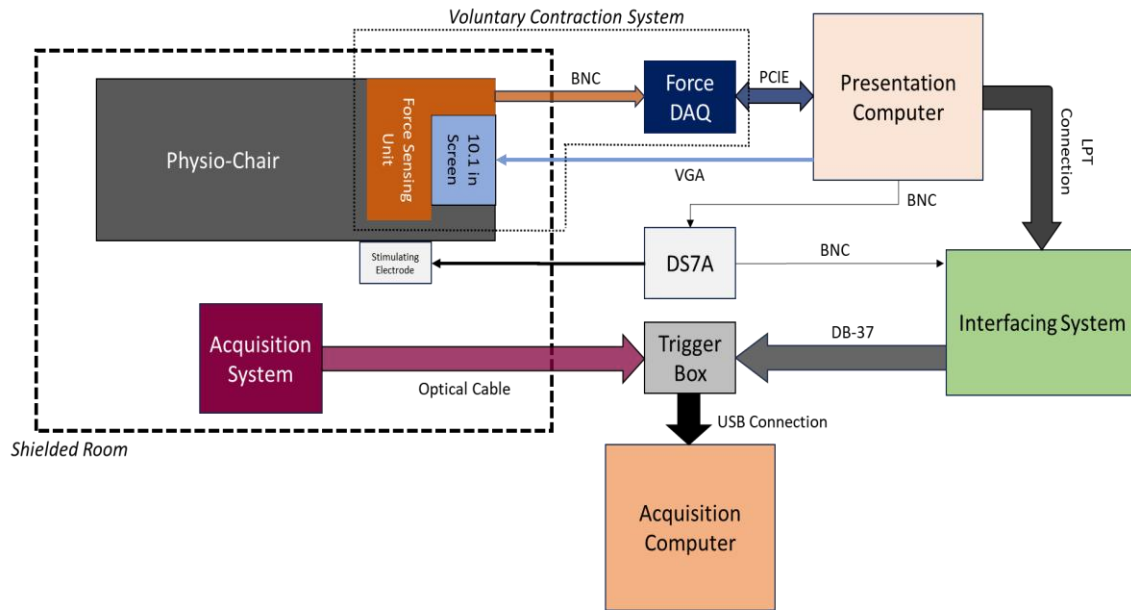
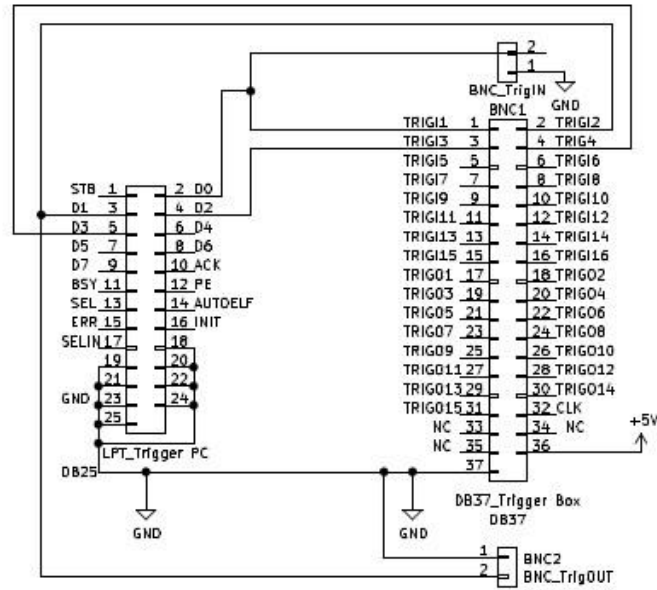


Figure 7: The schematic of the experiment setup showcasing the connections between the instruments and their respective locations.

The experiment setup consisted of Biosemi ActiveTwo acquisition system, presentation computer, biosemi trigger box, DS7A constant current stimulator, 10.1 inch display monitor, physio-chair, NI-DAQ (force acquisition), force sensing unit, and an interfacing system as shown in the Figure 7: The schematic of the experiment setup showcasing the connections between the instruments and their respective locations.. The interfacing system and the voluntary contraction system were engineered and developed in-house. The in-house developed systems are described below.

2.1. Interfacing System



integrates the electrophysiology data received via optical cable and 16-bit trigger information via DB37. This consolidated information of electrophysiology signals marker with trigger is sent out to acquisition computer by trigger box via USB connection. The acquisition computer records these signals using Biosemi Actiview software while providing real time visual representation of the electrophysiology signals and trigger markers.

The interfacing system's input (BNC and DB25) is pulled down by a 1kOhm resistor. This minimizes the risk of receiving accidental information from a floating pin.

2.2. Voluntary Contraction System

The voluntary contraction system was used to sense and record visually guided voluntary pinch grip force contraction. The system consisted of a force-sensing unit, NI Data Acquisition system (DAQ), and a 10.1 display screen. The force-sensing unit converted the pinch grip force applied to the unit to an analog voltage signal. This NI DAQ converted the analog signal received from force-sensing unit to digital signal. The digital force values were sent to the presentation computer via PCIE connection. This computer received the force information and displayed it on the 10.1-inch screen to provide visual guidance to the participant.

a. Force Sensing Unit

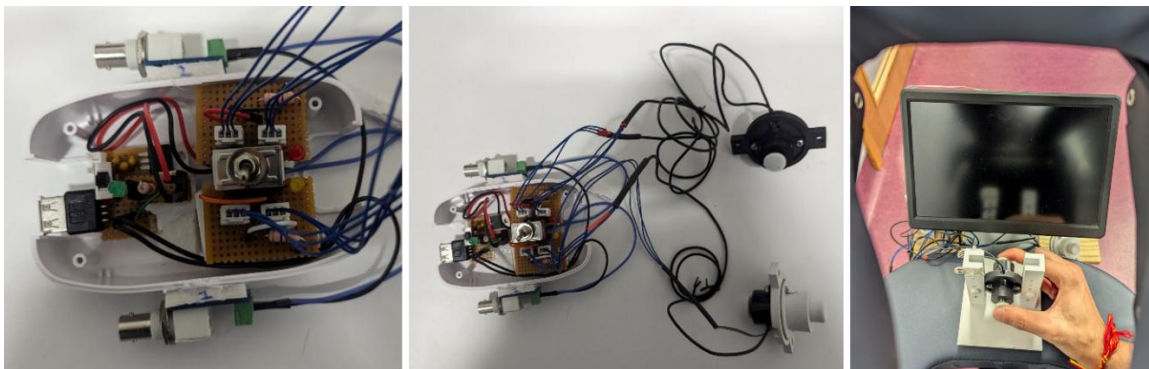


Figure 9: The developed force sensing unit and pair of force sensors are shown. Left, force sensing units. Centre, force sensing unit and pair of force sensors. Right, the force sensor is placed in a structure during the pinch-grip task.

The force sensing unit, built in-house, converts the pinch-grip force into a voltage analog signal and sends it to the NI-DAQ via a BNC connection. The unit features two pairs of force sensors, selectable by a toggle switch. Different coloured LEDs indicate the selected

pair. Each sensor pair displays a linear voltage versus force profile and measures the force exerted by the finger and thumb during a pinch-grip contraction. The first sensor pair uses a rigid contact-based load system with a maximum force range of 25lbf (~110 N). Meanwhile, the second pair employs a compression spring-based load system, offering improved force feedback control and a maximum force range of 10lbf (~50 N).

The unit can be powered by either a 9V battery or a USB (5V), with the power source chosen via a slider switch. The battery power is connected to an LM78 voltage regulator, which steps-down the voltage to a stable 5V before supplying power to the rest of the circuitry. The force sensors and spring system are housed in a 3D-printed enclosure.

The force sensing unit provides normalized zero and span. It also features thermal compensation to adjust for changes in zero and span due to temperature variations. The unit maintains an accuracy, taking into account non-linearity, hysteresis, and repeatability, of $\pm 1\%$ of the span. This makes it a robust sensing unit for both static and dynamic force contraction tasks.

3. Experimental Procedure

3.1. Ethical Approval and Informed Consent

The ethical approval was obtained from the from Tallaght Hospital/St. James's Hospital Joint Research Ethics Committee for St. James's Hospital, Dublin, Ireland (Project Reference: 0493). All participants were informed about the experiment paradigm and signed consent was obtained before the recording session.

The procedure for obtaining informed consent before scheduling involves sending an information leaflet. This leaflet explains the experimental paradigm, participant rights, data protection information, and associated risk (no to minimal risk). On the day of recording, the informed consent procedure involved a brief recap of these details, highlighting that the participant will not directly benefit from the study and reaffirming their right to discontinue the experiment at any time without repercussions.

3.2. Experimental Paradigm

Following informed consent, the participants were led to the shielded room and were helped onto the physio-chair. The participant sat conformably on the physio-chair as the

experimenters started preparing for the setup. First, the ECG electrodes were placed on the chest of the participant as described in section 1.2 of chapter III, following which the participants were asked to change to hospital gown to provide adequate access to the upper back and neck. Upper back, neck, and right hand was scrubbed using nuprep gel to prepare the surface for better skin-to-electrode contact. The anatomical locations listed in section 2.2 of chapter IV and muscles of interest were marked using skin-friendly marker. Following this, EEG cap and ESG patch was placed and secured as described in section 1.2 of chapter III. The electrodes were placed over the muscles of interest and external electrodes as described in section 1.2 of chapter III. Conductive gel was used to fill the electrode holders on the EEG cap and conductive paste was used to fill the electrode holders on the ESG patch to increase conductivity and minimize electrode impedance. Finally, labelled pin-type electrodes were inserted in the corresponding electrode holders on the cap and patch. The cables from positioned electrodes were inserted in the Biosemi box. Upon completion of the acquisition setup, the electrode offsets were checked via Actiview software. The active electrode offsets were kept between $\pm 25\text{mV}$, and signals from the electrodes were quickly checked to assess the recording quality. The needed improvements were made to reach satisfactory recording quality.



Figure 10: A. The setup with the physio-chair and 10.1-inch screen that was used during the experiment. B. A participant sitting in an inclined position on the physio-chair. The participant's chest was supported by the chest rest while their head rested on the headrest and hands rested on the handrest respectively.

The participant was instructed to lean forward and sit comfortably in an inclined position on the physio-chair, as depicted in Figure 10. The participant's lower torso and chest were supported by the chest rest. In this position, the legs formed an approximately 60-degree angle at the knee, and the chest rest was positioned at roughly 30 degrees from the vertical plane. Throughout the recording session, the participant's head rested on the chair's headrest, and their hand was placed on the hand-rest, forming a 90-degree angle at the

elbow, as illustrated in the Figure 10. The position thus described above limited the activation of back and neck muscles during the recording session and improved signal to noise ratio. The recording session lasted around 1 hour and 30 minutes and included the following paradigms.

a. Median Nerve Stimulation

Peripheral Nerve Stimulation: The DS7A constant current stimulator is used for peripheral nerve stimulation at the wrist. This medically certified device, which complies with MDD CE standards, can deliver high voltage pulses of up to 100mA constant current. The pulse duration can be adjusted within a range of 50us to 2ms. The device offers three types of pulse polarity for stimulation: positive, negative, or alternating. The stimulator can be either manually triggered or via TTL pulse. During the intervention, the stimulator was set to a positive pulse polarity configuration with a pulse duration of 200us to deliver the intended stimulation.

The initial site for median nerve stimulation was determined by placing the cathode of the stimulating electrode 8 cm from the ABP muscle, following the guidelines (Neal and Katirji, 2008; Nestor and Nelson, 1990). This site was then stimulated, gradually increasing the intensity until visible thumb movement was observed. Once the thumb moved visibly, the stimulation electrodes were repositioned to various nearby locations, maintaining the same intensity to identify the optimal stimulation site for the median nerve. The optimal location was identified by visually observing for the maximum muscle twitch at the set stimulation intensity. This accommodated for inter-individual variability and assisted in pinpointing the ideal stimulation location.

The stimulation electrodes were positioned at the optimal site, and the motor threshold was determined. To do this, stimulation was manually triggered and the intensity was gradually increased from 0mA. The intensity was increased in incremental steps of 0.25mA, until a noticeable twitch was observed in the thumb. Once a noticeable twitch was observed, the stimulation intensity was fine-tuned by decreasing it in 0.1mA decrements, until the corresponding muscle twitched at the lowest possible intensity. This intensity was marked as the motor threshold, and the EMG signal checks were performed.

During the recording session, the median nerve was stimulated at 1.75 times the motor threshold. The session consisted of 35 blocks, each lasting 30 seconds. Each block was composed of a 20-second stimulation phase followed by a 10-second rest phase, as shown

in Figure 11. In the stimulation phase, a train of stimulation pulses at an average frequency of 2Hz was delivered to the participant. The session recorded a total of 1400 trial responses (35 blocks x 40 stimulations per block) to peripheral stimulation. The simulator was triggered using TTL pulses, MATLAB was used to control and send out a train of TTL pulses from the presentation computer.

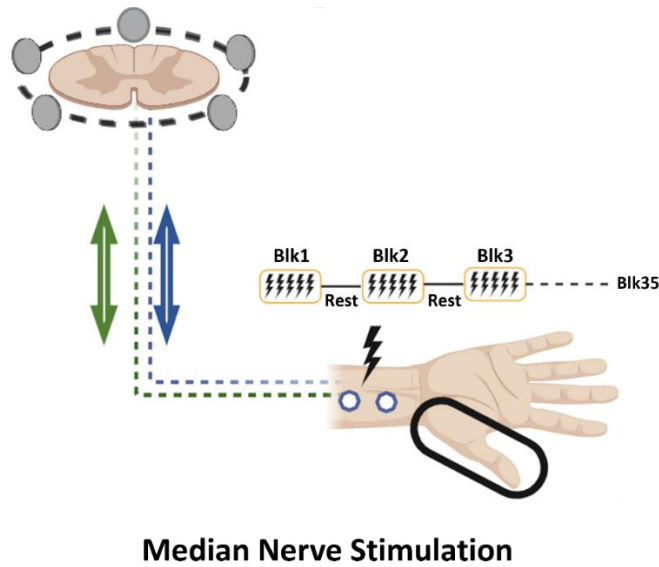


Figure 11: Illustration of the median nerve stimulation paradigm: The stimulation was applied at the wrist of the participant to illicit thumb movement. The paradigm had a total of 35 sessions (blocks) separated by a 10-second rest period.

b. Resting State

The resting state paradigm consisted of two sessions: eyes-open and eyes-closed. Each session consisted of two blocks of 200s recordings, the two blocks were separated by short rest breaks (~15s). This resulted in 400s of recorded data for each session (eyes open and eyes closed).

During eyes open recording session, the participants were requested to keep their eyes open and softly fixate their eyes on the letter X (6x8 cm², printed black on white background) presented on the display screen. The participants were asked not to constrain their blinking and blink naturally while letting their mind wander. During eyes closed recording session, the participants were instructed to keep their eyes closed and let their mind wander. The lights were switched off during the eyes closed session.

c. Pincer-Grip Voluntary Motor Contraction

The voluntary motor paradigm consisted of two distinct visually-guided tasks. Participants were directed to use their right hand regardless of their dominant hand (right-handed or left-handed). Their hand was positioned over the force-sensing unit in a pincer-grip stance, requiring them to apply force using their index finger and thumb, as shown in Figure 12. The tasks are as follows:

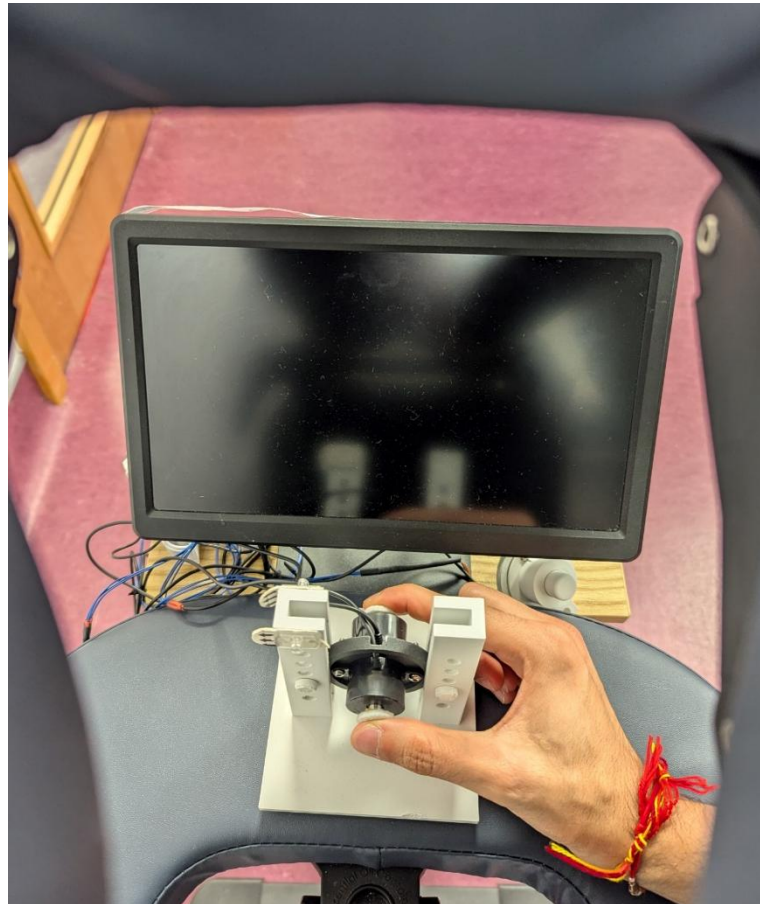


Figure 12: The photo of the force sensor system and the 10.1-inch screen used during the task, from the perspective of the participant (photo taken through headrest).

Task 1: This task aimed to record the maximum voluntary contraction (MVC) during isometric pincer-grip contraction. Participants applied maximum force for 3 seconds followed by 30 seconds of rest, guided by arrow based visual cues. The arrow at the top signifies the maximum force condition, where the participant was required to apply maximum isometric contraction corresponding to the peak force. Meanwhile, the arrow at the bottom represents the rest condition. Real-time visual force feedback was provided to the participants in the form of a filled rectangle, with the rectangle's height representing the force level. The task consisted of a total

of 5 trials, each involving a 5-second isometric-contraction period followed by a 30-second rest period. The average peak of the three trials, which were within 10% of each other was used to calculate the MVC force.

Task 2: This task aimed to record the electrophysiology signals during visually-guided isometric pincer-grip contraction at 10% MVC force level. The task consisted of a total of 3 sessions of 10 trials each, resulting in a total of 30 trials. Each trial lasted around 15 seconds and consisted of three 5 seconds visually guided phases: rest phase (5s), followed by isometric contraction phase (5s), followed by recovery phase (5s). The visual guidance was provided using an empty rectangular box, whose height represented the target isometric force level. Real-time visual force feedback was provided to the participants in the form of a filled rectangle, whose height represented the force level.

During the isometric contraction phase, an empty rectangular box was displayed, with its height representing the target 10% MVC. Participants were asked to match the target force by pinching the force-sensing unit, thereby increasing the filled rectangle's height until it reached the target. They were then required to maintain this contraction for about 5 seconds. The absence of an empty rectangular box (height = 0, target force = no force) visually represented the rest and recovery phases.

Participants were provided with visual feedback on the correct level of exerted force. This was done by changing the colour of a filled rectangle from grey to green if the difference between the target force and the real-time exerted force was within 10% of the range.

Task 1 was performed using a rigid contact-based load force sensor pair, as described in Section 2.2, with a maximum force range of 110N. Task 2 was performed using a compression spring-based load force sensor pair (Section 2.2), but with a lower maximum force range of 50N. The spring-based load sensor offers better force feedback and increased control. As the force increases, the spring compresses, thereby providing enhanced sensory feedback and control. The lower maximum force range of the spring-based load force sensing pair also provides increased granularity for force ADC. The required force sensing pair was selected by toggling the switch after task 1 and physically switching the force sensing pair.

4. Signal Pre-Processing

The signal pre-processing pipeline was implemented similarly for the multimodal signals collected during the resting-state and pincer-grip contraction paradigm. The preprocessing of median nerve stimulation involved an additional step to remove stimulation artifacts before proceeding to the pre-processing pipeline used for the other paradigms. Signal Processing was performed using MATLAB software (The MathWorks, 2023), the fieldtrip toolbox (Oostenveld et al., 2011), the noisetool toolbox (de Cheveigné et al., 2018), and the EEGLAB toolbox (Delorme et al., 2011).

4.1. Stimulation Artifact Removal

The EEG, ESG, ECG, and EMG signals showcased stimulation artifact for each stimulation trigger that lasted for 1-2 ms. This artifact was removed by interpolating the signals for all recorded channels between 0.5 ms before the stimulation trigger and 2.5 ms after the stimulation trigger. Piecewise cubic interpolation was performed based on the prior signal information from 3.5ms-0.5ms before the stimulation trigger and 2.5ms - 5 ms after the stimulation trigger for each channel. The piecewise cubic interpolation is a shape-preserving method that reconstructs the signal for the time of interest based on the signal information from neighboring time points. The resultant signals were free of any stimulation artifact.

4.2. ESG Pre-Processing

The 64-channel ESG signals were highpass filtered above 1Hz (dual-pass Butterworth filter, 3rd order) to remove signal drift. The ESG signals were visually inspected, and the detected bad channels were removed.

ECG artifact was seen as a prominent artifact in the ESG signals due to the close proximity of the heart to the lower thoracic and cervical spinal cord. The canonical correlation analysis (CCA) was adopted to remove ECG artifacts from the ESG signals (de Cheveigné et al., 2018; Hotelling, 1936). Canonical Component Analysis (CCA) is a statistical method used to identify and analyze relationships between two sets of variables. It seeks to find linear combinations (components) of the variables in each set that are maximally correlated with each other. The CCA was applied to the remaining ESG channels, and the 7 ECG leads as

input variables. The ESG channels and ECG leads used as the input were bandpass-filtered between 5 Hz and 45 Hz (dual-pass Butterworth filter, 4th order) and subjected to CCA to obtain highly correlated components corresponding to the ECG artifact. Canonical components with a correlation greater than 0.8 between ESG and ECG were marked as ECG artifact-related components.

To obtain ESG-artifact removed ESG signals, the high-pass ESG was transformed to CCA space, and the marked canonical components were removed. The remaining components were backtransformed to the original ESG space, resulting in reconstructed ESG signals with little to no ECG artifact. Before further processing of the ESG signals, bad channels were interpolated based on the weighted average method using neighboring channels (neighbor distance $\leq 50\text{mm}$). The signals obtained after artifact rejection and bad channel interpolation were re-referenced using ‘common average referencing’. A zero-phase comb-notch filter was then used to remove 50Hz harmonic line noise.

4.3. EEG Pre-Processing

The 128-channel ESG signals were bandpass filtered above 1Hz (dual-pass Butterworth filter, 3rd order) to remove signal drift. The EEG signals were resampled to 1024 Hz and band-pass filtered between 1Hz-345Hz (dual-pass Butterworth filter, 4th order). The EEG signals were visually inspected to determine the bad channels, these bad channels were removed before further processing. The remaining EEG channels were subjected to ICA decomposition, and the ICs related to the blink artifact were marked. The marked ICs were removed, and the remaining ICs were back-transformed to the original EEG space resulting in blink artifact rejected EEG signals. The resultant ESG signals were used to reconstruct the bad channels that were rejected earlier. The reconstruction of signals for the bad channels was performed using weighted average interpolation of the neighboring channels (Perrin et al., 1989). The EEG signals obtained after artifact rejection and bad channel interpolation were re-referenced using ‘common average referencing’. A zero-phase comb-notch filter was then used to remove 50Hz harmonic line noise.

4.4. EMG Pre-Processing

The unipolar EMG signals were re-referenced to the electrode positioned at the wrist bone. The re-referenced EMG signals were bandpass filtered between 10-500 Hz (Butterworth filter, 4th order). The bipolar EMG signals were obtained for the three muscles: FDI (first

dorsal interosseous); APB (Abductor Pollicis Brevis); and FPB (Flexor Pollicis Brevis). The bi-polar signals were obtained by subtracting the relevant bandpass filtered re-referenced unipolar EMG signals.

5. Data Analysis

The data analysis pipeline differed between the task paradigms (nerve stimulation, resting-state, pincer-grip contraction). This section explains the data analysis pipeline for each task paradigm separately. Furthermore, the data analysis pipeline for each paradigm type is further divided into sub-sections to explain the analysis protocols for different types of analysis.

5.1. Median Nerve Stimulation

The ESG and EMG signals were segmented into 0.5-second epochs and were baseline-corrected. Each 0.5s epoch started 0.1s before the stimulation and ended 0.4s after the stimulation.

The epoched ESG trials were visually and automatically inspected for bad trials. The selected bad trials were removed and the remaining trials were bandpass filtered between 10Hz – 1500Hz (Akaza et al., 2020), dual-pass Butterworth filter.

a. Spatial Filtering

The epoched ESG signals were spatially filtered to increase signal-to-noise ratio using task-related component analysis (TRCA) (Nakanishi et al., 2018; Tanaka et al., 2013). TRCA was proposed by Tanaka et. al. to extract the task-related components that maximize the covariances between trials during the specified time window. The TRCA-spatial filtering increases inter-block consistency and reproducibility. This results in an improved signal-to-noise ratio where the task-relevant spinal signals are retained.

Full-band TRCA-Spatial Filter: A full-band TRCA-spatial filter was designed for evaluating spinal somatosensory evoked potential in response to median nerve stimulation. The spatial filter was obtained by choosing the 15ms window between 7ms-22ms after stimulation as the time block to maximize the inter-trial consistency using TRCA. The time block was selected based on the prior knowledge of the expected latencies for the spinal SEPs (Cruccu et al., 2008; Desmedt and Cheron, 1981; Mauguière, 2003a). A total of 64

task-related components were obtained for the 64-channel ESG data subjected to the TRCA algorithm. The significant task-related components were selected by comparing the resultant eigenvalues against the eigenvalues obtained via null-hypothesis. A null hypothesis was considered, where randomized 15ms time-blocks sampled uniformly across the entire trial duration were used to calculate the eigenvalues (Tanaka et al., 2013). This was repeated three times to obtain a normal distribution of null hypothesis eigenvalues. Task-related components with eigenvalues outside the 95% confidence interval of the null distribution were chosen as the statistically significant components.

The trials were full-banded TRCA-spatial filtered. During full-banded TRCA-spatial filtering, the signal was transformed using task-related component analysis, and the non-significant components were removed.

High-Frequency TRCA-Spatial Filter: A High-Frequency TRCA-spatial filter was designed for evaluating very-high-frequency oscillatory amplitude modulation in response to the median nerve stimulation. The ESG signals were band-pass filtered between 300 Hz - 1500 Hz, the 300Hz - 1500Hz band is referred to as the high-frequency band. These signals were subjected to the high-frequency TRCA-spatial filter. The spatial filter for the high-frequency band was obtained by choosing the 10ms window between 7.5 ms - 17.5 ms after stimulation as the time block to maximize the inter-trial consistency using TRCA. The time block was selected based on the prior knowledge of the expected latencies for the evoked spinal HFOs (Insola et al., 2008; Prestor et al., 1997). Similar to the full-band TRCA-spatial filter design, the High-frequency task-related components were obtained for the 64-channel high-frequency bandpass filtered ESG signals. The significant task-related components were selected by comparing the resultant eigenvalues against the eigenvalues obtained via null-hypothesis for high-frequency band TRCA.

The ESG trials in the high-frequency band were high-frequency TRCA-Spatial filtered. During full-banded TRCA-spatial filtering, the signal was transformed using task-related component analysis, and the non-significant components were removed.

b. Somatosensory Evoked Potential

The somatosensory evoked potentials in response to the median nerve stimulation were obtained for both spatially filtered ESG signals and ESG signals without spatial filtering. Both sets of ESG signals were re-referenced using the surface Laplacian method (Hjorth, 1975), to improve the locally relevant signals further and minimize the effect of volume

conduction (Bradshaw and Wikswo, Jr., 2001). The re-referenced ESG epochs were averaged across all good trials resulting in event-locked spinal activity featuring spinal SEPs for each ESG type (spatially-filtered, non-spatially-filtered). The characteristics (latency, peak amplitude etc.) of the resulting SEPs were calculated for the participant.

The obtained event-locked spinal activity between -0.1s (pre-stimulation) to 0.4s (post-stimulation) was further averaged over all the participants resulting in grand-mean evoked responses for the participant population. The spatial distribution of the evoked spinal activity was analyzed by mapping the grand-mean evoked activity across the SC10X/U electrode space for the time window of interest.

c. Low-Amplitude High-Frequency Oscillatory Response

The epoched (trials) ESG signals were bandpass filtered between 300 – 1500Hz and was subjected to the high-frequency banded TRCA-spatial filtering. Each trial of the ESG signals thus obtained was normalized before further analysis. A baseline window of 250ms between 0.3s and 0.05s before the stimulation trigger was used to normalize the signal. The normalization for each trial was performed by removing the mean baseline value and dividing the resulting signal by the standard deviation of the baseline. The normalized trials were averaged across all the trials to obtain event-locked high-frequency oscillatory spinal activity (HFOs) for each participant. The envelope of the resultant trial-averaged HFOs for each participant was calculated using the Hilbert transform. The envelope was used to calculate the onset of evoked modulation of the HFOs (evoked-HFO). The onset of evoked-HFOs was determined when the envelope magnitude first exceeded the threshold after the stimulus trigger. The threshold was determined as $5 \times \text{standard deviation of the baseline envelope magnitude above the mean envelope magnitude during baseline}$. To determine the peak magnitude and duration of the HFOs, the envelope was smoothened using a low-pass filter (75Hz, 4th-order Butterworth filter). The peak of the HFOs was determined as the peak of the smoothen envelope of the evoked-HFOs. The duration of evoked-HFO response was calculated by the determining time duration when smoothen-envelope signal magnitude stayed above the calculated threshold ($\text{mean}_{\text{baseline}} + 5 \times \text{SD}_{\text{baseline}}$). The characteristics of the evoked-HFOs were calculated for each participant. The characteristics included onset latency, peak magnitude, latency at peak magnitude, and evoked duration.

The evoked-HFOs envelope was further averaged over all the participants resulting in an average evoked HFOs envelope for the participant population. The spatial distribution of

the evoked HFOs envelope was analyzed by mapping the grand-mean evoked activity across the SC10X/U electrode space for the time window of interest.

Time-Frequency Analysis: Event-locked and non-phase-locked time-frequency (TF) analysis was performed for the normalized 64-channel ESG data in the high-frequency band. Both time-frequency analyses were performed using complex wavelet convolution method ('mtmconvol' Fieldtrip) with Hanning taper, at a time resolution of 25ms, and a frequency resolution of 10Hz in the 300 - 1250Hz frequency range. The time-frequency domain was adopted to evaluate the event-locked and non-phase-locked spectro-temporal characteristics of the high-frequency oscillatory activity in response to the stimulation.

The non-phase-locked spinal activity was obtained by subtracting the event-locked high-frequency oscillatory spinal activity (evoked-HFOs) from every trial for each channel. The time-frequency analysis was performed on the resultant non-phase-locked spinal activity for every trial. The resultant trial-wise TFs were averaged across all trials for each channel to obtain the non-phase-locked time-frequency spectrum. The event-locked time-frequency spectrum was obtained by performing TF analysis on the event-locked signals obtained by averaging the high-frequency banded spinal activity across all trials.

The resultant event-locked and non-phase-locked time-frequency spectrum for each channel were baseline-corrected for every 10Hz frequency bin, -500ms to 25ms was used as the baseline window.

5.2. Pincer-Grip Voluntary Motor Contraction

The pre-processed ESG and EEG signals during task 2 of the pincer-grip motor contraction were segmented into 30 trials of 15 seconds, starting 3 seconds before the start of the contraction phase till 7 seconds after the end of the contraction phase.

The ESG trials were visually and automatically inspected for bad trials. The selected bad trials were removed and the remaining trials were bandpass filtered between 2Hz - 250Hz dual-pass 4th-order Butterworth filter. The resultant band-pass trials were spatially filtered using the full-banded TRCA-spatial filter designed during the median nerve stimulation analysis, resulting in increased quality of spatially relevant signals while removing the irrelevant noise. Furthermore, the ESG signals were re-referenced using the surface Laplacian method (Hjorth, 1975), to improve the locally relevant signals further and minimize the effect of volume conduction (Bradshaw and Wikswo, Jr., 2001).

a. Spectral Analysis

Spectral analysis was performed during the sustained period of the task 2. The re-referenced ESG signals were further segmented into Hanning windowed 1s epochs with 50% overlap (Bayram et al., 2023; Polat and Güneş, 2007) during the sustained contraction phase (0.75s - 4.75s after the contraction cue). The spectral power (Welch's method) was calculated at every integer frequency value between 5 Hz and 95 Hz for each 1s segment. The average power spectral density (PSD) across all segments for each participant was computed for the three frequency bands: beta (13 – 30 Hz), low gamma (31 – 60 Hz), and high gamma (61 – 90 Hz). The PSD for each band was normalized by the average band-specific baseline power. A two-second time-period between 8.5s – 10.5s after the contraction cue was chosen as the baseline window.

The normalized band-specific PSD was grand-averaged (gPSD) over all the participants and the PSD magnitude was converted to decibel for better visualization. A topological plot of the gPSD was plotted over the SC10X/U cervical electrode space.

b. Event-Related Spectral Perturbations (ERSP)

ERSP analysis was performed on the epoched and re-referenced ESG signals during task 2 of the pincer-grip paradigm. The signals from each participant were segmented into trials between 2 seconds before the contraction cue and 2.5 seconds after the end of the contraction cue i.e. between -2.5 to 8 seconds where $t=0$ s signifies the start contraction cue. The bad trials were visually and automatically rejected. All channels for each trial underwent time-frequency analysis between the frequency range of 10Hz – 95Hz. The time-frequency analysis was performed using complex wavelet convolution method ('mtmconvol' Fieldtrip) with Hanning taper, at a time resolution of 200ms, and a frequency resolution of 2Hz. The output time-frequency power was normalized w.r.t the average baseline power calculated across all trials for each 2Hz frequency block. The normalization utilized a two-second time widow between 8.5 s – 10.5s after the contraction cue to calculate the average baseline power across all trials for each 2Hz frequency block.

The statistically significant normalized time-frequency spectrum blocks were identified and referred to as event-related spectrum perturbation (ERSP). The ERSPs were averaged across all trials and in frequency bands to calculate band-specific ERSPs. Beta (13 – 30 Hz), low gamma (31 – 60 Hz), and high gamma (61 – 90 Hz) frequency bands were used

to calculate band-specific ERSPs. Both band-specific and un-banded ERPS were group-averaged across all participants. The group-average band-specific ERSPs (banded gERSP) were 10log10 transformed for better visualization.

c. Cortico-Spinal Coherence

Coherence analysis was performed on 1s non-overlapped segmented ESG signals recorded during the sustained contraction phase of the voluntary motor task paradigm. The coherence spectrum between EEG-ESG (Cortico-Spinal Coherence) was calculated using the method described by Bista et. al. (Bista et al., 2023; Coffey et al., 2021). The coherence analysis was applied to 1s EEG, and ESG epochs, epochs with either bad ESG signal, bad EEG signal, or bad force were rejected during the analysis. Three EEG channels (C3, Cz, C4) and three ESG regions (ipsi-anterolateral, midline, and contra-anterolateral) were used for the analysis. The ML5 and ML6 ESG channels were selected as the midline region at the lower cervical level; LL8 and AL2 ESG channels were selected as the ipsi-anterolateral region at the lower cervical level; and LL7 and AL1 were selected as the contra-anterolateral region at the lower cervical level. The selected ESG channels were resampled to 1024 Hz.

Coherence for each combination of channels and each participant was estimated by applying spatial median to the auto-spectra and cross-spectra in 2-Hz frequency resolution (2Hz banded-coherence). The resultant was normalized with respect to the corresponding auto-spectra (Bista et al., 2023; Coffey et al., 2021).

6. Statistical Analysis

Statistical analyses were performed using MATLAB software (The MathWorks, 2023).

6.1. Parametric Statistics

Parametric statistics are based on the concept that the data follow a specific probability distribution, typically the normal distribution, with fixed parameters.

The Student t-test was applied in this study to determine whether there is a significant difference between the means of two groups (Student, 1908). It assesses whether observed differences are likely due to chance or reflect true underlying differences in populations. The test was operated under the assumption of normally distributed data and compared the

sample means to see if they differ significantly. In this study, the paired t-test was used to compare the same group under different conditions. A statistical significance was reached if the null hypothesis, of no significant difference between the groups, was rejected.

The t-test is a simple and widely used statistical method for comparing the means of two groups, offering flexibility for small sample sizes and clear interpretation of results. However, it assumes normality and (in some cases) equal variances, making it less reliable with non-normal data or outliers.

6.2. Non-Parametric Statistics

Non-parametric statistical tests offer several advantages for analyzing electrophysiological signals. They are robust to non-normal distributions and outliers, making them well-suited for the often complex and skewed nature of data, such as EEG, EMG or ESG. These tests can be effectively applied to small sample sizes, which is expected in the exploratory stage of development of a non-invasive spinal cord neuro-electro-imaging method. Non-parametric tests focus on medians rather than means, allowing for a more accurate representation of central tendency.

The Mann-Whitney U test, also known as the Wilcoxon unsigned rank test, was used as a non-parametric statistical method for independent samples (Mann and Whitney, 1947). It tested the null hypothesis that two samples come from identical distributions and can be viewed as a test of whether the medians of the two groups are equal. For paired observations, the Wilcoxon signed rank test served as the non-parametric equivalent to the paired t-test, assessing whether the median difference between paired samples was zero (Mann and Whitney, 1947). The Kruskal-Wallis test, a non-parametric alternative to one-way ANOVA, was applied when comparing more than two independent groups, allowing for the analysis of medians across multiple samples without assuming a normal distribution (Kruskal and Wallis, 1952).

6.3. Multiple Comparisons

When conducting studies that involve multiple comparisons, the likelihood of encountering errors increases as the number of inferences rises, which can lead to misleading conclusions if not properly managed. Two primary types of statistical errors must be considered: Type I errors, which occur when a null hypothesis is incorrectly rejected (false positives), and Type II errors, which happen when a null hypothesis is not rejected when it is false (false

negatives). As the number of tests increases, the chances of committing Type I errors also rise, necessitating robust correction methods.

To address this issue, various controlling procedures have been developed, one of the most well-known being the Bonferroni correction (Bonferroni, 1936). This method adjusts the significance threshold by dividing it by the number of comparisons and was applied in this project to reduce the probability of Type I errors. However, while effective, the Bonferroni correction can be overly conservative, leading to a higher risk of Type II errors, especially in studies with many comparisons. To account for this limitation, the Bonferroni correction was applied after a principal component analysis (PCA) which reduced dimensionality while maximising variances.

As an alternative approach, the False Discovery Rate (FDR) method was also performed (Benjamini and Hochberg, 1995). This procedure estimates the expected proportion of false discoveries among the rejected hypotheses, allowing for a more balanced control of Type I errors while maintaining higher statistical power. The FDR, implemented in the micompm toolbox, has been utilized in this project to correct for multiple comparisons effectively (Fachada and Rosa, 2018).

IV. Result: SC10X/U electrode placement system - description and demonstration

1. Introduction

Spinal cord (SC) is an integrated part of the sensorimotor nervous system, with a crucial role in forming the neural communication between the cortical/sub-cortical regions and periphery, which is underpinned by complex neural circuits within the spinal cord (Pierrot-Deseilligny and Burke, 2012). These neural circuits are responsible for transmitting and processing sensorimotor signals and for functional connections (Hochman, 2007). Although the SC plays an essential role in the communication of sensorimotor networks, neurophysiological and imaging studies have largely treated it as a wire that relays information between the brain and the peripherals, ignoring the neural complexity of the spinal cord. Similarly, neuroelectric imaging studies on sensorimotor communication have primarily focused on brain-muscle communication using Electroencephalography (EEG) – Electromyography (EMG) based analysis (Bao et al., 2021; Bista et al., 2023; Boonstra,

2013; Coffey et al., 2021; Conway et al., 1995); hence limiting our understanding of the neuro-electrophysiology of the role of spinal cord.

Non-invasive recording of spinal neural activity is necessary to assess and analyse the (dys)function of spinal cord in neurodegenerative diseases (e.g. Amyotrophic Lateral Sclerosis, ALS), other neurological conditions (e.g. stroke), as well as across different physiological conditions in healthy population at large. The use of spinal functional magnetic resonance imaging (fMRI) in spinal cord assessment is limited due to the structure and location of the spinal cord (Bede et al., 2012; Bede and Hardiman, 2014; Cohen-Adad, 2017). Non-invasive neuroelectric and neuro-electromagnetic imaging techniques provide high temporal resolution that is necessary for assessing the spinal cord function, communication, and fast neural oscillations (Pierrot-Deseilligny and Burke, 2012).

In earlier electrophysiological studies, the function of the spinal cord was assessed by recording evoked potentials at lower cervical vertebral levels in response to electrical stimulation (Cioni and Meglio, 1986; Desmedt and Huy, 1984; Fujimoto et al., 2001; Prestor et al., 1997; Shimoji et al., 1971). More specifically, both invasive (Ertekin, 1976; Insola et al., 2008; Jeanmonod et al., 1991; Kaneko et al., 1998; Prestor and Golob, 1999) and non-invasive (Emerson et al., 1984; Restuccia and Mauguière, 1991; Schabrun et al., 2012; Zanette et al., 1995) recording methodologies were used to record somatosensory evoked potentials (SEPs) at spinal level in response to peripheral nerve stimulation to either understand spinal (dys)function or develop clinical biomarkers. Non-invasive short latency cervical spinal SEP's at C6 vertebrae (Cv6) level were reported at average latencies of 9ms (P9), 11ms (N11), and 13ms (N13) from the posterior region (Crucchi et al., 2008; Mauguière, 2003a). The N13 potentials were reported to be generated by post-synaptic signalling in the dorsal horn of the spinal cord (Desmedt and Cheron, 1981; Jeanmonod et al., 1989; Urasaki et al., 1990).

The earlier electrophysiological studies have primarily relied only on a limited number of channels to record the spinal activity from surface or from epidural sites. In the early 1980s, ring electrode placement system (Desmedt and Huy, 1984; Emerson et al., 1984; Restuccia and Mauguière, 1991) was proposed to record spatial spinal evoked activity at a pre-selected cervical vertebral level. The ring electrode placement standardized the system for recording both posterior and anterior spinal activity from a particular cervical vertebra. This system still remains an active standard to record the neuro-electric activity from a select

lower cervical level that has been used in more recent studies (Bankim Subhash Chander et al., 2022).

Advancements in recording equipment have increased our capability to record neural spinal activity from large number of channels. This has led to high-density non-invasive recording of spinal activity becoming more common, where the high-density (HD) electrode montages increase the spatial resolution of recordings. Recent studies of HD-magnetospinography (MSG) (Akaza et al., 2020; Mardell et al., 2022; Sumiya et al., 2017) and “multi-channel electrospinography (ESG)” (Nierula et al., 2022) exploited multiple electrodes to record spinal activity, with the number of electrodes ranging from 16 to 44. Despite the rise of multi-channel spinal studies, there is no standardized electrode placement system for localizing the electrode space w.r.t. the spinal space. The absence of a recognized electrode placement standard has resulted in considerable variation in electrode locations across studies (Akaza et al., 2020; Mardell et al., 2022; Nierula et al., 2022).

In the late 1950s the need for a standardized electrode location for EEG recordings was recognized, and the 10-20 electrode system was proposed by Jasper, HH. (H, 1958). This later advanced to the 10-10 (Chatrian et al., 1985) and 10-5 system (Oostenveld and Praamstra, 2001) as the resolution of EEG recording increased (Jurcak et al., 2007). We believe that high-density ESG will substantially benefit from a recognized placement system: a standardized electrode placement system that allows for compatible, comparable, and reproducible methods across research groups, participant groups, and clinicians. Moreover, this should promote standard practices that can be combined across large-scale neurophysiological and clinical studies on neurological conditions.

In this paper, we propose a high-density electrode placement system for non-invasive recording of neuroelectric signals from cervical and upper thoracic spinal cord. The proposed electrode placement system (SC10-X/U) can incorporate up to 76 channels aimed at standardizing electrode space with respect to the spinal space. This system was realised using a flexible patch for holding the electrodes. Here, we also show how we tested and demonstrated the utility of the SC10-X/U placement system by recording and analysing the evoked spinal potentials in response to median nerve stimulation from a 64-channel HD-ESG montage derived from the new SC10X/U system.

2. The SC10-X/U Electrode System

There has been no recognized standard electrode placement system for recording electrical activity over the cervical and upper thoracic spinal cord. The description of the proposed SC10-X/U is explained in this section, following the statement of the design requirements and criteria. SC10-X/U system can incorporate up to 76 channel electrodes for non-invasively recording spinal activity.

We used the basic principles of the EEG 10-10 system and extended it to spinal cord space to design a high-density ESG electrode placement system, based on the rationale that these principles defined for the brain, are also applicable for the other neural mass in the central nervous system, i.e. SC. The principles include defining 2D representation of the region of interest (e.g., brain, spinal cord), finding reference anatomical landmarks, and dividing the 2D space in proportional distances. In the EEG 10-10 system the 2D space is represented as a circle; anatomical references include nasion, inion, left and right pre-auricular points (LPA and RPA); and the space is divided into 10% proportional distances. These principles are extended to the spinal space, resulting in the electrode placement for spinal space. The electrode placement system is named SC10X/U system (SC: Spinal Cord).

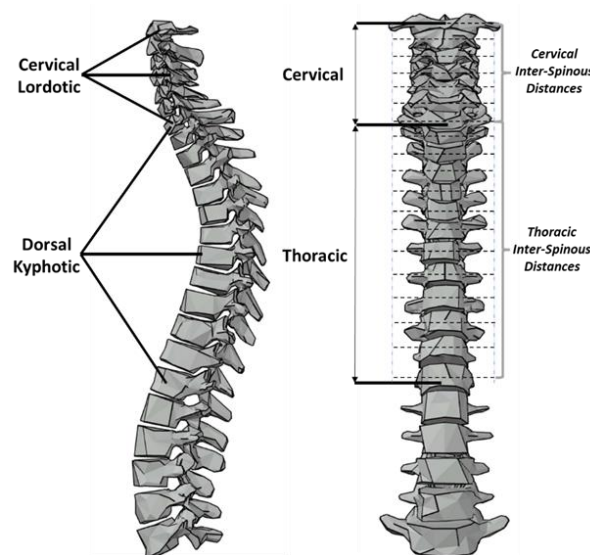


Figure 13: Spinal Cord model for the cervical and thoracic regions designed using AutoCAD. The left figure shows lordotic and kyphotic curvatures of the spinal cord. The right figure represents inter-vertebral spinous distances.

2.1. The Design Criteria and Principles of ESG electrode placement:

1. *Two-dimensional representation of the space:* The cervical-thoracic spinal space can be envisioned as two curved cylinders joined together. The cervical curvature (lordotic) and the thoracic curvature (kyphotic) can be characterized as concave and convex respectively, as shown in Figure 13. The 2D representation of the curved surface of the cylinders is described by a rectangle with curved top and bottom.

2. *Anatomical Landmarks:* A total of 16 anatomical references for representing cervical and upper thoracic spinal space were selected. These include the occipital protrusion, C2 spinous process, cervical (C5 and C6) and thoracic (T1) spinous processes, left and right pre-auricular points, transverse spinous process of atlas (left and right), carotid tubercles (left and right), root of scapula (left and right), intersection of transverse plane through C5 spinous process and sagittal plane passing through inferior angle of scapula (left and right). These anatomical points encompass the cervical and upper thoracic spinal space as shown in Figure 14.

3. *Division of 2D space:* The medial-transverse space is divided into 10 equal segments each accounting for 10% of the total lateral-medial-lateral distance, Figure 15. The medial-sagittal space along the length and direction of the spinal cord is divided in proportion to the inter-vertebrae spinous distances contained in the electrode space (Figure 13 shows the inter-vertebral spinous distances). The medial sagittal space encompassing the cervical spine region is divided in proportion to the average cervical intervertebral spinous distance relative to the total medial sagittal distance. Similarly, the space that encompasses the thoracic region is divided in proportion to the average thoracic intervertebral spinous distance relative to the total medial sagittal distance. The division of medial sagittal and transverse space is highlighted in Figure 15.

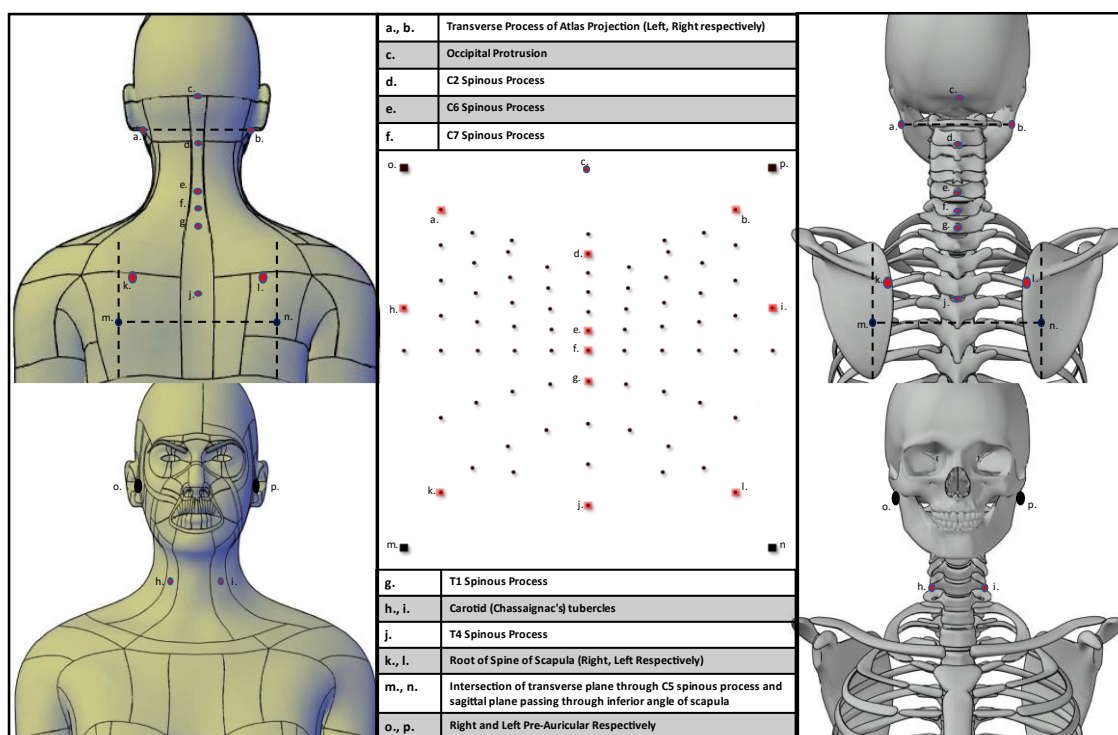


Figure 14: Anatomical References selected as surface landmarks are shown on the human model, skeleton model, and the SC10-X/U Electrode Placement System. The anatomical points encompassing spinal space are labeled from a. to p. and are shown over the surface for the human model (left), skeleton model (right), and electrode system (centre).

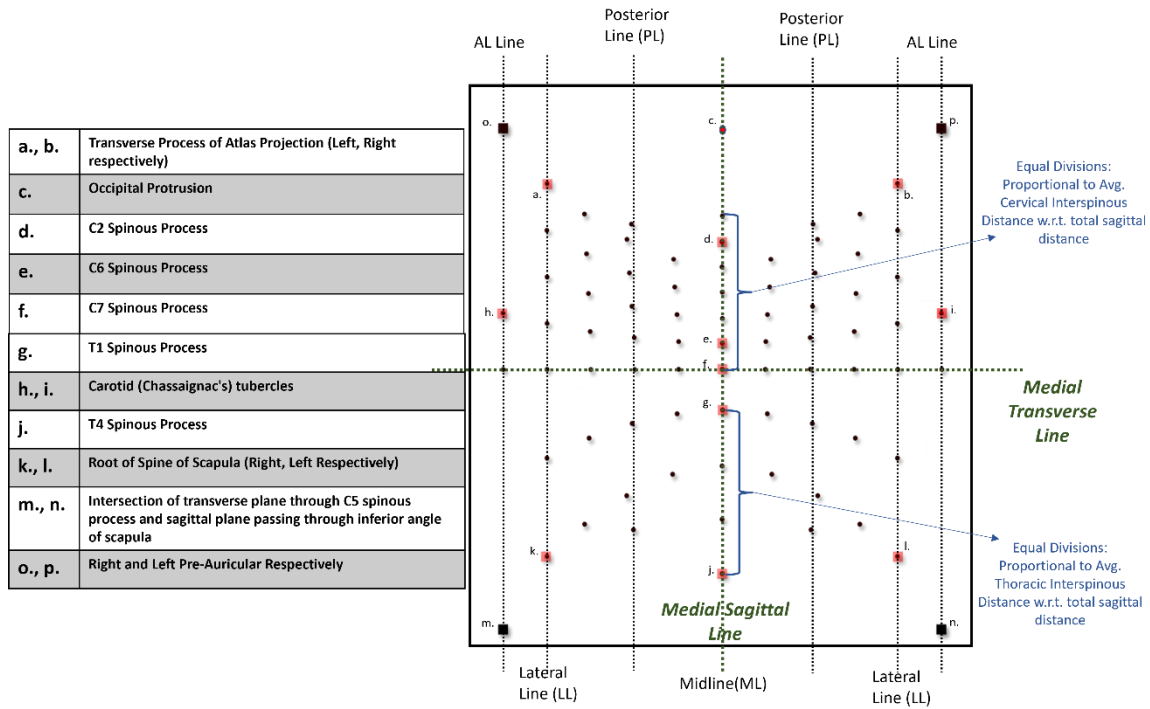


Figure 15: Electrode placement system (SC10-X/U) representing spinal space for high-density surface electrospinography (HD-ESG). The SC10-X/U system for the 76-electrode locations is shown along with anatomical reference points and spatial features, which are utilized for 2D space division.

2.2. The Design and Characteristics of the SC10-X/U Electrode Placement System

The electrode placement system shown in Figure 15 is designed to cover the cervical and upper thoracic spinal cord space for surface neuro-electro-physiological recordings. The boundary of the space is based upon the anatomical reference points to standardize the electrode placement system. The upper boundary points are defined by the right pre-auricular and left pre-auricular points (LPA and RPA). The bottom boundary points of thoracic region are defined by the intersection of transverse plane at T5 spinous level and sagittal planes passing through the inferior angle of scapula (marked as m. and n., Figure 14). The lateral end points of the cervical region are defined by left and right C6 anterior tubercles (marked as h. and i., Figure 14). The boundary is thereby defined by connecting the marked boundary points as shown in Figure 16.a. The transverse plane passing through the C7 spinous process is divided in 10% proportional segments (ten equidistance points) between left and right lateral ends as shown in Figure 15 and Figure 16.

A line is subsequently drawn along the C2, C7, T1, and T4 spinous processes to define the midline (ML). The midline running along the spinal cord is divided into ten segments

resulting in electrode positions labelled ML1 to ML11 (Figure 16.a). The inter-electrode distance between cervical midline electrodes (ML1-ML7) and upper thoracic midline electrodes (ML8-ML11) is in proportion to the average cervical inter-vertebral spinous distances and average thoracic inter-vertebral spinous distances relative to the total medial-sagittal distance (refer to figure 3 and figure 1). The midline between ML1 and ML7 spanning cervical vertebrae levels are divided in equal proportions corresponding to the average cervical inter-spinous distance. Similarly, ML8 and ML11 spanning upper thoracic vertebral levels are divided in equal proportions corresponding to the average thoracic inter-spinous distance (Figure 16.a). As a result, the defined electrode positions (ML1-ML11) along the midline roughly align with their respective vertebral levels.

Furthermore, the outer perimeter contour is defined by connecting LPA, RPA, ML1, ML11 position markers and AL lines (defined by connecting pre-auricular points, m., n., and C6 anterior tubercles on either side), encompassing the complete electrode space. The electrode positions one level below ML1, one level above ML11, and one level beside RPA and LPA on the lateral plane are marked as ML2, ML10, LL1, and LL2 respectively. This defines the inner-perimeter contour (Figure 16.a). The electrodes LL1 and LL2 reside at the level of the transverse process of the atlas (marked as a. and b., figure 2). The left and right lines of the inner- perimeter contour are defined as lateral lines (LL). The lateral line (LL) on either side of contour is divided into six segments, four equal segments on cervical side and two equal segments on the thoracic side. The top and bottom segment of the left contour connecting LL1 to ML2 and LL13 to ML10 respectively are divided in two halves each. Similar divisions are made on the right top and bottom segment connecting LL2 to ML2 and LL14 to ML10. This division result in ten divisions on each half of the inner- perimeter contour. The division of inner- perimeter contour define the electrode position markers, labelled LL1, PL1, ML2, PL2, LL2, LL4, LL6, LL8, LL10, LL12, LL14, PL18, ML10, PL17, LL13, LL11, LL9, LL7, LL5, and LL3 as shown in Figure 16.b.

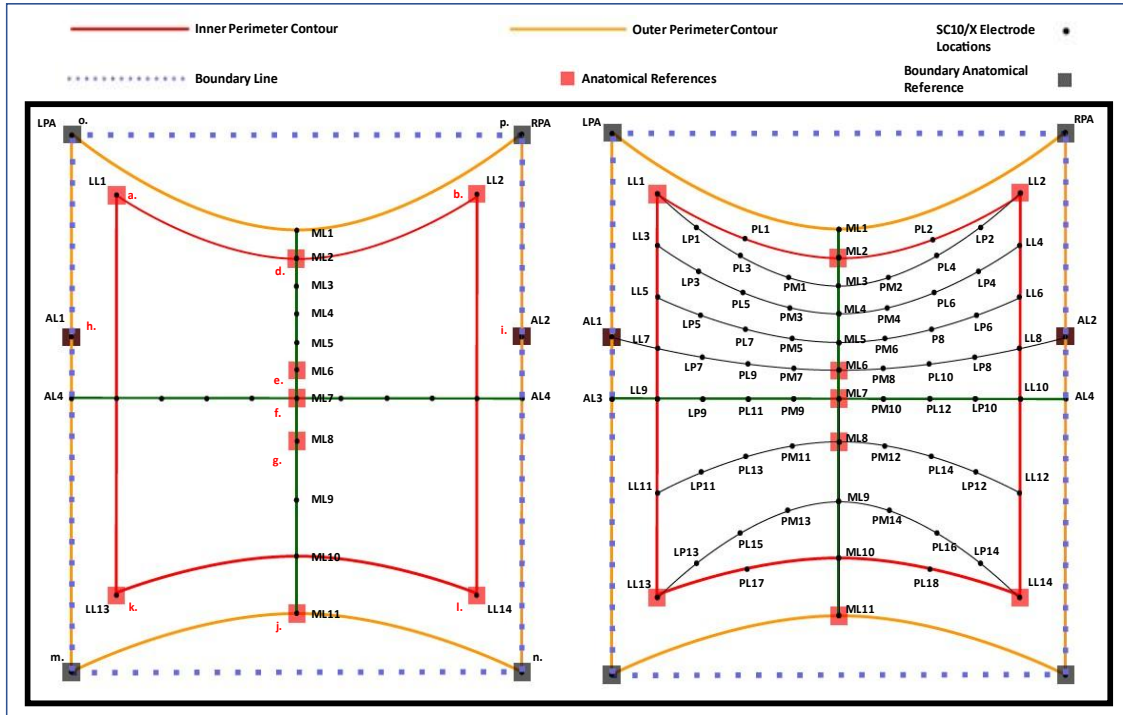


Figure 16: Design description of the SC10-X/U electrode placement system with electrode locations and labels (right). The left figure showcases the reference boundary, inner and outer perimeter contour. The right figure showcases all 76-electrode locations and labels. The labels are defined as, ML: Midline, PM: Posterior Midline, PL: Posterior Line, LP: Lateral Posterior, LL: Lateral Line, AL: Anterior Line

Finally, a transverse curve is defined by connecting LL1 to LL2 and passing through the midline at ML3 (LL1-ML3-LL2). The defined transverse curve is divided in 8 equal fractions, resulting in positions marked by LP1, PL3, PM1, PM2, PL4, and LP2 as shown in Figure 16.b. This is achieved by bisecting the curve twice on either side of the midline, dividing it into halves and quarters. Similar transverse curve are determined along the electrode markers LL3-ML4-LL4, LL5-ML5-LL6, LL7-ML6-LL8, LL9-ML7-LL10, LL11-ML8-LL12, and LL13-ML10-LL14 positions. These transverse curves are similarly divided in 1/8 fractions to define the remaining electrode position markers.

These steps define the resultant SC10-X/U electrode placement system (Figure 16b) determined to cover the spinal space encompassing cervical and upper thoracic spinal cord.

2.3. SC10-X/U Electrode Labels Nomenclature

The labels used in the SC10-X/U nomenclature for the electrode locations are defined by two uppercase letters followed by a number. The numbering system of electrode locations follows EEG convention where the locations on the left are odd-numbered and the locations on the right are even-numbered. The nomenclature of two uppercase letters is adopted to

differentiate SC10-X/U labels from EEG system labels that use single uppercase letter (optionally followed by a lowercase letter) to identify the electrode location. The label letters for the electrode positions (SC10-X/U) are defined based on the virtual vertical lines (figure 3) dividing the electrode space, symmetrical on either side of the spinal cord. The electrodes are sequentially numbered for each line. Sequence of odd numbers for lines that reside on the left side, sequence of even numbers for lines that reside on the right side, and sequence of consecutive numbers for lines that reside in the center.

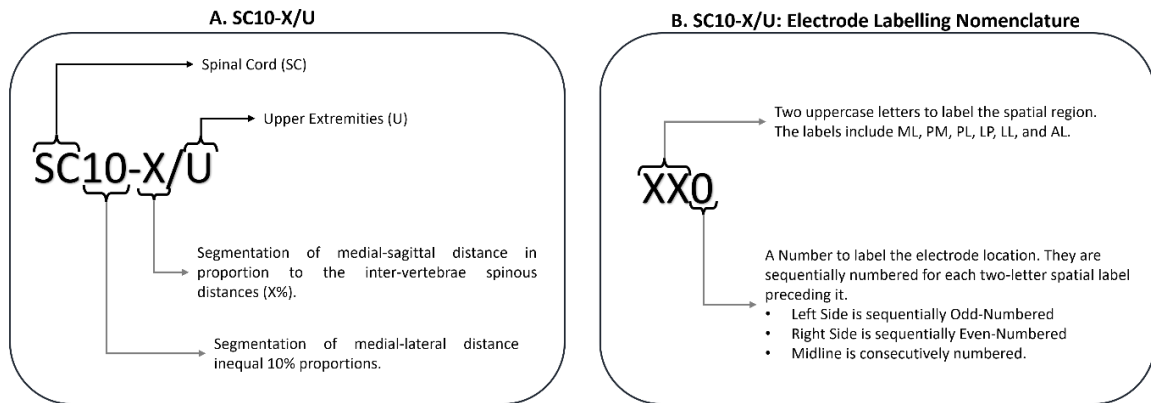


Figure 17: A. The SC10-X/U electrode placement system acronym. B. The SC10-X/U electrode location labelling nomenclature.

The line that traces the anatomical midline of the spinal cord is defined as midline (ML) and encompasses the reference spinous processes; the label ML is used for the electrode locations on the midline. The virtual line named posterior line (PL) is defined by the electrode locations that divides the transverse curves in half on either side, label PL is used for the electrode locations on the posterior line. The virtual line that is defined along the left and right boundary of the inner-perimeter contour is referred to as lateral line (LL); hence, the label LL is used for the electrode locations on the lateral line. Label PM is used for the locations that reside between the posterior line and midline and label LP is used for the locations that reside between the lateral line and posterior line. These labels are defined by the combination of the letters from adjacent lines, where the first letter signifies the adjacent lateral line, and the second letter is defined by the adjacent medial line. Finally, the label AL is used to define the electrode locations that rest on AL and are in the region of C6 anterior tubercles.

2.4. Implementation of SC10-X/U using an Elastic Electrode Patch

An in-house non-invasive data recording elastic electrode patch was developed to realise the SC10-X/U electrode placement system. The electrode patch is designed to record spinal electrophysiological signals over spinal space covering cervical (neck) and upper thoracic (upper back) region up to T4 level. The designed elastic electrode patch can hold up to 76-channel electrodes for recording high-density electrospinography (ESG).

The electrode patch was realized in six sizes to incorporate the anthropometric (inter-individual) spinal cord length variability. The average inter-cervical spinous distance for each electrode patch is determined based upon the study by Ernst, M. J., et al. (Ernst et al., 2019); average inter thoracic spinous distance is based upon an earlier study (Ernst et al., 2013). The electrode patch was developed to offer: flexibility to accommodate for curvature around neck, shoulder, and back; vertical, and horizontal stretchability to accommodate inter-individual height and width variability; and comfort for long recording sessions.

The developed electrode patch is shown in Figure 18. The extended lateral fins are used to hold the patch firmly against the neck (corresponding to the C4-C7 cervical spinal cord) using Velcro. The top part of the patch is held firmly against the participant by elastic bands around the head and ears, while elastic suspenders are used to hold the electrode patch against the upper back (corresponding to the thoracic spinal cord). The electrode patch placed over the spinal cord of a participant can be seen in Figure 18.

3. Demonstration in Experiments: **Methodology**

3.1. Participant and Ethics

Healthy participants ($n=10$, age: 28 ± 4 , 4 Males) were recruited to record ESG, EMG, and ECG signals during the experimental paradigm of peripheral nerve stimulation. The experiment protocol was explained to the participants and an informed consent (signed) was taken before the recording. Ethical approval was obtained from Tallaght Hospital/St. James's Hospital Joint Research Ethics Committee for St. James's Hospital, Dublin, Ireland (Project Reference: 0493).

3.2. Participant Exclusion Criteria

The exclusion criteria for this study include:

- (i) History of neuromuscular, neurological, or active psychiatric disease.
- (ii) Diagnosis of chronic cervical back pain or any other neck or upper back muscle stiffness, wear, and tear.
- (iv) Recent history of severe backpain, suffering with spondylosis or spondylitis or any other severe back pain / spinal degeneration.

3.3. Experimental Design

The experimental part of the study was observational and aimed to observe spinal potentials in response to peripheral stimulation, using multimodal technique involving Electrospinogram (ESG). ESG and multi-channel Electrocardiogram (ECG) signals.

The signals were acquired with no randomization or blinding since signals cannot be influenced by the experimenter or the data collection/analysis process. The participants were instructed to keep their eyes closed during the experiment.

a. Experimental Paradigm

The experiment paradigm sought to record the spatial spinal activity evoked in response to the peripheral nerve stimulation. During the experiment, the participant laid in prone position on a physio-therapy chair and multimodal signals were recorded during median nerve stimulation. While lying in the prone position, the participant's hand rested in a comfortable position and made approximately 30° angle between the body and the arm, as shown in Figure 10.

The experiment consisted of a single session of median nerve stimulation, the session lasted for about 20 mins. Each session consisted of 35 blocks, each block lasting for 30 seconds. Each block consisted of 20 seconds of stimulation phase followed by 10 seconds of rest phase. In the stimulation phase, a train of stimulation pulses at an average frequency of 2Hz were given to the participant. The stimulation intensity was set to 1.5 times the motor threshold and the pulse width was set to 200µs. The motor threshold was defined as the minimum intensity at which prominent thumb movement was observed with consistency. In the session, a total of 1400 trial responses (35 block x 40 stimulations per block) to peripheral stimulation were recorded.

Peripheral Nerve Stimulation: Constant current stimulator DS7A (Digitimer Ltd., UK) was used for median nerve stimulation at the wrist. DS7A is MDD CE medically certified and can provide up to 100mA constant current high voltage pulses.

b. Data acquisition

The data collection system consisted of a high-density biosignal amplifier that was used for high density ESG, and multi-channel ECG. The electrical activity of 64 (ESG) + 5 (ECG) + 8 (EXG) channels were recorded simultaneously using high-speed Biosemi-ActiveTwo system (Biosemi B.V., Amsterdam, The Netherlands) at a sampling frequency of 8 kHz, with a bandpass filter over the range of 0Hz - 1600Hz. Recent studies on spinal cord activity using Magnetospinography (MSG) (Adachi et al., 2013; Akaza et al., 2020; Sumiya et al., 2017) have shown that high sampling frequency is required to process the signal condition across the spinal cord for inter-electrode distance of less than 2 – 4 cm. All electrode channels were visually inspected for signal quality prior to recording and the electrode offset was kept between $\pm 25\text{mV}$.

ESG: The ESG signals were recorded using 64-channel high-density active electrode system. The ESG electrode placement was based upon the SC10-X/U electrode system (Figure 18a). The active electrodes were connected to the electrode holder and electrolyte gel was used to enhance skin-electrode connection.

ECG: ECG was recorded in accordance with 5-electrode ECG system with flat active sintered Ag-AgCl electrodes.

EXG: Eight external channels were connected using flat active sintered Ag-AgCl electrodes. The position of external electrodes were— (i) left and right earlobe, (ii) to the left of left eye and to the right of right eye, (iii) left and right clavicle, (iv) below left eye, (v) Erb's point (brachial plexus).

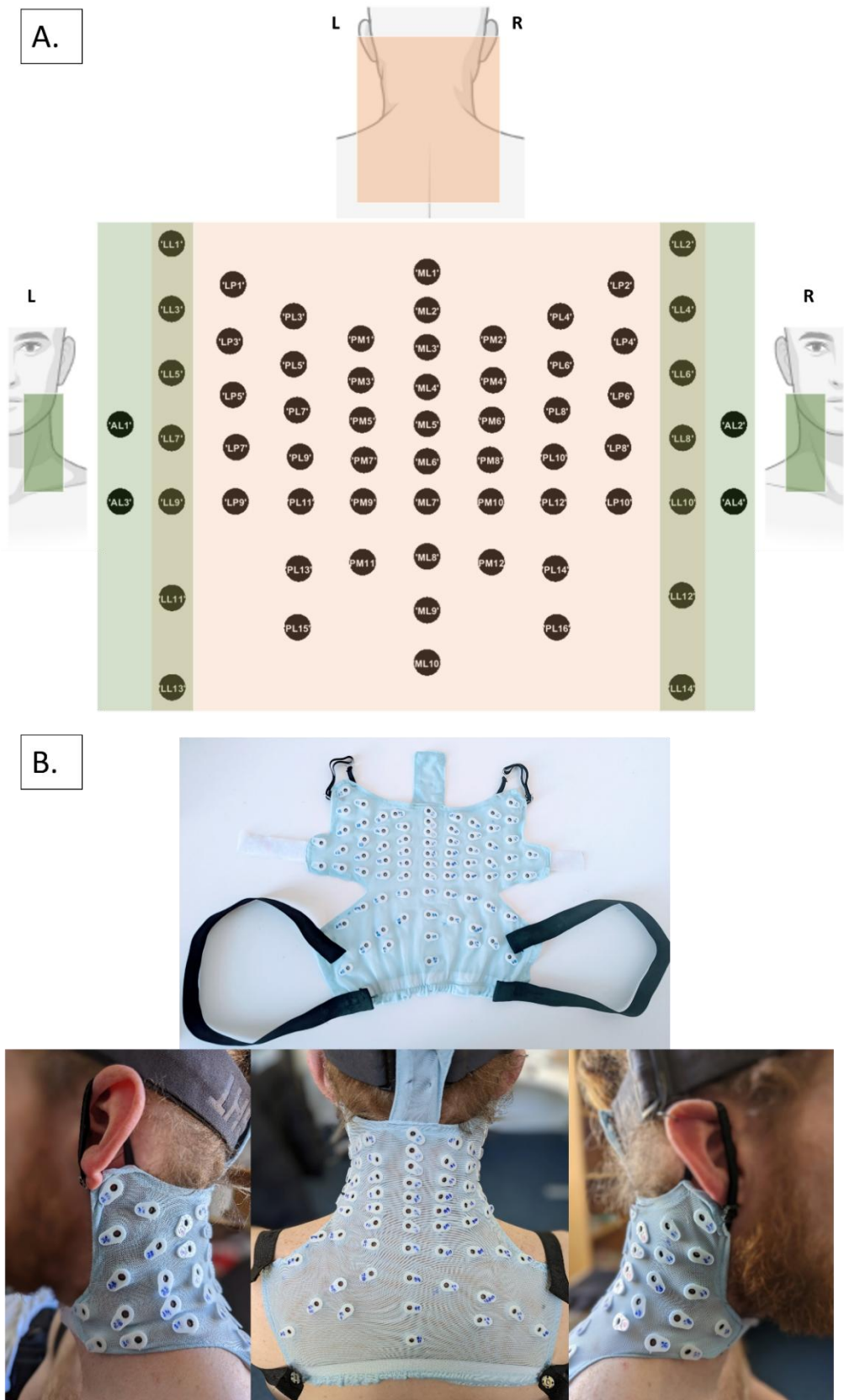


Figure 18: In-house developed electrode patch based upon the SC10-X/U electrode system. .a. The 64-electrode channels used for the recording and its corresponding electrode space. .b. The electrode patch (up

to 76 electrode channels) placed over the surface of the cervical and upper thoracic spinal cord space of a participant.

3.4. Data Analysis

a. Signal pre-processing

Two main contaminating artifacts were identified in the recorded ESG signals following the visual inspection and preliminary analysis: stimulation and ECG artifacts. The stimulation artifact lasted approximately 1-2ms; this artifact was identified and the signal during this period was interpolated using piecewise cubic interpolation. ECG artifact from the ESG signal was removed by applying canonical correlation analysis (CCA) (de Cheveigné et al., 2018; Hotelling, 1936). This was achieved by isolating and removing the two canonical components that represented the highest correlation between ESG data (64 channels) and ECG leads (7 leads). First two canonical components (CC) were selected for removal, as the correlation (r) score after first two CC's demonstrated sharp decrease in magnitude.

The remaining CC's were back-transformed to the original space to provide the denoised ESG signal. The signal post artifact rejection was re-referenced using 'common average referencing'. The re-referenced signals were baseline corrected and segmented into epochs of 0.5 seconds, starting 0.1s before the stimulation until 0.4s after the stimulation. Before further processing of the signal, bad channels were visually determined and interpolated (weighted average) based on the signal from neighbouring channels (neighbour distance $\leq 50\text{mm}$). The epoched trials were visually inspected and the bad trials were rejected. The remaining trials were bandpass filtered between 10 and 1500 Hz (Akaza et al., 2020), dual-pass Butterworth filter.

b. Event-Locked Activity

The pre-processed epochs from the ESG channels underwent time-locked analysis. The epochs were averaged over all the good trials. Surface Laplacian referencing, based on Hjorth's method (Carvalhaes and de Barros, 2015; Hjorth, 1975) was applied to the averaged signals. The resulting signals were analyzed to identify characteristics of the event-locked (time-phase locked) evoked activity and spatial distribution of the evoked activity.

c. Statistical Analysis

Statistically significant difference from zero of the evoked response was identified at each time of the recorded signal across all participants, using Wilcoxon signed-rank non-parametric test (Iyer et al., 2017; Nasseroleislami et al., 2014). To account for multiple comparisons in 64 channels and several time points (-0.001s – 0.03s), we retained the dominant principal components that explain 85% of the total variance, plus an additional component. Bonferroni correction was subsequently applied at $\alpha = 0.01$.

4. Demonstration of HD-Electrospinography: Results

High-density electrospinography using non-invasive 64-channel surface electrodes was used to record spinal activity in response to median nerve stimulation. The event-locked activity was evaluated for the recorded signals.

4.1. Average Evoked Potential

The grand mean of evoked potentials across all participants was calculated for each electrode channel. Average (across trials) evoked potential for individual participants and the grand-mean signal across participants at channel ML6 (C6 vertebral level) are shown in Figure 19 (respectively in grey and black). Significant evoked potential peak P9 and N13 were recorded at ML6 (C6 cervical level); marked in red in Figure 19. Observed latency of the evoked potential N13 and P9 across individual participants was $13.2 \pm 1.1\text{ms}$ (mean $\pm$ sd) and $9.7 \pm 1.2\text{ms}$ (mean $\pm$ sd) respectively.

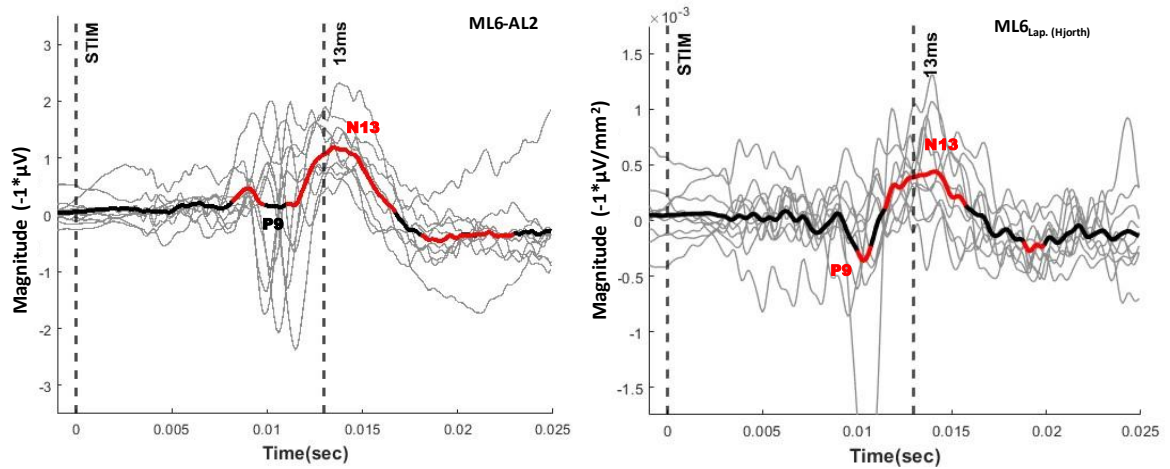


Figure 19: Grand-mean of the evoked potentials across all participants at ML6 electrode. Grey lines represent individual participant evoked potentials, and thick black line correspond to the grand mean evoked potential

across all participants. The duration of significant evoked response is highlighted by red lines. The figure indicates significant N13 and P9 evoked potentials. 6a. ML6 response in reference to AL2 electrode (left). 6b. surface Laplacian re-referenced ML6 response (right).

Traditionally, ring electrode placement (Restuccia and Mauguière, 1991) at C6 spinal level is used to record spinal evoked potential in response to median nerve stimulation. Electrode channels AL1, LL7, LP7, PL9, PM7, ML6, PM8, PL8, LP8, LL8, and AL2 from SC10-X/U system are comparable to the traditional ring electrodes. The grand mean signals for the channels used to define the ring electrodes system is shown in Figure 20. In Figure 20, evoked potential at 13ms has highest amplitude at channel ML6 and this response reverses its polarity at the anterior-lateral electrode channel (AL2).

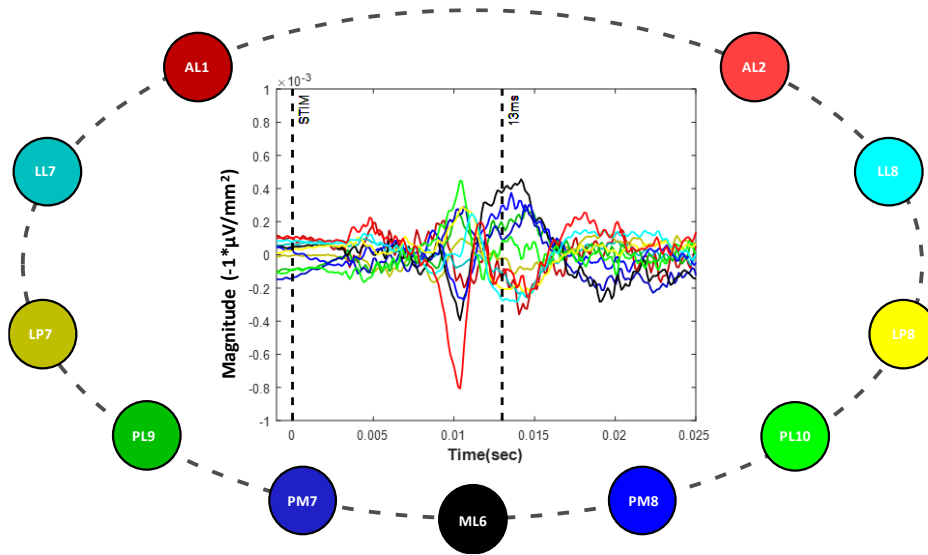


Figure 20: Grand-mean signals plotted for SC10-X/U channels at Cv6 compatible with ring electrode placement system at Cv6. (Surface Laplacian re-referenced)

4.2. Topographic Map of Evoked Spinal Potentials

High density spinal activity recorded using the SC10X/U electrode placement system was used to map the spatial distribution of the electrical activity across the spinal space. The topographical plot of grand-mean spinal evoked response during the time frame 12-14ms (N13) after the median nerve stimulation is shown in Figure 21. The region around midline indicates higher spinal response activity with its epicentre around the ML4-ML6 channels. Another epicentre of spinal response with reversed polarity is also indicated in Figure 21 near lower cervical right-anterior-lateral and right-lateral region.

The grand-mean response of the midline electrodes in the cervical region (ML2-ML7) can also be seen in Figure 21. The lower cervical level channels on the right side of the Topography plot (Figure 21) indicate reversed polarity of the evoked potentials in the region.

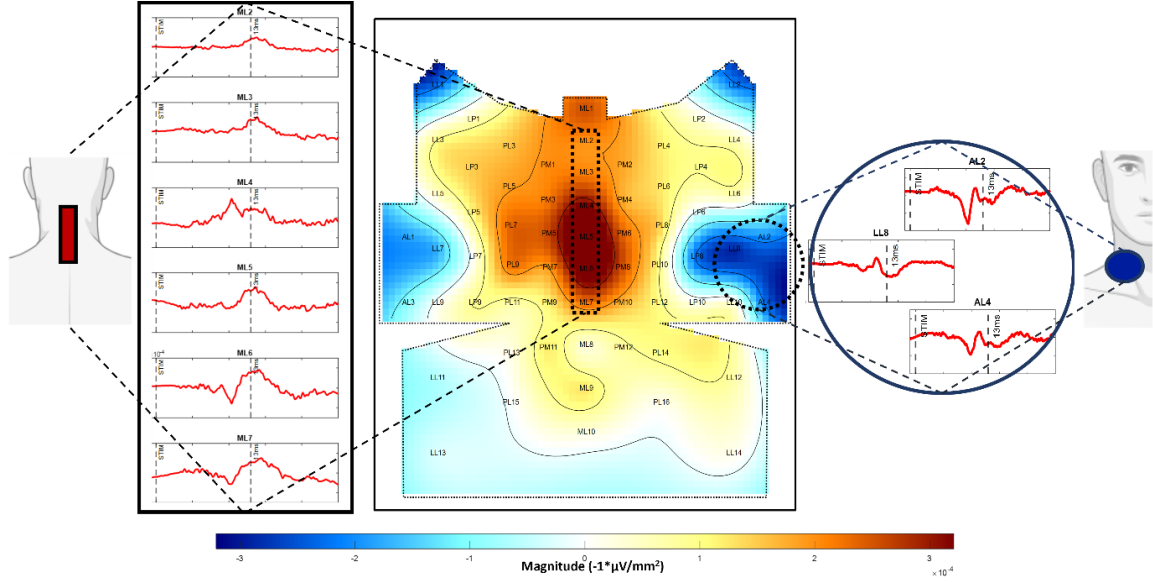


Figure 21: **Topographic Map of Evoked Spinal Potentials.** Centre Figure: Topographic Map of the grand-mean spinal evoked response over the SC10-X/U sensor space, time-window (12ms-14ms). Left figure: corresponds to the spinal activity recorded from channels ML2-ML7 (midline, posterior) for the time frame between 0.001s before stimulation and 0.025s after stimulation. Right figure corresponds to the spinal activity recorded from lateral-anterior channels for the time frame between 0.001s before stimulation and 0.025s after stimulation.

5. Discussion

Recent studies have utilized non-standardized high-density recordings for estimating spinal signals in response to external stimulation (Akaza et al., 2020; Mardell et al., 2022; Sumiya et al., 2017) and to develop spatial filters for accessing single trial evoked potentials (Nierula et al., 2022). These studies indicate the potential benefits of high-density (HD) spinographic techniques over non-HD recordings. In this study, an electrode placement system (SC10-X/U) was designed and validated for high-density surface ESG recording. The proposed system maps the cervical and upper thoracic region to the electrode space for non-invasive neuroelectric imaging and can incorporate up to 76 channels as shown in Figure 15. This is especially suitable for the sensorimotor studies of the upper extremities in health and disease.

SC10-X/U electrode placement system aims at the standardization of future studies, leading to compatible, comparable, and reproducible methods across research groups, participant groups, and clinicians. EEG electrode placement systems (10/20, 10/10, 10/5) have proven to be an invaluable tool in the field of Neuro-electro-physiology, enabling standardized electrophysiology research resulting in applications such as biomarkers (de Aguiar Neto and Rosa, 2019; McMackin et al., 2019), brain-computer interface (Lotte et al., 2007; Zhang et al., 2018), resting-state analyses (Dukic et al., 2022; Metzger et al., 2023). Similarly, standardized high-density electrospinography presents huge potential in uncovering the role of spinal cord in central nervous system and move away from treating SC as a “wire” relay between brain and periphery.

The SC10-X/U system was demonstrated using an in-house 64 channel electrode patch based on the proposed system to record evoked spinal activity in response to the median nerve stimulation. Short latency SEP's P9, and N13 were recorded as shown in Figure 19, and corroborate previous studies (Cruccu et al., 2008; Mauguière, 2003a) with consistent SEP measures and the anatomically valid location of responses representative by the corresponding dermatome (Cramer et al., 2014). Posterior cervical channels showed negative evoked response around 13ms (N13) after stimulation. The N13 potential is thought to be generated by the dorsal horn and to have a post-synaptic (Desmedt and Cheron, 1981; Jeanmonod et al., 1989; Urasaki et al., 1990). We also observed reversed polarity of N13 response when moving from the posterior (ML6) to right-anterior electrodes (AL2) at an approximate C6 vertebral level, as demonstrated in previous studies (Desmedt and Huy, 1984; Restuccia and Mauguière, 1991).

The spatial distribution of the spinal activity was mapped using the HD electrode channels. The topographic map indicated that high cervical spinal activity is localized around midline with its epicenter at ML4-ML6 level (Figure 21). This epicentre could correspond to fibres from roots of spinal nerves C5-C7, innervation of which is contained in the median nerve. Moreover, high activity epicenter was also seen around anterior and lateral region at lower cervical levels, through the polarity is reversed. The observed evoked potentials and their characteristics are compatible with the existing literature (Cruccu et al., 2008; Mauguière, 2003a). The results from previous studies can easily be extended and interpreted using the comparable electrode locations from the proposed SC10-X/U system. Here, we demonstrated in Figure 20 that the evoked activity recorded at the selected electrode

channels are comparable to the evoked activity reported in studies using ring placement electrode system.

The future direction of studies will be to analyze the evoked responses using combined spatial, spectral, and temporal techniques. Source localization techniques (Akaza et al., 2020; Sumiya et al., 2017) will improve the understanding of the functioning of spinal activity and allow to further investigate the dysfunction of spinal cord in neurological conditions.

6. Conclusion

The SC10-X/U electrode system described in this paper provides full coverage of the cervical and upper thoracic region by closely and evenly spaced electrodes applied at standardized locations w.r.t. accessible anatomical locations. The system can incorporate up to 76 electrode positions, certain studies may require only a subset of electrodes for covering the required spinal space. The utilization of this system in studies of spontaneous and evoked ESG activities is expected to facilitate compatibility and reproducible research. In theory, information derived from the surface-recorded ESG could be projected onto anatomical space and communicated through the use of a standardized atlas across different research and participants groups, promoting identification of sources in spinal cord. This should promote standard practices across large-scale neurophysiological and clinical studies of neurological conditions and healthy populations.

V. Result: Spinal neural oscillatory characteristics and cortico-spinal coherence during motor control

1. Introduction

Understanding the neurophysiology behind motor control is essential for evaluating (dys)function in health and disease, leading to better care for people with motor disabilities. Non-invasive electrophysiology and neuroimaging techniques have played a major role in uncovering the neural basis for motor control. The spinal cord (SC) has been largely neglected and treated as a passive relay between the brain and periphery despite being an essential component of motor control and sensorimotor integration (Nielsen, 2004; Pierrot-Deseilligny and Burke, 2012; Wolpert et al., 2011). The spinal neuronal circuits are

responsible for transmitting and processing of sensorimotor signals and brain-spine-muscle functional connection (Hochman, 2007), highlighting the importance of spinal neurophysiology. The need to understand the role of the SC in movement is further exemplified by recent studies indicating that the spinal cord plays a role in motor learning (Brüel et al., 2024; Vahdat et al., 2015) and has modulatory effects during sensorimotor communication (Moreno-López et al., 2016; Stachowski and Dougherty, 2021).

Non-invasive recording of spinal activity presents significant challenges due to the spinal cord's complex structure and location (Bede et al., 2012; Nawal Kinany et al., 2023), making it extremely difficult to record fMRI or neurophysiological signals from SC. Previously, electrophysiology techniques like transcranial magnetic stimulation and peripheral stimulation have been used to assess the upper motor neuronal and lower motor neuronal spinal (dys)function respectively (de Carvalho and Swash, 2016; Fatehi et al., 2018; Fernández, 2021; Šoda et al., 2023). These indirect methods though helpful largely neglect the neural complexities of the SC and remain dependent on stimulation-based techniques, limiting their effectiveness in understanding the complex interactions between sensory afferents and motor efferents during daily motor-control tasks. Functional connectivity measures like cortico-muscular coherence (CMC) have been utilized to evaluate the topographical and frequency-dependent interactions between brain and muscle (Bao et al., 2021; Bista et al., 2023; Coffey et al., 2021; Conway et al., 1995) during motor-control tasks. These studies evaluated the corticospinal tract integrity by highlighting the functional coupling between the brain and periphery have largely left out the crucial role of the spinal cord.

Supraspinal studies (Baker, 2007; Muthukumaraswamy, 2010; Pfurtscheller et al., 2003) during motor activity have highlighted the frequency band-dependent role of neuronal signaling during a motor control task. Frequency bands including beta, low-gamma, and high-gamma have been reported during motor activity. These frequency-band activities have demonstrated specific spatial and temporal characteristics during isometric contraction (Crone et al., 1998a, 1998b). Previous cortical electrophysiology studies have highlighted that different frequency-banded neurophysiology signals might indicate an indirect or direct representation of distinct neural functionality (Engel and Fries, 2010; Kilavik et al., 2013; Nowak et al., 2018; Ulloa, 2022). The beta band activity has been observed in a generalized spatial area spanning the contralateral sensorimotor cortex and ipsilateral primary motor cortex (Baker, 2007; Chakarov et al., 2009; Engel and Fries, 2010;

Kilner et al., 1999) during isometric contraction and has been broadly associated with descending pathways. The low-gamma band activity has a more localized activation pattern over sensorimotor areas, featured around the anterior and posterior central sulcus (Pfurtscheller et al., 2003; Szurhaj et al., 2005). This activity has been associated with sensory afference and sensorimotor integration (Aoki et al., 1999; Ihara et al., 2003). The high-gamma band is observed to be both spatially and temporally localized during isometric contraction, the high-gamma activation is majorly observed during the onset of motor activity and has been associated with information processing (Ball et al., 2009, 2008; Muthukumaraswamy, 2010). These supraspinal studies indicate spatial, spectral, and temporal neuronal characteristics highlighting distinct neurophysiological functionalities during motor control.

In our study, we aim to investigate spectral, spatial, and temporal characteristics of spinal electrical activity and its interaction with brain activity during isometric contraction and in response to median nerve stimulation. To our knowledge, this is the first study to investigate the complex spatial-temporal-spectral spinal activation during motor activity using non-invasive electrophysiology. In recent years, advancements have been made in recording high-resolution spinal neurophysiology signals (Akaza et al., 2020; Mardell et al., 2022; Nierula et al., 2022; Sumiya et al., 2017) using multi-electrode systems. Due to the challenging location of SC, these high-resolution systems have only been utilized to study evoked spinal activity in response to peripheral stimulation. Here we utilized novel task design and analysis techniques to investigate spinal activation characteristics and its coherence with the brain during isometric contraction using high-density electrospinography (ESG) and high-density EEG recording.

2. Methods

2.1. Participant and Ethics

Ten healthy participants (n=10, age: 28 ± 4 , 4 Males) were recruited to record multimodal (ESG, EEG, ECG) electrophysiology signals during the experiment. All participants were informed about the experiment paradigm and signed consent was obtained before the recording session. The ethics for the study was approved by ‘Tallaght Hospital/St. James’s Hospital Joint Research Ethics Committee, Project Reference: 0493, and the study was performed in accordance with the declaration of Helsinki.

2.2. Participant Exclusion Criteria

The exclusion criteria are as follows:

1. No history of neuromuscular, neurological, and or psychiatric condition.
2. No diagnosed chronic cervical and upper back condition (affecting spinal nerves, and or muscles)
3. No history of spondylosis or spondylitis or any other back pain / spinal degeneration.

2.3. Experimental Paradigm

This observational study recorded multimodal signals during two different experimental paradigms, voluntary motor task paradigm, and peripheral nerve stimulation paradigm. The participants performed voluntary task experiment which was followed by peripheral nerve stimulation paradigm. During the voluntary task, participants were instructed to keep their eyes open. However, during the peripheral nerve stimulation, they were instructed to keep their eyes closed.

a. Voluntary Motor Task

The voluntary motor task experimental paradigm is described in detail in the section III.3.2.c In brief, the participant performed isometric pincer-grip contraction during the voluntary task paradigm. The task was performed using a force sensor block held between the thumb and the index finger of their right hand (Figure 22c). During this experiment paradigm the participant performed 30 sessions of the visually guided voluntary task. Each session lasted around 15 seconds and consisted of three guided phases; rest phase (5s) followed by isometric contraction phase (5s) followed by relaxation phase (5s). During the isometric contraction phase, the participant maintained a pincer grip force at 10% of their maximum voluntary contraction (MVC). This was visually guided by displaying fixed target force and real-time applied-force feedback on the screen (Figure 22c). The MVC was determined at the start of the paradigm as the average peak force during three short maximal contractions trials (3s each) with 30s inter-trial rest period.

b. Peripheral Nerve Stimulation

The peripheral nerve stimulation experiment paradigm is described in detail in the section III.3.2.a In brief, the peripheral nerve stimulation paradigm consisted of 35 sessions of

median nerve stimulations performed on the wrist at an intensity of 1.5 times the motor threshold and 200 μ s pulse width. Each session lasted for 30 seconds; 20 seconds of the stimulation phase followed by 10 seconds of rest phase. In the stimulation phase, the median nerve was stimulated by a train of pulses at an average frequency of 2Hz, producing 40 stimulation responses per session. During this paradigm, a total of 1400 responses (35 sessions x 40 trials per block) to peripheral stimulation were recorded.



Figure 22: A. The setup with the physio-chair and 10.1-inch screen that was used during the experiment. B. A participant sitting in an inclined position on the physio-chair. The participant's chest was supported by the chest rest while their head rested on the headrest and hands rested on the handrest respectively. C. The photo demonstrates the hand position during the voluntary motor task. The photo also showcases the force sensor system and the 10.1-inch screen used during the task, from the perspective of the participant (photo taken through headrest).

2.4. Experiment Setup

The participant sat comfortably in a physio-chair during the length of the experiment as shown in Figure 22b. The participant's head rested comfortably on the headrest of the physio-chair and the hand rested on the handrest while making a 90-degree angle at the elbow (Figure 22b).

Peripheral Nerve Stimulation: Constant current stimulator DS7A was used for median nerve stimulation at the wrist. DS7A is MDD CE medically certified and can provide up to 100mA constant current high voltage pulses.

2.5. Data Acquisition

A total of 210 channels of data were recorded simultaneously using the high-speed Biosemi-ActiveTwo System (Biosemi B.V., Amsterdam, The Netherlands) at a sampling frequency of 8kHz. This system was employed to collect high-density EEG, high-resolution ESG, ECG, and ExG signals. Visual inspection was conducted on all EEG and ESG channels, electrode offset maintained between $\pm 20\text{mV}$ and $\pm 25\text{mV}$ respectively. The data acquisition protocol and the electrode positions for ESG, EEG, ECG, and EXG are explained in section III.1.2. The Figure 23 showcases HD-EEG and HD-ESG electrode positioning over the participant's head, neck, and upper back.



Figure 23: The HD-EEG and HD-ESG electrodes positioned over the participant's head, neck, and upper back. The photos highlight the electrode locations used for recording the HD-EEG and HD-ESG signals during the experiment paradigms.

2.6. Signal Processing

a. ESG Processing

I. Pre-Processing

The stimulation artifact recorded during peripheral stimulation paradigm lasted 1-2ms after stimulation trigger and was removed by interpolating (piecewise cubic interpolation) the signal between 0.5ms before the stimulation trigger and 2.5ms after stimulation. The stimulation artifact removal process is described in the section III.4.1.

The ESG preprocessing pipeline explained in section III.4.2. was implemented on the ESG signals recorded during the voluntary motor task paradigm as well as on the stimulation artifact removed ESG signals recorded during the peripheral stimulation paradigm. In brief, bad channels were visually detected and removed. Canonical component analysis (CCA) was utilized to remove ECG artifacts from the remaining ESG channels. The bad channels were interpolated. The resulting ESG signals were re-referenced using ‘common average referencing’. A zero-phase comb-notch filter was then used to remove 50Hz harmonic line noise.

The pre-processed ESG signals during the peripheral stimulation paradigm were segmented into 1400 trials of 0.5 seconds, -0.1 seconds before stimulation and 0.4 seconds after stimulation. The pre-processed ESG signals during voluntary motor task were segmented into 30 trials of 15 seconds, starting 3 seconds before the start of the contraction phase till 7 seconds after the end of the contraction phase. The resulting trials for each paradigm were visually and automatically inspected for bad trials. The identified bad trials for each paradigm were rejected. The remaining trials were bandpass filtered between 2Hz - 250Hz and 10Hz - 1500Hz (Akaza et al., 2020) for voluntary task ESG and peripheral stimulation ESG signals respectively.

II. Spatial Filtering

The pre-processed trials during the peripheral stimulation paradigm were subjected to task-related component analysis (TRCA) (Nakanishi et al., 2018; Tanaka et al., 2013). The design of the full-band TRCA-spatial filter is described in the section III.5.1.a

The trials during the peripheral nerve stimulation paradigm were TRCA-spatial filtered. During TRCA-spatial filtering, the signal was transformed using task-related component analysis, and the non-significant components were removed. The remaining components were back-transformed to the original ESG space, resulting in a TRCA-spatial filtered signal. TRCA-spatial filtering resulted in an improved signal-to-noise ratio where the task-relevant spinal signals were retained, and the noise was removed. The trials during the voluntary motor task paradigms were also TRCA-spatially filtered

using the spatial filter obtained using the evoked spinal signals during the stimulation paradigm. This resulted in increased quality of spatially-relevant signals while removing the irrelevant noise.

The signals obtained after TRCA-spatial filtering for both paradigms were re-referenced using the surface Laplacian method (Hjorth, 1975), further improving the locally relevant signals for analysis and minimizing the effect of volume conduction (Bradshaw and Wikswo, Jr., 2001).

b. EEG Processing

The EEG preprocessing pipeline explained in the section III.4.3. was implemented on the EEG signals obtained during the voluntary motor task. In brief, the 128-channel EEG signals were bandpass filtered between 1Hz – 345 Hz (dual-pass Butterworth filter) and the bad channels were visually identified and repaired using neighbouring channels. The eye-blink artifact was removed using ICA. The signals obtained after artifact rejection and bad channel interpolation were re-referenced using ‘common average referencing’. A zero-phase comb-notch filter was then used to remove 50Hz harmonic line noise.

The resulting EEG signals were segmented into 30 epochs (trials) of 15 seconds, starting 3 seconds before the start of the contraction phase till 7 seconds after the end of the contraction phase. The epoched signals were resampled at 1000Hz, and channels corresponding to ‘Cz’, ‘C3’, and ‘C4’ 10-5 locations were selected for further processing. The selected channels were band-pass filtered between 2-125 Hz (dual-pass Butterworth) and re-referenced using surface Laplacian (Hjorth, 1975). The signals from the resulting three channels were then divided into 1-second epochs during the sustained phase, between 0.5 and 4.5 seconds after the visual cue. This provided four 1-second epochs for each good trial. All 1-second epochs were subjected to automated artifact rejection routines (Fieldtrip Toolbox) (Oostenveld et al., 2011) to discard data contaminated by any other noise.

2.7. Data Analysis

a. Evoked Response Analysis

The section III.5.1.b describes the analysis pipeline utilized to obtain the evoked responses. In brief, the processed ESG signals during the peripheral nerve stimulation

paradigm were averaged across all remaining epochs. The latency characteristics of the resulting evoked spinal response potentials were evaluated. The evoked ESG responses were further averaged over all the participants resulting in grand-mean evoked responses for the participant population. The grand-mean evoked activity between 12ms-15ms was mapped across the SC10X/U electrode space to analyze the spatial distribution of the evoked spinal activity.

Statistics: Statistical significance was determined at each sample point of the evoked response waveform. The significant evoked response (each electrode) was determined by comparing the difference between the evoked amplitude and zero at each sample point of the recorded signal across all participants, using the Wilcoxon signed-rank non-parametric test (Iyer et al., 2017; Nasserolelami et al., 2014). To account for multiple comparisons, the statistical test was applied to the reconstructed ESG signal that retained dominant principal components explaining 85% of the total variance, plus an additional component. PCA was applied between -0.001s – 0.03s time-window and 64 channels. Bonferroni correction was subsequently applied at $\alpha = 0.05$.

b. Spectral Analysis

Spectral analysis was performed on the processed ESG signals recorded during voluntary motor task paradigm. The section III.5.2.a describes the analysis pipeline utilized for spectral analysis.

The signals during the sustained contraction phase (0.75s - 4.75s after the contraction cue) were segmented into Hanning windowed 1s epochs with 50% overlap (Bayram et al., 2023; Polat and Güneş, 2007). The spectral power (Welch's method) was calculated at every integer frequency value between 5 Hz and 95 Hz for each epoch. The average power spectral density (PSD) across all epochs for each participant was computed for three frequency bands: beta (13 – 30 Hz), low gamma (31 – 60 Hz), and high gamma (61 – 90 Hz). The PSD for each band was normalized by the average band-specific baseline power. A two-second time-period between 8.5s – 10.5s after the contraction cue was chosen as the baseline.

The normalized band-specific PSD was grand-averaged (gPSD) over all the participants and the PSD magnitude was converted to decibel for better visualization. A topological plot of the gPSD was plotted over the SC10X/U cervical electrode space.

Statistics: The difference in spectral power between ipsi-anterolateral, midline, and contra-anterolateral regions of the lower cervical levels was analyzed. The electrode positions LL8 and AL2 are referred to as ipsi-anterolateral location, ML5 and ML6 as midline location, and LL7 and AL1 as contra-anterolateral location. The significant differences between the regions for each band were determined using the paired-sample student's t-test. Spectrum power at each integer frequency in the specific band was used to calculate the statistically significant differences. Adaptive false discovery rate (FDR) at $q = 0.05$ was used to correct for multiple comparisons (Benjamini et al., 2006).

c. Event-Related Spectral Perturbations (ERSP)

ERSP analysis was performed on the processed ESG signals during the voluntary motor task. The section III.5.2.b describes the analysis pipeline utilized for ERSP analysis.

The signals from each participant were epoched between 2 seconds before the contraction cue and 2.5 seconds after the end of the contraction cue i.e. between -2.5 to 8 seconds where $t=0s$ signifies the start contraction cue. The bad trials were visually and automatically rejected. All channels for each trial underwent time-frequency analysis between the frequency range of 10Hz – 95Hz. The time-frequency analysis was performed using complex wavelet convolution method ('mtmconvol' Fieldtrip) with Hanning taper, at a time resolution of 200ms, and a frequency resolution of 2Hz. The output time-frequency power was normalized w.r.t the average baseline power calculated across all trials for each 2Hz frequency block. The normalization utilized a two-second time widow between 8.5 s – 10.5s after the contraction cue to calculate the average baseline power across all trials for each 2Hz frequency block.

The statistically significant normalized time-frequency spectrum blocks were identified and referred to as event-related spectrum perturbation (ERSP). The ERSPs were averaged across all trials and in frequency bands to calculate band-specific ERSPs. Beta (13 – 30 Hz), low gamma (31 – 60 Hz), and high gamma (61 – 90 Hz) frequency bands were used to calculate band-specific ERSPs. Both band-specific and un-banded ERPS were group-averaged across all participants. The group-average band-specific ERSPs (banded gERSP) were $10\log_{10}$ transformed for better visualization.

Statistics: Statistically significant time-frequency (TF) blocks for each electrode were determined by comparing the difference between the TF magnitude and zero for each block across all trials and participants, using the Wilcoxon signed-rank non-parametric

test (Iyer et al., 2017; Nasserolelami et al., 2014). To account for multiple comparisons, the statistical test was applied to the reconstructed time-frequency spectrum that retained dominant principal components explaining 85% of the total variance, plus an additional component. PCA was applied for each time-frequency block across all trials and ESG channels. FDR correction was subsequently applied at $\alpha = 0.01$.

d. Coherence Analysis

Coherence analysis was performed on 1s non-overlapped segmented ESG and EEG signals recorded during the sustained contraction phase of the voluntary motor task paradigm. The coherence spectrum between EEG-ESG (Cortico-Spinal Coherence) was calculated between three EEG channels (C3, Cz, C4) and three ESG regions (ipsi-antrolateral, midline, and contra-antrolateral). The section III.5.2.c describes the analysis pipeline utilized for ERSP analysis. The analysis resulted in the cortico-spinal coherence (CSC) for each cross-combination between EEG channels and ESG regions. The banded coherence between EEG and ESG was calculated for frequencies between 8-100 Hz at a 2-Hz frequency resolution.

Coherence for each combination of channels and each participant was estimated by applying spatial median to the auto-spectra and cross-spectra in 2-Hz frequency resolution (2Hz banded-coherence). The resultant was normalized with respect to the corresponding auto-spectra (Bista et al., 2023; Coffey et al., 2021) to obtain 2-Hz banded coherence for each combination.

Statistics: The non-parametric one-sample spatial-signed rank test for spectral coherence (Nasserolelami et al., 2019; Oja, 2010; Oja and Randles, 2004) was applied to compare each banded coherence value against zero, resulting in individual p-values for each band per channel-combination per participant. The respective p-values represented the strength of the normalized coherence. Stouffer's method (Stouffer et al., 1949; Westfall et al., 2014) was used to combine individual p-values across all participants to derive group-level average p-values. Adaptive false discovery rate (FDR) at $q = 0.05$ was used to correct for multiple comparisons (Benjamini et al., 2006). The resulting group-level strength (p-value) for each coherence value per combination was visualized by taking the negative log of the p-values.

3. Results

3.1. Evoked Potentials

The average evoked response for individual participants and the grand-mean evoked response across all participants for the electrode positions ML6 and AL2 are shown in Figure 24.a. Evoked potentials P9, N13, and P13 were observed in response to the median nerve stimulation. Latency of $13.2 \pm 1.1\text{ms}$ (mean $\pm$ sd) and $9.7 \pm 1.2\text{ms}$ (mean $\pm$ sd) for evoked potentials N13 and P9 respectively was observed across all participants. Significant evoked response activity is highlighted in Figure 24.a with discrete red circles.

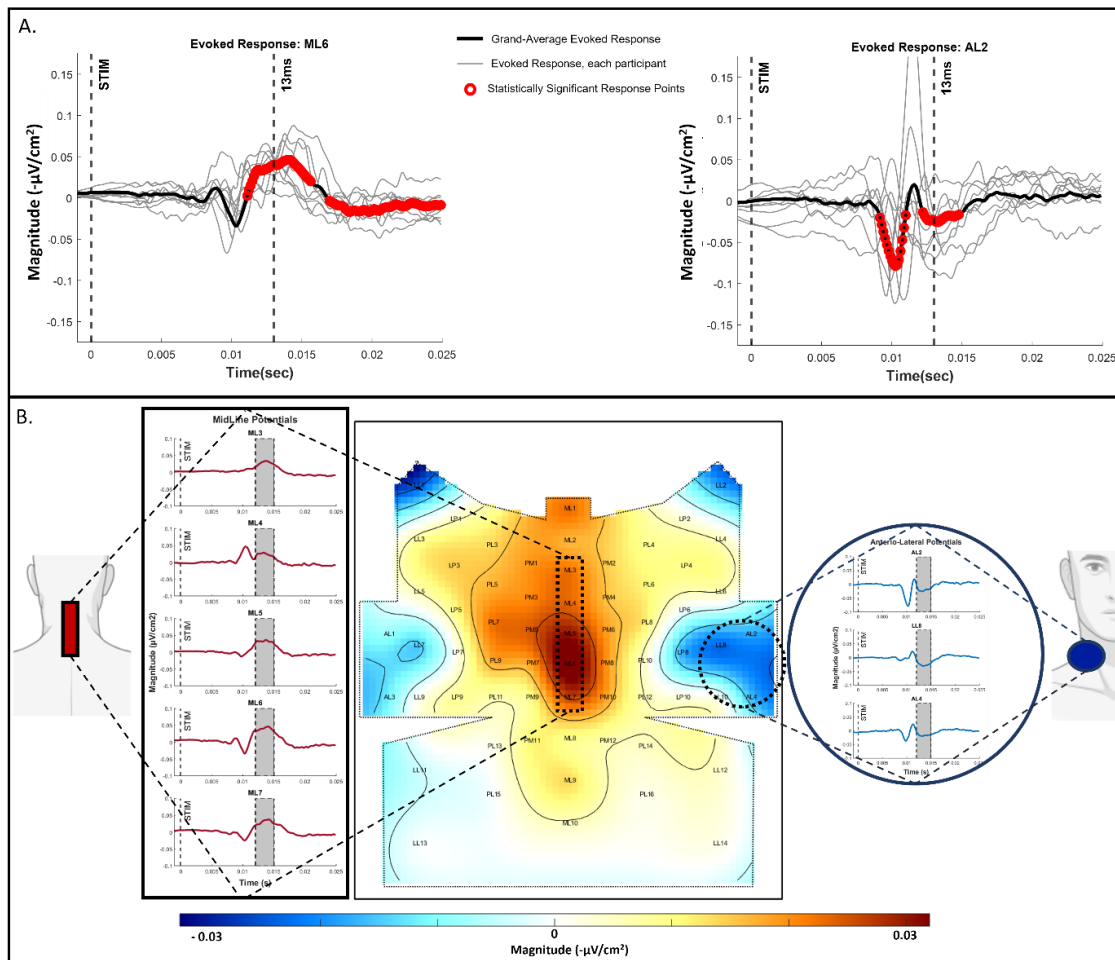


Figure 24: A. Grand-mean of the evoked potentials across all participants at ML6 and AL2 electrode positions. Grey lines represent individual participant evoked potentials, and thick black line correspond to the grand mean evoked potential across all participants. The statistically significant evoked response sample points are illustrated as hollow red circles. B. Topographic Map of Evoked Spinal Potentials: Centre Figure: Topographic Map of the grand-mean spinal evoked response over the SC10-X/U sensor space, time-window (12ms-15ms). Left figure: corresponds to the spinal activity recorded from channels ML3-ML7 (midline, posterior) for the time frame between 0.001s before stimulation and 0.025s after stimulation. Right figure

corresponds to the spinal activity recorded from lateral-anterior channels for the time frame between 0.001s before stimulation and 0.025s after stimulation. This figure demonstrate dorsal epicentre of activity at channel locations ML5-ML6.

The topological spatial distribution of the grand average evoked spinal activity between 12ms-15ms (N13, P13) after stimulation for SC10X/U electrodes is shown in Figure 24.b. The midline cervical region demonstrated higher spinal response with epicenter at the ML5-ML6 location. The ipsi-anterolateral electrode (LL8, AL2, AL4) also showcased increased spinal activity with reversed polarity. Figure 24.b also showcases the evoked response at midline electrodes (ML3-ML7) and ipsi-anterolateral electrodes (LL8, AL2, AL4) w.r.t the corresponding surface region.

3.2. Spinal spectral power during isometric muscle activity

The topological spatial distribution of the grand average PSD (gPSD) for three bands (beta, low-gamma, and high-gamma) is shown in Figure 25. The spatial distribution of gPSD (Figure 25) indicated increased gPSD on the same side of the spinal cord as the active muscle in the beta band. This was supported by the significant spectral power increase in the beta-band for ipsi-anterolateral electrodes as compared to both midline and contra-anterolateral electrodes ($p < 0.05$). The low-gamma gPSD spatial distribution (Figure 25) indicated higher PSD at dorsal electrodes, epicenter around ML5. The midline electrodes showed a significant increase when compared to contra-anterolateral electrodes ($p < 0.05$) but no significant difference was observed compared to ipsi-anterolateral electrodes. The high-gamma gPSD spatial distribution (Figure 25) demonstrated more bi-lateral activity, though the contra-lateral side indicated higher gPSD as compared to the ipsi-lateral side. There was no significant difference between the three regions (midline, ipsi-anterolateral, and contra-anterolateral) for the high-gamma band, but a marginally significant difference was observed between midline and ipsi-anterolateral spectral power ($p = 0.0631$).



Figure 25: This figure shows the topographic map of the grand-averaged normalized band-specific PSD in beta, low-gamma, and high-gamma bands for the cervical electrode locations. The bottom plot demonstrates the box plot of the spectral activity for the frequencies in each frequency band and three ESG regions marked

in the topography map: Midline, Ipsi-anterolateral, and Contra-anterolateral. Beta band-specific PSD demonstrated highly ipsilateralized activity and showcased significantly increased spectral activity in ipsi-anterolateral region as compared to midline and contra-anterolateral. Low-gamma band-specific PSD demonstrated significant increased activity in midline as compared to contra-anterolateral region. Bi-lateral activity was observed for high-gamma band-specific PSD and no-significant differences were observed between the three regions.

3.3. Event-Related Spectral Perturbations during Voluntary Motor Task

The gERSP at ML5, ML6 electrodes were averaged to get gERSP for the dorsal midline ESG region. Similarly, gERSP was calculated for the ipsi-anterolateral and contra-anterolateral regions comprised of channels AL2, LL8 and AL1, LL7 respectively.

The banded gERSP for the three ESG regions (ipsi-anterolateral, midline, and contra-anterolateral) at the lower cervical spine is shown in Figure 26. The figure plots the banded-ERSP averaged over 0.75s time-window (smoothing) for each region during the pre-motor, contraction, and post-motor phases of voluntary motor activity.

In the beta band, increased gERSP was observed in the ipsi-anterolateral region during the sustained contraction period. An increased gERSP in the contra-anterolateral region was also observed during the termination period.

In the lower-gamma band, midline (dorsal) region demonstrated decreased gERSP during the pre-motor period. The ipsi-anterolateral region demonstrated a monotonic increase in gERSP during the sustained contraction period. Increased gERSP was observed during the contraction phase in both midline and contra-anterolateral regions. The Contra-anterolateral region also indicated higher gERSP during the termination period.

In the high-gamma band, an increase in gERSP was observed for all three regions during the execution period from relatively low gERSP during the pre-motor period. Similarly, increased gERSP was observed in all three regions during the termination period as compared to the middle of the post-motor phase. Additionally, a decrease in gERSP was observed during the post-motor period in the contra-anterolateral region.

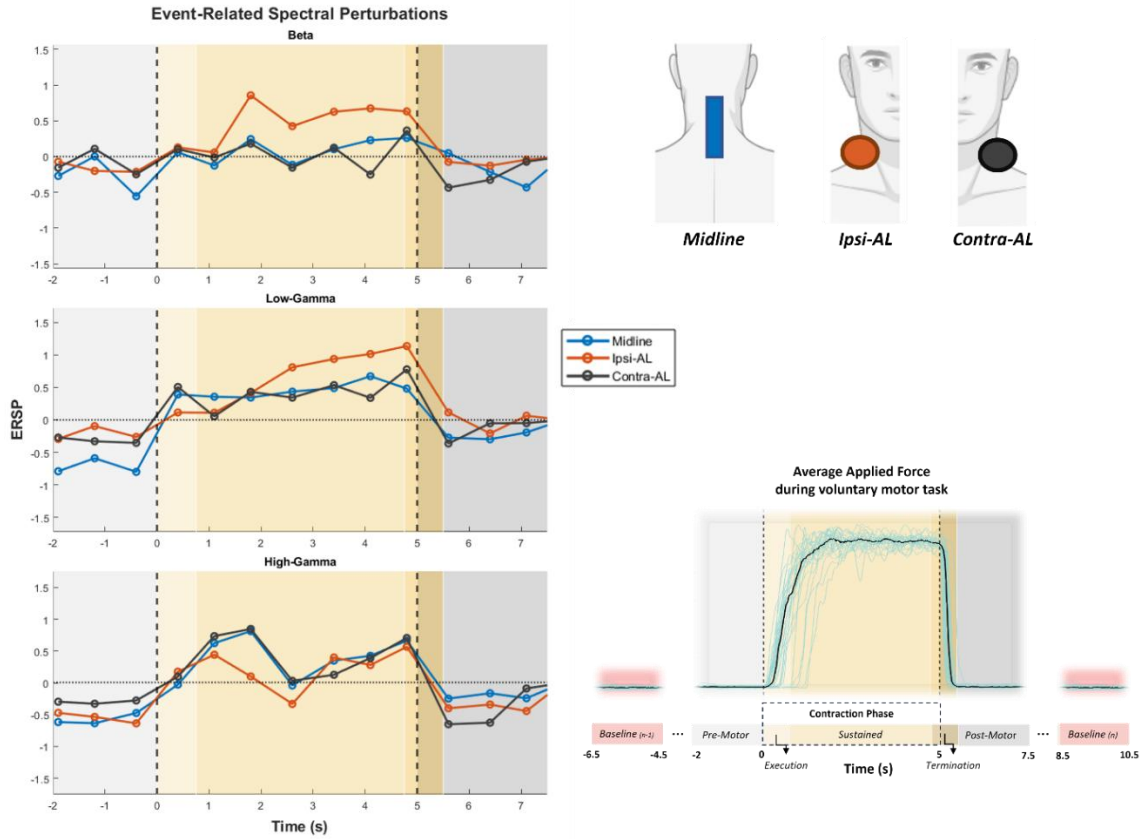


Figure 26: The group-average band-specific ERSs (beta, low-gamma, high-gamma band) for ipsi-antrolateral (red), midline (blue), and contra-lateral (black) spinal regions at the lower cervical cord are shown in the figure. Smoothened (averaged) over 0.75s time-window. The figure showcases the gERSPs in the pre-motor, execution, sustained, termination, and post-motor period of the motor task. gERSP in beta band showcased pre-dominant ipsi-antrolateral activity during the sustained period of the isometric contraction. In low-gamma band, dorsal midline region showcased increased gERSP for the duration of the sustained period, whereas ipsi-antrolateral region indicated a monotonic increase in gERSP during the sustained period. Increased gERSPs was observed for all three regions for the execution and termination period of the isometric contraction phase.

3.4. Cortico-Spinal coherence during isometric muscle activity

The coherence between EEG electrodes (contralateral motor (C3), central midline (Cz), and ipsilateral motor (C4)) and ESG regions (ipsi-antrolateral, midline, contra-antrolateral) in the lower cervical spine is shown in Figure 27.

In beta band, the C3 EEG electrode demonstrated significant coherence with the ipsi-antrolateral ESG, indicating spinal laterality in featuring cortico-spinal coherence with contralateral primary motor cortex in the beta band. Contrasting, ESG from all three regions demonstrated significant coherence with Cz EEG electrode indicating spinal bi-laterality

but '20-Hz' frequency specificity in featuring cortico-spinal coherence with central midline in the beta band.

In the low-gamma band, Cz EEG electrode demonstrated significant coherence with the midline ESG. The C3 EEG electrode also demonstrated significant coherence with the contra-anterolateral signal ESG while the C4 EEG electrode demonstrated significant coherence with ipsi-anterolateral ESG and marginally significant coherence with midline ESG in the low-gamma band.

Additionally, we also observed significant cortico-spinal coherence in the mu-band for midline ESG and contra-anterolateral ESG, but its evaluation was outside the scope of this paper.

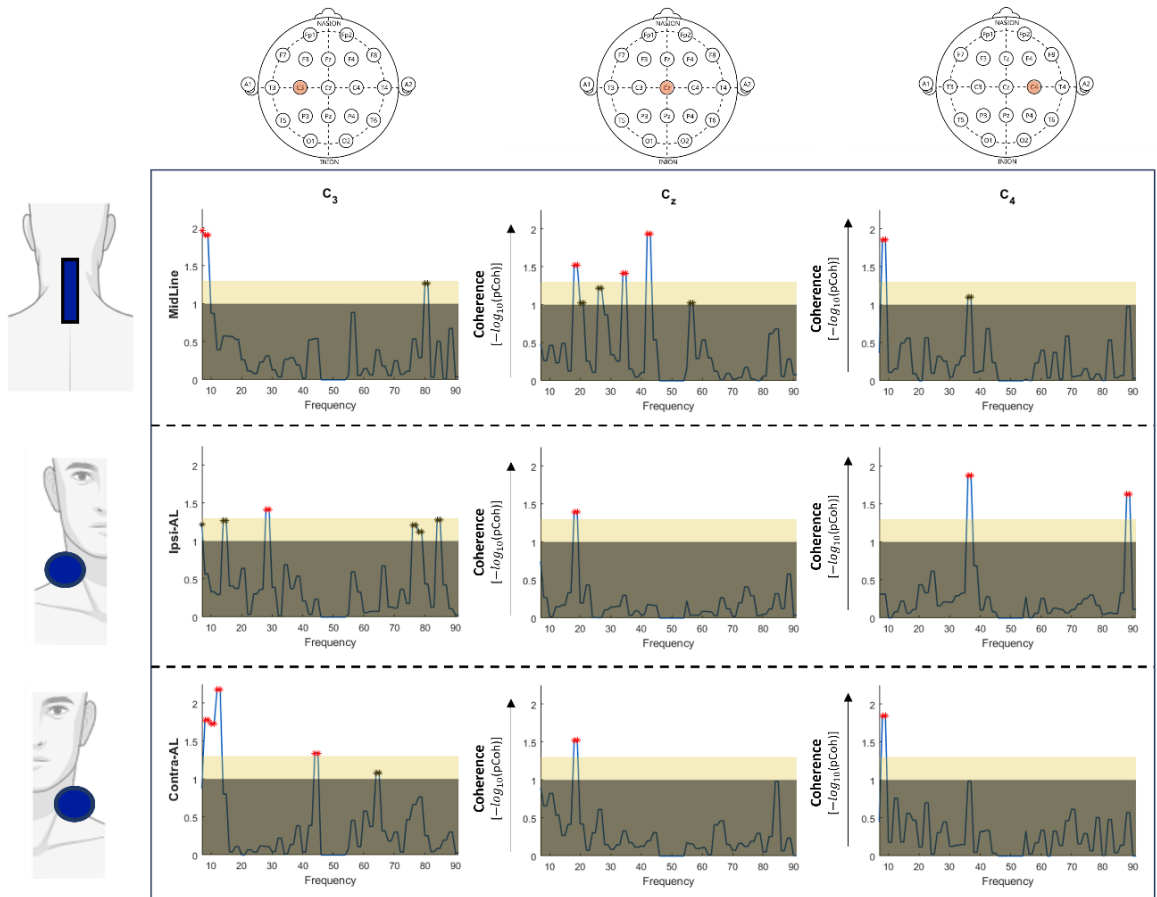


Figure 27: Corticospinal Coherence (CSC) between three spinal regions (ipsi-anterolateral, dorsal-midline, contra-anterolateral) and three EEG electrodes (C3, Cz, C4). The red-star indicates significant CSC at the corresponding frequency. The shaded area relates to the significance level, dark shade represents significance >0.1 and light shade represents significance >0.05 & <0.1 , while the unshaded region represents significant CSC values (<0.05).

4. Discussion

This study evaluated the neural oscillatory characteristics in the spinal cord during isometric voluntary contraction and in response to the external stimulus (median nerve). The results demonstrated that the neural signals of the spinal cord have spatial (topological) as well as frequency-specific responses during voluntary motor tasks. To our knowledge, this is the first study to non-invasively evaluate the spatio-spectral characteristics of spinal neural activity and its coherence with the brain activity. We use PSD, ERSP, and coherence analysis techniques to uncover spinal activity spatial lateralization, spatial-temporal-spectral changes, and cortico-spinal coherence respectively. The results indicate that there is a frequency band-specific lateralization and coherence with brain activity during the voluntary motor. The ERSP analysis indicated characteristic frequency-band-specific spatiotemporal changes during the pre-motor phase, isometric contraction phase, and post-motor phase of the voluntary motor task.

A significant evoked P9, N11, and N13 responses were recorded in response to the median nerve stimulation. The spatial topology of evoked spinal activity across SC10X/U electrode space during N13 response latency indicated high activity at dorsal midline channels with its epicenter at ML5-ML6 which roughly corresponds to cervical levels C6 and C7. A reversed polarity epicenter was also observed around ipsi-anterolateral channels at C6-C7 levels. The location of the evoked responses and its polarities are consistent with earlier low-density electrophysiology studies (Desmedt and Huy, 1984; Mauguière, 2003a; Restuccia and Mauguière, 1991). Figure 24.b shows a predominantly dorsal and ipsilateral activity in response to the median nerve stimulation, this is consistent with the reported fMRI studies that indicate dominant lateralization of the evoked spinal activity in response to external stimulation (Landelle et al., 2021). The N13 response is thought to be generated by the post-synaptic activity at the dorsal horn based on invasive recordings (Desmedt and Cheron, 1981; Jeanmonod et al., 1989; Urasaki et al., 1990) and is also suggested by the increased activity in the dorsal region observed using high-density electrophysiology, shown in Figure 24.b. These observations indicate that we are successfully recording spinal activity and can characterize the spatial topology of the activity.

Dominant lateralization (ipsi) was observed in the beta-band spinal gPSD during sustained isometric phase, shown in Figure 25a. The beta-band gPSD was significantly higher in the ipsi-anterolateral region as compared to the dorsal midline and contra-anterolateral region.

This increase was observed approximately at C6-C7 cervical levels. Increased beta-band activity during sustained contraction has been widely reported in electrophysiology studies investigating supraspinal motor regions (Baker, 2007; Chakarov et al., 2009; Kilner et al., 1999; Klostermann et al., 2007) and is a dominant feature of sensorimotor pathways during voluntary motor tasks. Our results indicate that beta-activation during isometric activity can also be observed at lower cervical levels and is highly localized over ipsi-anterolateral region, suggesting ventral horn activity. The ipsi-anterolateral region also showcased significant coherence with contralateral motor cortex. These oscillations could represent activation of the ipsi-spinal grey matter area that is involved in motor control and has direct motor-neuronal links to the skeletal muscles as well as supraspinal systems (Haines et al., 2018). The ERSP indicated a constant increase in beta-banded spectral perturbation during the sustained contraction period. Similar to our observation in spinal gERSP, a pronounced constant beta band activation has been readily reported in the primary motor and sensorimotor cortex during sustained contraction (Baker, 2007; Kilner et al., 1999). Beta band activation is proposed to signify a signature of promoting the existing active contraction while inhibiting other movements (Gilbertson et al., 2005; Swann et al., 2015), suggestive of an ‘active akinetic’ process (Brown and Williams, 2005; Engel and Fries, 2010). According to the supraspinal studies, the characteristic beta-band activation might be responsible for driving motor unit activity for muscle contraction (Baker et al., 1997; Denker et al., 2011; Kilavik et al., 2013), similarly our observation of an exclusive ipsi-anterolateral (ventral horn) beta-banded gERSP increase can indicate beta-band’s direct involvement in motor unit activation.

Unlike the beta band, no significant lateralization in spectral spinal activity was observed in the low-gamma band, but an increase in dorsal spectral activity at ML5 (roughly C6 level) was observed, as shown in Figure 25b. The dorsal midline demonstrated significantly higher low-gamma banded gPSD than the contra-anterolateral region while no difference between ipsi- anterolateral and contra-anterolateral region was seen. This suggested an increased activation of dorsal spinal in the low-gamma band which was not observed in the beta-banded gPSD. The dorsal midline also showcased significant coherence with the central midline brain region (Cz) in the low-gamma band, demonstrating coherence between dorsal-spinal and sensorimotor cortex activity. Low gamma activity has been observed in sensorimotor cortex (Crone et al., 1998a) during voluntary tasks and has been associated with sensorimotor integration (Aoki et al., 1999; Ihara et al., 2003) and sensory

afferent feedback (Szurhaj et al., 2006, 2005). The increase in dorsal spinal activity seen in our study could indicate the activity of proprioceptive afferent feedback indicative of somatosensory afference contributing to low-gamma activation. Our dorsal gERSP results showcased increased low-gamma for the duration of the contraction phase, further supporting the role of low-gamma in constant sensory afferent feedback. The low-gamma spectral perturbation changes in the ipsi-anterolateral region showed a gradual monotonic increase over time during the sustained isometric period, as shown in Figure 26. This suggests an increase in the required efferent drive over time to maintain the isometric force during the sustained period (Brown, 2000; Salenius et al., 1996). Interestingly, both left and right anterolateral regions showcased significant coherence with the same side of the motor cortex, as shown in Figure 27. The demonstrated cortico-spinal coherence between the ipsi-anterolateral spinal cord and the ipsilateral motor cortex suggests the ipsilateral motor cortex's role in maintaining task performance of a visually-guided isometric contraction. The ipsilateral motor cortex has been reported to complement the contralateral motor cortex for maintaining high-task performance (Mayhew et al., 2017; Perez and Cohen, 2008; Porcaro et al., 2021; Shibuya et al., 2014). TMS-based electrophysiology studies have shown that ipsilateral motor cortex can influence interhemispheric inhibition and corticospinal tract output to modulate task-dependent motor activity (Davare et al., 2007; Diedrichsen et al., 2013; Perez and Cohen, 2008; Stinear et al., 2001).

Bi-lateral activation was observed in the high-gamma frequency band, as shown in Figure 25.c. No significant differences in gPSD were observed between the dorsal midline, ipsi-anterolateral, and contra-anterolateral regions. The ERSP analysis indicated an increase in gamma-banded spectral perturbation during the execution and termination phase of the isometric contraction for all the regions. High-gamma gERSP showed focused activity during the execution and termination but did not remain constantly active during the sustained phase. A transient high-gamma activity close to movement onset has been reported in previous studies observing cortical neural oscillation during motor tasks (Crone et al., 1998a; Miller et al., 2007; Muthukumaraswamy, 2010; Pfurtscheller et al., 2003). Additionally, high-gamma activity has been described as prokinetic during motor execution (Brown, 2003; Joundi et al., 2012). This could explain the focused high-gamma spectral perturbation observed in our study and supports the previous cortical observations (Ball et al., 2008), where execution and termination can be considered two separately triggered movements. Though there isn't a consensus behind the neurophysiological significance of

high-gamma activity during motor activity, previous studies have reasoned that this could be associated with the processing of motor and sensory information to encode kinematic parameters (Ball et al., 2009; Muthukumaraswamy, 2010; Ulloa, 2022). This rationale is supported by our observation of increased high-gamma spectral activity at ML2 electrode (roughly C3 level, Figure 25c), which could indicate the involvement of propriospinal neurons. The studies investigating the role of propriospinal neurons during motor control in humans and non-human primates (Alstermark et al., 2007; Isa et al., 2006; Pierrot-Deseilligny, 2002), suggested that spinal activity at C3-C4 level could be indicative of propriospinal interneuronal activity during voluntary control. However, further investigation of spinal activity at upper cervical levels was outside the scope of the current study.

In our study, the observed frequency band-specific spinal lateralization could in part explain the conflicting results reported in spinal-fMRI studies during motor activity (Landelle et al., 2021). Spinal-fMRI studies have observed contrasting degrees of lateralization during movement, with some studies reporting bi-lateral spinal activity (Bouwman et al., 2008; Ng et al., 2008) while others reported significant ipsi-lateralization (Maieron et al., 2007; Weber et al., 2016). Our gPSD results indicate that lateralization in the spinal cord is frequency band dependent which could account for the observed variance in lateralization between fMRI results. This could inform future spinal-fMRI studies to explore the effects of motor tasks and analysis methods on spinal-fMRI lateralization and indirectly infer its effects on band-specific spinal activity.

VI. Result: Characteristics of evoked high-frequency oscillations in health and neurodegeneration (MS)

1. Introduction

Very high-frequency oscillations (>300 Hz) have been observed in response to the median nerve stimulation at subcortical and cortical levels (Bankim Subhash Chander et al., 2022; Hanajima et al., 2004; Insola et al., 2008; Prestor et al., 1997; Tanaka et al., 2013). In 1997 Prestor et al. (Prestor et al., 1997) recorded spinal oscillatory activity in response to the median nerve stimulation using invasive epidural electrodes at lower cervical levels. These oscillations demonstrated low amplitude responses in the high-frequency band (>300) with peak activity at a latency of 11ms post-stimulation. Multiple studies (Bankim Subhash

Chander et al., 2022; Insola et al., 2008) following that have also reported HFO activity in the spinal cord after median nerve stimulation. The N11 response (Desmedt and Cheron, 1981; Mauguière, 2003b) observed as the spinal somatosensory evoked potential after median nerve stimulation has been attributed to signify the low-amplitude high-frequency response (Insola et al., 2008; Prestor et al., 1997). The high-frequency oscillations have been observed at ~500Hz and ~1200Hz in the cervical spinal cord, the amplitude at ~500Hz did not show any gating effect and is proposed to be of presynaptic origin (Insola et al., 2008). The HFOs observed in the somatosensory cortex and thalamus have demonstrated HFO activity at ~600Hz and ~1000Hz respectively (Curio et al., 1994; Hanajima et al., 2004; Hashimoto et al., 1996; Ozaki and Hashimoto, 2011). The HFOs demonstrate localized activation and are deemed to have different generators than the ones responsible for somatosensory evoked potentials (Curio, 2000; Hanajima et al., 2004; Insola et al., 2008), though the exact origin and neurophysiology still remain debatable.

HFOs at supraspinal have been investigated in neurological conditions that affect the motor system and have shown differences between patients and control participants, these patient groups included participants from Parkinson's (Inoue et al., 2001), cervical dystonia (Inoue et al., 2004), amyotrophic lateral sclerosis (Hashimoto et al., 1996), etc. In 2003 Gobbele et. al. (Gobbelé et al., 2003) investigated somatosensory cortical HFOs in response to median nerve stimulation in people with multiple sclerosis and observed significant disturbed HFOs. The HFos are proposed to be synchronous in nature (Gobbelé et al., 1998) and the demyelination of axons in multiple sclerosis could lead to disruption in HFOs as observed by Gobbele et. al.. Here, we evaluated the evoked spinal HFOs in people with multiple sclerosis (pwMS) in response to the median nerve stimulation. This aimed to investigate if the HFOs are disrupted at the spinal cord level in multiple sclerosis or if disruption is primarily a supraspinal observation in pwMS. The potential disruption in HFOs and its characteristics could provide a marker of synchrony at the spinal level in pwMS.

2. Methods

2.1. Participants and Ethics

Fourteen people with MS (pwMS) (age: 40 ± 8 , 6 Males) and eighteen (age: 33 ± 15 , 5 Male) age-matched ($p = 0.18$, independent t-test) control participants were recruited for this

study to perform an experimental paradigm featuring median nerve stimulation. All participants were informed about the experiment and informed signed consent was taken before the recording session. The study was approved by the ‘Tallaght Hospital/St. James’s Hospital Joint Research Ethics Committee, Project Reference: 0493, and the study was performed in accordance with the declaration of Helsinki.

2.2. Exclusion Criteria

The exclusion criteria are as follows:

1. Any history of neuromuscular, neurological, and or psychiatric condition. [Except multiple sclerosis for people with MS]
2. Diagnosed chronic cervical and upper back condition (affecting spinal nerves). [Except due to MS]
3. Any history of spondylosis or spondylitis or any other back pain due to injury or external impact.

Table 1: Demographic Details of the people with MS who were recruited for the study,

Patient Code	Type of MS	Gender	Age	Height	Handedness (R/L)	Clinical Score (EDSS)
	relapsing-remitting (RR) /primary-progressive (PP) /secondary-progressive (SP)	(F/M)	40 ± 8 (years)	(in cm)	Edinburgh Handedness	1.39 ± 0.68
1	RR	M	39	183	R	1
2	RR	M	39	182	R	1.5
3	RR	F	28	167	R	1
4	RR	F	51	165	R	2.5
5	RR	M	50	178	L	2
6	RR	M	35	180	R	1.5
7	RR	F	39	176	R	2.5
8	RR	F	28	163	R	1
9	RR	M	53	180	R	1.5
10	RR	M	40	175	R+L	1
11	RR	F	38	163	R	1
12	RR	F	36	165	R	1
13	RR	F	36	169	R	0
14	RR	F	51	161	R	2

2.3. Experiment Setup

The participant sat comfortably on a physio-chair during the experiment paradigm with their head resting comfortably on the headrest and the hand resting on the handrest of the

physio-chair, as shown in Figure 10. The arm made a 90-degree angle at the elbow. During the task, participants were instructed to keep their eyes closed.

2.4. Data Acquisition

High-density Electrospinogram (ESG), multi-channel Electrocardiogram (ECG), and EXG signals were recorded while the participant rested comfortably on the physio chair and performed the experiment. The signals were recorded using a high-speed Biosemi-ActiveTwo System (Biosemi B.V., Amsterdam, The Netherlands) at a sampling frequency of 8.196kHz, and active electrode offsets were visually inspected to be kept between $\pm 25\text{mV}$. The data acquisition protocol and the electrode positions for ESG, ECG, and EXG are explained in section III.1.2.

a. Experiment Paradigm

This study aimed to record High-Density Electrospinogram in response to peripheral stimulation (median nerve stimulation) to observe evoked high-frequency oscillations. The median nerve stimulation experiment paradigm is described in detail in the section III.3.2.a. In brief, median nerve stimulations were performed on the wrist at an intensity of 1.5 times the motor threshold and $200\mu\text{s}$ pulse width. A total of 1400 responses in 35 sessions were collected. Each session lasted for 30 seconds; 20 seconds of the stimulation phase followed by 10 seconds of rest phase. In the stimulation phase, the median nerve was stimulated by a train of pulses at an average frequency of 2Hz, producing 40 stimulation pulses (trials) per session.

Non-invasive electrophysiology signals were recorded during the experiment with no applied randomization or blinding since signals cannot be influenced by the experimenter or the data collection process. The participants were instructed to keep their eyes closed during the experiment.

2.5. Signal Processing

a. Pre-Processing

The recorded ESG signals showcased stimulation artifact that lasted for 1-2 ms after stimulation trigger and was removed by interpolating (piecewise cubic interpolation) the signal between 0.5ms before the stimulation trigger and 2.5ms after stimulation. The stimulation artifact removal process is described in the section III.4.1.

The ESG preprocessing pipeline explained in section III.4.2. was implemented on the stimulation artifact removed ESG signals. In brief, bad channels were visually detected and removed. Canonical component analysis (CCA) was utilized to remove ECG artifacts from the remaining ESG channels. The bad channels were interpolated. The resulting ESG signals were re-referenced using ‘common average referencing’. A zero-phase comb-notch filter was then used to remove 50Hz harmonic line noise.

b. Processing

The pre-processed ESG signals were segmented into 1400 trials of 0.5 seconds, -0.1 seconds before stimulation, and 0.4 seconds after stimulation. The bad trials were automatically detected and rejected based on three criteria: z-value, standard deviation, and maximum absolute z-value across time. The cut-off z-value was above $4 \times \text{SD}(\text{z-value})$ above the median z-value across all epochs, the cut-off for standard deviation was $3 \times \text{SD}(\text{standard deviation})$ above the mean standard deviation across all epochs, and the cut-off for maximum absolute z-value was set to 8. These cut-off parameters were chosen based on the visual inspection of the preliminary data of 5 participants.

The resultant ESG signals were bandpass filtered between 355 Hz – 1500 Hz to evaluate spinal high-frequency oscillation.

c. Spatial-Filtering

The bandpass ESG signals were subjected to task-related component analysis (TRCA) (Nakanishi et al., 2018; Tanaka et al., 2013). The design of the high-frequency TRCA-Spatial Filter is described in the section III.5.1.a

The bandpassed ESG signals were TRCA-spatial filtered. During TRCA-based-spatial filtering, the signal was transformed using task-related component analysis, and the non-task-relevant components were removed. The remaining components were back-transformed to the original ESG space, resulting in a TRCA-spatial filtered ESG signal with optimized SNR.

The signals obtained after TRCA-spatial filtering were re-referenced using the surface Laplacian method (Hjorth, 1975), further improving the locally relevant signals for analysis minimizing the effect of volume conduction (Bradshaw and Wikswo, Jr., 2001).

2.6. Data Analysis

a. High-Frequency Oscillatory Response

Each trial of the ESG signals obtained post-signal processing was normalized before further analysis. A baseline window of 250ms between 0.3s and 0.05s before the stimulation trigger was used to normalize the signal. The normalization for each trial was performed by removing the mean baseline value and dividing the resulting signal by the standard deviation of the baseline. The normalized trials were averaged across all the trials to obtain time-phase locked very high-frequency oscillatory spinal activity (HFOs) for each participant. The envelope of the resultant trial-averaged HFOs for each participant was calculated using the Hilbert transform. The envelope was used to calculate the onset of evoked modulation of the HFOs (evoked-HFO). The onset of evoked-HFOs was determined when the envelope magnitude first exceeded the threshold after the stimulus trigger. The threshold was determined as $5 \times \text{standard deviation of the baseline envelope magnitude above the mean envelope magnitude during baseline}$. To determine the peak magnitude and duration of the HFOs, the envelope was smoothened using a low-pass filter (75Hz, 4th-order Butterworth filter). The peak of the HFOs was determined as the peak of the smoothen envelope of the trial-averaged HFOs. The duration of evoked-HFO response was calculated by the determining time duration when smoothen-envelope signal magnitude stayed above the calculated threshold ($\text{mean}_{\text{baseline}} + 5 \times \text{SD}_{\text{baseline}}$). The resultant evoked characteristics: onset latency, peak magnitude, latency at peak magnitude ($\text{Latency}_{\text{peak}}$), and evoked-duration were calculated for each participant, and compared between pwMS and control group. The topographical map of the evoked-HFOs for group-averaged activity for pwMS and control was visualized.

b. Time-Frequency Analysis

Event-locked and non-phase-locked time-frequency (TF) analysis was performed for the normalized 64-channel ESG data in the high-frequency band. Both time-frequency analyses were performed using complex wavelet convolution method ('mtmconvol' Fieldtrip) with Hanning taper, at a time resolution of 25ms, and a frequency resolution of 10 Hz in the 300 - 1250Hz frequency range. The time-frequency domain was adopted to evaluate the event-locked and non-phase-locked temporo-spectral characteristics of the high-frequency oscillatory activity in response to the stimulation.

The non-phase-locked spinal activity was obtained by subtracting the event-locked high-frequency oscillatory spinal activity (evoked-HFOs) from every trial for each channel. The time-frequency analysis was performed on the resultant non-phase-locked spinal activity for every trial. The resultant trial-wise TFs were averaged across all trials for each channel to obtain the non-phase-locked time-frequency spectrum. The event-locked time-frequency spectrum was obtained by performing TF analysis on the event-locked signals obtained by averaging the high-frequency banded spinal activity across all trials.

The resultant event-locked and non-phase-locked time-frequency spectrum for each channel were baseline-corrected for every 10Hz frequency bin, -500ms to 25ms was used as the baseline window.

2.7. Statistical Analysis

The statistical difference in the peak amplitude of the envelope evoked-HFO response between the two groups was calculated using the Mann-Whitney U test for each electrode location. The results were corrected for multiple comparisons using adaptive false discovery rate (Benjamini et al., 2006) at $q = 0.05$.

For time-frequency analysis, the statistical differences between the two groups were calculated for each channel and for each time-frequency block using the Mann-Whitney U. The results were corrected for multiple comparisons using adaptive false discovery rate (Benjamini et al., 2006) at $q = 0.05$.

3. Results

3.1. Evoked HFOs Temporal Characteristics

A lateralized evoked HFOs modulation was observed at the C6 cervical level in response to the median nerve stimulation for both groups, as shown in Figure 28. The onset latency of HFOs evoked modulation was observed as 8.0 ± 0.94 ms (mean $\pm$ sd) and 7.8 ± 1 ms (mean $\pm$ sd) for pwMS and control group respectively. The onset latency did not show any significant difference between the two groups. The evoked HFOs at LL8 lasted for 7.2 ± 1.5 ms and 6.7 ± 0.75 ms duration for pwMS and control group respectively. The HFOs duration did not show significant differences between the two groups. The LL8 channel demonstrated maximum high-frequency activity for both groups. Normalized peak

amplitude of 0.26 ± 0.11 and 0.4 ± 0.2 at an average latency of 11.5 ± 2 ms and 10.4 ± 0.081 ms was observed at LL8 for pwMS and control group respectively. The pwMS and control groups showed a significant difference ($p=0.021$) in peak amplitude at LL8, with pwMS demonstrating significantly lower amplitude than controls. No significant difference in latency of peak amplitude was observed between the groups. Figure 28 also demonstrates focused ipsilateral activation over the lateral line and posterior lateral line for both groups for the time-window of 9 ms – 13 ms after stimulation.

Figure 30.A showcases the statistically significant differences in the peak spinal amplitude between the two groups in the very-high frequency band for SC10X/U electrodes. Significantly lower evoked very-high frequency oscillatory amplitude modulation was observed in pwMS for 4 spinal regions as highlighted in the Figure 30.A.

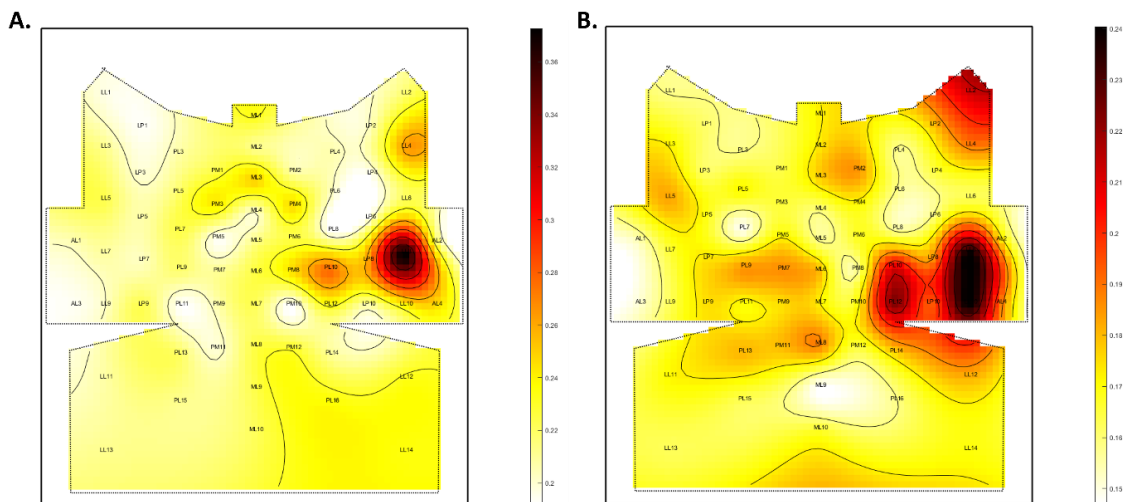


Figure 28: The figure illustrates the topographical map of the group averaged enveloped-HFO activity for controls (A.) and pwMs (B.). The figure demonstrates a highly localized activity over ipsilateral electrodes of the lower cervical levels for both groups.

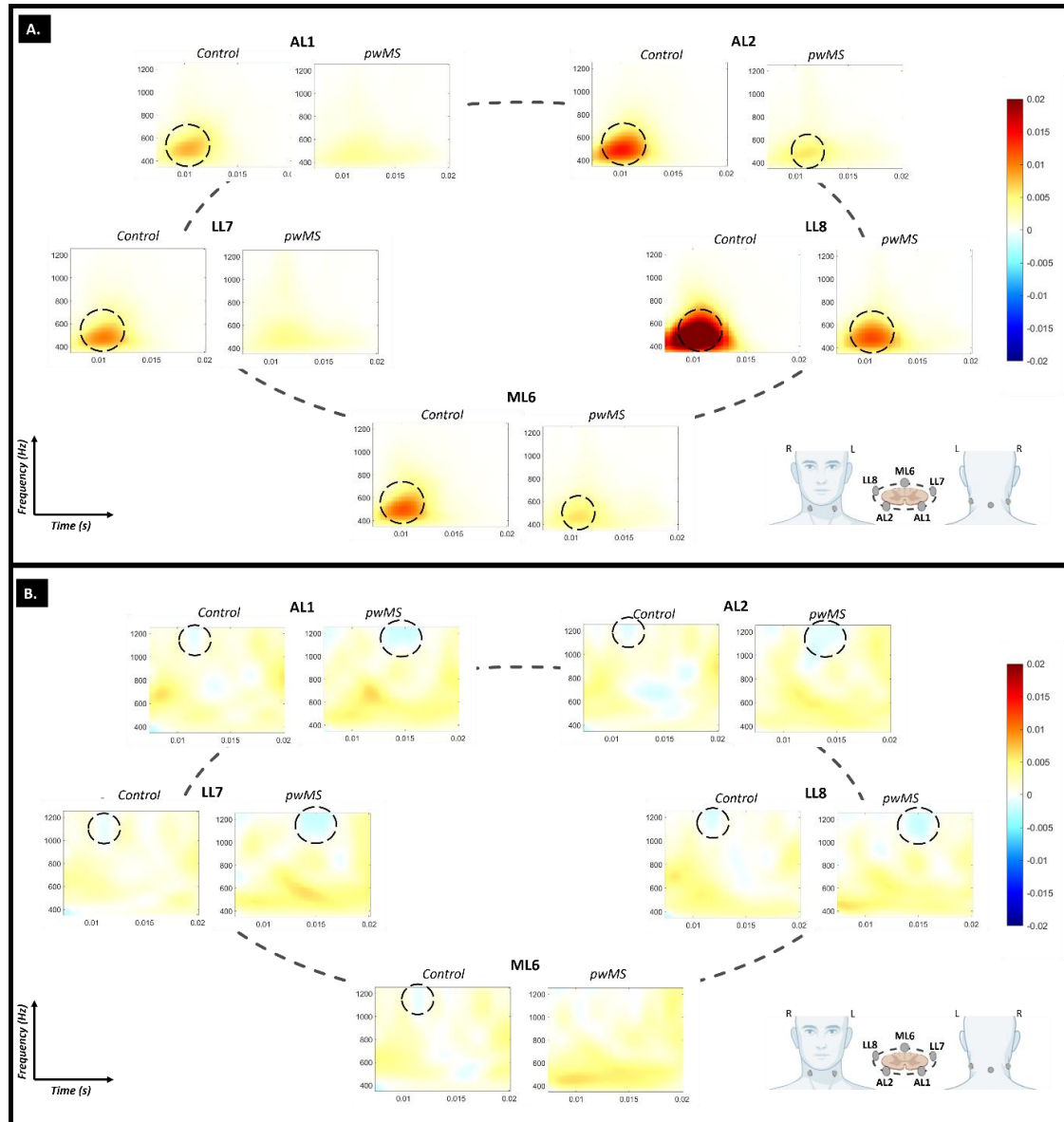


Figure 29: The figure illustrates the temporal-spectral activity of controls and pwMS for event-locked (top) and non-phase locked (bottom) spinal signals at the C6 vertebral level. The activity at contra-anterolateral electrode (AL1), contralateral electrode (LL7), dorsal-midline electrode (ML6), ipsi-anterolateral electrode (AL1), and ipsilateral electrode (LL7) is shown in the figure. A. Event-Locked Temporal-spectral activity: An increase in spectral activity was observed in the 400 Hz – 600 Hz frequency band between 8 ms–14 ms post-stimulation for both groups. The increased activity was most prominent at the ipsilateral electrode position for both pwMS and controls. B. Non-phase locked temporal-spectral activity: A decrease in spectral activity was observed in the 1000 Hz – 1200 Hz frequency band for both groups, though the latency of the observed response was delayed in pwMS.

3.2. Evoked HFOs Spectral Characteristics

The temporo-spectral characteristics of HFOs for the dorsal-midline electrode (ML6), ipsilateral electrode (LL8), contralateral electrode (LL7), ipsi-anterolateral electrode

(AL1), contra-anterolateral electrode (AL2) at Cv6 (C6 vertebral level) for both group is shown in Figure 29. Both groups demonstrated event-locked spectral activity in 350-600 Hz with a peak frequency of ~500 Hz between 8ms – 15ms after stimulation as shown in Figure 29.A. The ipsilateral electrode demonstrated significantly ($p<0.01$) higher peak event-locked spectral activity in 350-600 Hz than dorsal-midline, and contra-lateral electrodes for both groups. Figure 29.B. demonstrates the non-phase locked time-frequency activity of HFOs. Both groups demonstrate a reduction in non-phase locked spectral activity between 1000-1250 Hz but at different time points. The pwMS group showed spectral reduction at a delayed average latency as compared to the control group. No significant difference in non-phase-locked spectral activity was observed between the electrodes.

The statistically significant differences in the event-locked temporal-spectral activity between the two groups for SC10X/U electrodes are shown in Figure 30.B. The channels showcasing significant differences in time-frequency signals closely overlapped with the channels that demonstrated significant differences in peak-envelope amplitudes as shown in Figure 30.A. and Figure 30.B. The difference in time-frequency signals between the two groups for the 4 spinal regions is shown in Figure 30.C. The regions encompassing lower cervical ipsilateral and dorsal-medial electrodes (region a., b.) indicated significantly different spectral activity at ~500Hz. The pwMS group showcased a significant decrease in the spectral activity in the 400-600 Hz wide frequency band between 8ms -15ms followed by an increase in spectral activity between 15 - 20ms after stimulation, as shown in Figure 30.C. Unlike regions b. and c., region c. (ipsilateral upper cervical electrodes) indicated significantly lower spectral activity for the pwMS group around 800Hz between 8ms - 15ms. Region d. indicated different spectral activity around both 500 Hz and 800 Hz. No significant differences in the non-phase-locked temporal-spectral activity between the two groups for SC10X/U electrodes were observed.

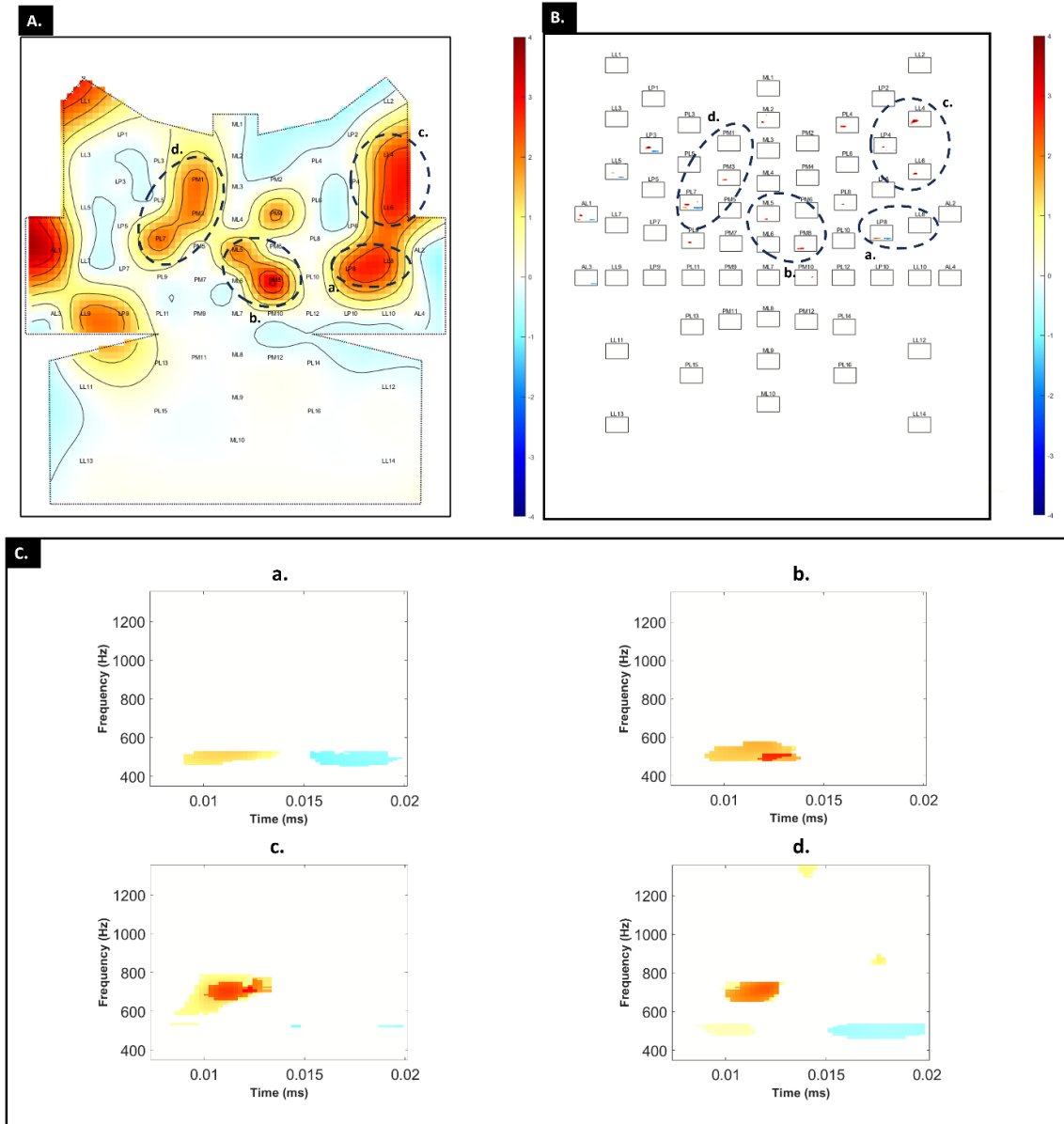


Figure 30: This figure showcases the significant differences between pwMS and control [control - pwMS]. A. Showcases the significant differences between control and pwMS at peak HFO amplitude (signal envelope). The differences are shown as T-values and a positive difference indicate higher signal envelope amplitude in control i.e. pwMS have a lower signal envelope amplitude. Significant differences were observed in 5 electrode regions labelled a.,b.,c.,d. B. Showcases significant differences between control and pwMS in the time-frequency domain. The differences are shown as T-values and a positive difference indicate higher spectral power in controls. Significant differences were observed in the similar 4 electrode regions (a.,b.,c.,d.). C. The C. plot showcases the difference between pwMS and controls averaged for the respective regions. The region a and b demonstrated significant differences in 400-600 Hz frequency while the regions c and demonstrated significant differences in 600-800Hz.

4. Discussion

In this study, high-density electrospinography was utilized to evaluate evoked spinal activity in the high-frequency band (350 - 1250 Hz) in response to the median nerve stimulation. Highly ipsilaterally localized spinal high-frequency oscillations were observed in response to median nerve stimulation in both healthy control participants and pwMS. The spectral activity of the localized HFOs indicated an event-locked increase of spinal activity in the wide high-frequency band of 400-600 Hz. In both groups, the ipsilateral electrode (LL8) located at approximate C5-C6 cervical level demonstrated the highest activity in the high-frequency band as compared to the contralateral and dorsal electrode locations. There was a significant difference in peak RMS amplitude at the LL8 electrode location between the two groups, with pwMS showing lower peak RMS amplitude as compared to the controls.

Earlier studies have reported low-amplitude high-frequency oscillations at lower cervical levels in response to the median nerve stimulation (Bankim Subhash Chander et al., 2022; Insola et al., 2008; Prestor et al., 1997). However, these studies employed either invasive recording methods (Insola et al., 2008; Prestor et al., 1997) or low-density non-invasive electrode montage (Bankim Subhash Chander et al., 2022) to record the spinal signals, resulting in a very limited spatial resolution. To address this limitation, we utilized a 64-channel high-density system to record the spinal signals. Our results revealed significantly ipsi-lateralized HFOs at lower cervical levels, indicating localized spinal activity in the high-frequency band. The sensor-level spinal spatial activity pattern in the high-frequency band differed from the spatial pattern of the N13 spinal somatosensory evoked potential (SSEP) in response to the median nerve stimulation as reported in Chapter IV. HFOs evoked in response to median nerve stimulation have been reported at the thalamus (Hanajima et al., 2004; Klostermann et al., 2002), cuneate nucleus (Insola et al., 2010), and somatosensory cortex (Hashimoto et al., 1996; Ozaki and Hashimoto, 2011) although the peak frequency differs between regions. This indicates that HFOs might be an important feature of the sensory pathway. Previous studies have suggested that the generators of HFOs differ from those of the slow postsynaptic somatosensory response (Hanajima et al., 2004; Katayama et al., 2010), possibly resulting from synchronous interneuronal pre-synaptic activity. This supports the distinct spatial pattern of HFOs activity compared to N13-SSEP observed in our study. The localized activity observed in our study may indicate the

involvement of spinal interneurons located in the gray matter in the middle of the dorsoventral axis, possibly in lamina IV-VI. The time-frequency analysis demonstrated an event-locked activity in the wide-frequency band of 400-600 Hz for the duration of the evoked high-frequency oscillations, indicating a time-phase locked activity. Earlier studies have reported that observed high-frequency oscillations result from synchronous activity of neurons (Gobbelé et al., 1998) . Additionally, we observed a decrease in the spinal activity at 1200 Hz, this activity was non-phase locked and was more temporally localized as compared to the activity observed in the 400 - 600 Hz frequency band. Previously, an invasive spinal study by Insola et. al. reported that the HFOs in the frequency range of 400-600 Hz might demonstrate presynaptic spinal activity whereas HFOs at 1200Hz might be related to postsynaptic activity. The study did not report the non-phase-locked characteristic of the HFO activity at 1200Hz.

No significant differences in latency characteristics of HFOs between pwMS and healthy controls were observed in the 400-600 Hz frequency band. Both groups showcased similar onset latency, latency at peak RMS amplitude, and duration. The peak RMS-amplitude was significantly different between pwMS and healthy control. The reduction in RMS-amplitude could be a result of reduced synchronization of the high-frequency activity because of the temporal dispersion caused by delay in conduction due to demyelination in pwMS, a similar reduction in RMS-amplitude was partly seen by Gobbelé et. al. in HFOs recorded from somatosensory cortex in pwMS (Gobbelé et al., 2003). Earlier studies (Curio et al., 1994; Hashimoto et al., 1996) have proposed that reduced synchrony of HFOs could result in phase-cancellation resulting in reduced activity of event-locked responses. The electrode that demonstrated significant differences between the two groups is shown in Figure 30.A, and are marked in four regions. The lateral electrodes at lower cervical levels as well as at upper cervical levels showcased the greatest differences. Interestingly, the time-frequency characteristics demonstrated distinct high-frequency wide bands for lower cervical regions (region a., b.) and upper cervical regions (region c., d.) that showed differences between the two groups. The lower cervical regions showcased differences in the 400-600Hz band whereas the upper cervical regions in the 600 – 800Hz band. In the 400 – 600 Hz band for lower cervical, pwMS group showcased decreased activity between 8ms – 15ms followed by an increased activity between 15ms – 20ms compared to healthy controls. This might further indicate the disruption of synchronization of high-frequency activity during the 8ms-15ms period and a prolonged non-synchronous activity that extends

till 20ms post-stimulation. Furthermore, the observed difference in the upper cervical region might indicate disrupted activation of interneurons between the lower and upper cervical area. This could indicate the potential activity of propriospinal premotor interneurons, which are known to communicate between upper cervical (C3-C4) and lower cervical (C5-C7) levels (Cramer et al., 2014; Haines et al., 2018). These interneurons might synchronize at the higher frequency band of 600 – 800 Hz, similar high-frequency band (600 Hz) activation has been observed in the somatosensory cortex (Curio, 2000; Gobbelé et al., 2003; Ozaki and Hashimoto, 2011).

The findings of this study suggest that the characteristics of high-frequency oscillations in the spine could serve as a potential biomarker for clinicians to examine the synchronization of neural activity. A decrease in peak RMS amplitude and changes in the spatio-spectral profile of spinal activity may indicate phasic and interneuronal communication disruptions that could uncover additional information during assessment in conjunction with the somatosensory evoked potentials. Further investigation of evoked HFOs is required to determine the underlying neurophysiological principles responsible for generating the synchronous high-frequency activity. Investigation of source-localized spinal activity could further provide additional information regarding the area of activity in the spinal grey matter.

VII. Discussion

1. Summary of the results

1.1. Standardized Electrode Placement System

In this project, we proposed an electrode placement system named SC10X/U to standardize the electrode location w.r.t. the spinal space. The standardization is essential due to the inherent anatomical differences between individuals based on their body dimensions. The location of an electrode in a non-standardized system can vary considerably based on the height, width, and other anatomical differences. In my opinion, this has been one of the biggest limitations of recent studies incorporating high-density electrode systems to record spinal signals, where electrodes with the same inter-electrode (eg. 2 cm inter-electrode distance) distance might not translate to the same location between individuals based on their body characteristics. This variability largely limits the group analysis between

individuals and study centres. In 1958 a need for standardization was recognized for EEG recording and 10-20 system was proposed by Jasper, HH. (H, 1958). Similarly, here we recognize the need for an electrode placement system for ESG and propose SC10X/U system.

The SC10X/U system defines the electrode positions of 76 electrodes that can be used to record the neuroelectric signals over the standardized space that encompasses the cervical and upper thoracic (up to T4) spinal cord regions. This standardized electrode space was established based on 16 predefined anatomical locations, as illustrated in Figure 14. In the SC10X/U system, the median transverse section of the electrode space is divided into 10 equal segments (10% proportion). On the other hand, the medial-sagittal space, aligning with the length and direction of the spinal cord, is divided in proportion to the inter-vertebral spinous distances contained within the electrode space (X%, signifying proportional to inter-vertebral distances).

A nomenclature for the electrode labels of the SC10X/U system was also established. The nomenclature utilized two capital letters followed by a number to define the electrode position. The letters signified approximate transverse locations to identify the position in relation to dorsal-midline, posterior, lateral, or anterior cross-sectional regions. The SC10X/U electrode placement system mapped the spinal cord (till T4 spinous process) to an electrode space and the electrode positions were standardized by systematically dividing the space.

1.2. Spinal Activity in response to median nerve stimulation

This section discusses the results obtained in response to the median nerve stimulation applied at the wrist at 1.5x MT. Spinal somatosensory evoked potentials and evoked high-frequency oscillatory activity were investigated for the high-density ESG channels recorded during the stimulation.

In healthy control group, spinal Somatosensory evoked potentials (sSEP) P9, N11, and N13 were recorded from the dorsal-midline electrodes at a latency of 9ms, 11ms, and 13ms respectively. A highly dorso-ipsilateral spatial activity was recorded for N13 response mapped using the SC10X/U electrode space, with a dorsal epicenter at M4-ML6 positions. The ML4-ML6 electrode position is indicative of the C5-C7 spinal levels, which is supported by previous literature (Cruccu et al., 2008; Mauguière, 2003). and the spinal

representation of the dermatomes for the upper extremities (Cramer et al., 2014). The N13 potential is proposed to be generated by the postsynaptic activity at the dorsal horn (Jeanmonod et al., 1989), this could be indicative of the reversal of polarity observed between the ML6 (dorsal-midline, Cv6) and A12 (anterior, Cv6) electrode positions. The N11 potentials are believed to signify the very high-frequency oscillations observed in the spinal cord (Prestor et al., 1997). We analyzed the high-frequency oscillations in the 300Hz - 1250Hz wide frequency band. A high-frequency oscillatory activity was observed with an onset latency of ~8ms and lasted for a duration of ~7ms. A peak HFO activity was observed at a latency of ~11ms, suggestive of the N11 potential observed during sSEP analysis. Increased spectral activity in the 400Hz - 600Hz frequency band was observed for the duration of the evoked HFOs as compared to the baseline, with the highest increase at ~11ms post-stimulation. Spinal HFOs showcased highly synchronized event-locked activity for the HFO duration, this has been observed in previous studies (Curio et al., 1994; Hashimoto et al., 1996). HFOs are proposed to be generated with high synchronization and phase-locking.

a. Evoked HFOs a marker for (dys)synchronous spinal activity?

The evoked spinal activity in the wide high-frequency band (300Hz - 1250Hz) was investigated in pwMS. No significant differences in onset latency, HFO duration, or latency of peak HFO activity were observed between pwMS and the control group. Significant difference in RMS amplitude at peak HFO activity was observed, pwMS showcased significantly decreased amplitude as compared to the control group. The reduced RMS amplitude might signify reduced synchronization of the high-frequency spinal activity, the time-frequency analysis indicated similar results. The difference in time-frequency signals between pwMS and control at lower cervical levels indicated reduced spectral power in 300Hz – 600Hz between 8ms-15ms but an increased spectral power was observed between 15ms-20ms. The 8ms-15ms corresponds to the duration of the synchronous HFO activity, a decreased power in this period with an increase in post 15ms might signify disruption in synchronization. This disruption in synchronization could be the result of demyelination resulting in varying delays in conduction, a similar observation was found in somatosensory HFOs in pwMS (Gobbelé et al., 2003).

b. sSEPs vs evoked HFOs

The N13 SEP and HFOs (300Hz - 600Hz) band demonstrated different spatial patterns indicating distinct generators. N13 SEP is proposed to be generated by postsynaptic dorsal root activity while the HFOs in 300Hz - 600Hz are suspected to be generated by presynaptic activity (Insola et al., 2008). In our study, the HFOs showcased localized lateral activity at lower cervical electrodes whereas N13 SEP demonstrated a more widespread dorsal-ipsilateral spatial pattern with its epicenter at lower cervical region. HFOs in the thalamus and somatosensory cortex are suspected to be generated in a localized area, similar localized activity was observed in the spinal cord as well. Additionally, the spatial pattern was indicative of the involvement of different spinal lamina for HFOs and N13 SEPs though further investigation is required.

1.3. Neural Oscillations in Spinal Cord

Neural oscillations in defined frequency bands like the beta band are reported to signify distinct neurophysiological characteristics in the sensorimotor cortex and descending pathways during motor control. We examined the neural oscillatory signals from the lower cervical spinal cord in the beta band, low-gamma band, and high-gamma band during the sustained phase of isometric contraction.

Beta-banded spinal oscillations demonstrated a significant ipsi-anterolateral activity, indicating the potential activation of the ventral horn for descending motor control. Sustained beta-banded activity was observed for the sustained phase of the isometric contraction in the ipsi-anterolateral electrode region. The dorsal midline and contra-anterolateral electrode region did not demonstrate the sustained beta band oscillatory activity during the sustained phase. The execution period and pre-motor phase did not indicate any sustained beta-banded oscillatory activity for any of the three regions. Beta-banded oscillations in ipsi-antrolateral region indicated a direct involvement in the motor unit activation and active descending control. In the low-gamma band, no significant ipsi-lateralized spinal activation was observed. The dorsal midline showcased high spectral activity, and a significant difference between the low-gamma band spectral activity of dorsal-midline and contra-anterolateral electrodes was observed. The dorsal gamma band indicated a high spectral activity for the contraction phase as compared to the pre-motor phase. Unlike dorsal-midline, a gradual increase of low-gamma banded spectral activity

was observed for ipsi-anterolateral electrodes, this activity gradually increased from zero to a significant spectral activity for the later part of the sustained activity.

Bi-lateral activation was observed for the high-gamma band oscillatory spinal activity. The high-gamma oscillations demonstrated transient activation during the execution and termination period of the contraction phase. No sustained high-gamma activity was observed for any of the three spinal regions.

a. Cortico-Spinal Coherence: A measure of functional connectivity

We investigated the functional connectivity between the brain regions (contralateral motor, central midline, and ipsilateral motor) and spinal cord regions (ipsi-anterolateral, dorsal-midline, and contra-anterolateral) at the lower cervical level during the sustained phase of the isometric contraction using coherence analysis.

The CSC between the contralateral motor region and the ipsi-anterolateral spinal region demonstrated a significant correlation in the beta band. The central midline and dorsal-midline spinal region demonstrated significant coherence in the low-gamma region. Additionally, both contra and ipsi anterolateral regions showcased significant coherence with the same-sided motor cortex, i.e. ipsi-ipsi CSC and contra-contra CSC in the low-gamma band.

Interestingly, the central midline showcased significant coherence with all three spinal regions at 20Hz. This indicated that the central midline demonstrates a frequency specificity but spinal bilaterality at 20Hz frequency.

To our knowledge, this is the first study to non-invasively evaluate the spatio-spectral characteristics of spinal neural activity and its coherence with brain activity.

2. Impact and Advantages of Investigating Spinal Cord using HD-ESG

2.1. Assessment of spinal (dys)function

As briefly described in the section I.5 of Chapter I, multiple indirect measures of assessing descending and ascending pathways and motor neurons have been designed to investigate the spinal integrity in people with motor disorders. These assessments include MUNIX and CMAP (Dengler et al., 2020; Nandedkar et al., 2004) for evaluating LMNs, and TMS and

F-waves (Jerath and Kimura, 2019; Zewdie and Kirton, 2016) for assessing the UMN. The current techniques for assessing spinal functionality rely heavily on indirect inferences, which have left much to be desired in our understanding of the neurophysiological characteristics that are altered along the spinal cord in motor disorders. The proposed HD-ESG directly records the spinal activity and its responses to evaluate the spinal function and spinal integrity. This provides a method that has the potential to uncover the neurophysiological understanding and enhance the information attained by the assessment techniques.

We utilized HD-ESG to evaluate the spinal activity and its spatio-spectral characteristics that highlight the neurophysiological basis of spinal activity in response to median nerve stimulation and during voluntary control. The results of spinal responses indicated sSEPs and HFOs might have distinct neural generators and their potential as markers of spinal function/dysfunction. The HD-ESG signals were analyzed during voluntary control in healthy individuals, which interestingly highlighted the role of neural oscillatory frequency during isometric contraction. The potential of being able to record and analyze spinal activity in response to external stimulation or during voluntary control can provide novel clinical features for the assessment of motor disorders.

The HD-ESG recorded in conjunction with other neurophysiological signals like HD-EEG or sEMG provides a way of better assessing the functionality of ascending and descending pathways. The CSC assessed the functional connectivity between the cortex and the lower spinal cord, providing a novel method of assessing the functional connectivity of the descending and ascending tracts between the brain and spinal cord. Earlier, CMC (Bista et al., 2023; Coffey et al., 2021) has been utilized to assess the descending pathways, this includes both UMN and LMN connections. The potential of incorporating spinal activity and calculating CSC or SMC (spino-muscular coherence) can provide much-needed independent information regarding the UMN and LMN functional connectivity. Additionally, CSC and SMC in non-beta frequency bands can provide neurophysiological information related to sensorimotor integration that could further improve our understanding of sensorimotor control in health and disease.

2.2. Uncovering oscillatory characteristics of SC and a step forward in understanding sensorimotor control

Neural oscillations in the cortex are proposed to be an important feature of information transfer and neural signalling (Buzsáki and Draguhn, 2004). During the motor activity, cortical oscillations in beta, low-gamma, and high-gamma frequency bands are proposed to signify the cortical representation of the underlying neurophysiological process. Beta band oscillation has been widely observed as a characteristic oscillatory pattern over the sensorimotor cortex and has been proposed to signify an ‘active akinetic’ neurophysiological process that promotes existing active contraction while inhibiting (Brown and Williams, 2005; Engel and Fries, 2010). Gamma oscillations are reported to be more localized and seem to be related to sensorimotor integration (Aoki et al., 1999; Ihara et al., 2003) and prokinetic during movement execution (Brown, 2003; Joundi et al., 2012). The neural oscillations in the cortex are comparatively widely studied in different domains including motor control, motor planning, motor learning, resting state, and cognition but its counterpart in the spinal cord has been very limited.

We non-invasively investigated the oscillatory characteristics of spinal activity during motor control. To our knowledge, this is the first of its kind to non-invasively evaluate the characteristics of spinal oscillatory activity during voluntary motor control. We demonstrated that spinal activity shows characteristic oscillatory properties in the lower cervical cord. The neural oscillations in the spinal cord demonstrated frequency band-dependent spatial and temporal properties during motor control. This could indicate that the oscillations might related to similar neurophysiological processes in the entire sensorimotor pathways including spinal cord. The oscillations that were observed to be an important feature of the cortex by Buzsáki and Draguhn (Buzsáki and Draguhn, 2004) might be an inherent property of neural communication and information transmission that includes the spinal cord. The frequency band-specific neurophysiological significance were observed to be intact in the spinal cord and feature similar significance as that of supraspinal regions, i.e. beta band closely related to the descending pathway while the low-gamma band was observed in the sensorimotor integration.

The understanding of neural communication in the spinal cord and its iterations with brain and brain could be a feature of a better understanding of sensorimotor control. A recent study of epidural recording of spinal activity during rest-state demonstrated distinct delta

and alpha band activity during eyes-open and eyes-closed paradigms similar to that of the cortex (Wang et al., 2021). Interestingly, a recent fMRI (N. Kinany et al., 2023) study indicated the role of the spinal cord during motor learning. We believe that HD-ESG and the investigation of neural oscillatory properties of the spinal cord be a novel step forward in our understanding of sensorimotor communication.

3. Limitations

In this section, general limitations that influenced the design of this project or limited the analyses/interpretation of results within this project are discussed.

3.1. Participant Recruitment and Small Sample Size

The project began during the peak of the COVID-19 pandemic, which made it difficult to recruit participants until 2022. The Clinical Research Facility (CRF) was operating at a limited capacity for a couple of months after the COVID-19 pandemic, which further restricted the number of participants as the study required in-person attendance. Additional challenges were encountered in recruiting participants due to administrative delays faced in setting up the study at the CRF, St. James Hospital.

In this thesis, the focus was on developing a new method for recording and evaluating spinal activity in both healthy and diseased states. Multiple preliminary participant recruitments were carried out at each stage of the project's development. However, in this thesis, only results from the final design are discussed, as the optimal standard for recording was achieved. This resulted in a lower sample size of participants who were assessed during the final phase of the project. To take into account the sample size, non-parametric measurements were used to evaluate the statistical significance (Nasserroleslami et al., 2019).

3.2. Non-Accessibility for highly impaired participants

The setup requires the participants to sit on a physio-chair and perform pincer-grip task. The overall setup including EEG, ESG, EMG, and EXG electrode placement takes around 1.5 hours. This requires the participant to have moderate lower limb functioning, moderate ability to balance, and moderate upper limb functionality to perform the task. This makes the experiment paradigm not suitable for highly impaired individuals and non-accessible to

the participants in wheelchairs. This could lead to a bias where highly impaired individuals are excluded from the study, resulting in the research being conducted only on mildly impaired individuals within a specific group of neurodegenerative conditions.

4. Future Work

4.1. Data Collection

As discussed above the small sample size as being one of the limitations. The EEG/ESG/EMG/ECG/ExG data collection will continue for the healthy control and pwMS group. This will make sure that in the future we have a reasonable sample size that can provide sufficient power to the analysis. Additionally, other neurological conditions that affect the spinal cord will be studied in the future. Particularly, the conditions that specifically affect either UMN in the case of PLS or LMNs in the case of SMA along with ALS in which both LMN and UMN are affected.

4.2. Spinal Neural Oscillations in pwMS

Chapter V discusses the neural oscillatory activity in different frequency bands during voluntary isometric activity in the healthy population. In the future, a similar analysis can be conducted for people with multiple sclerosis to evaluate their neural oscillatory characteristics. By examining neural oscillations in pwMS, it is possible to uncover neurophysiological changes in spinal activity that may serve as biomarkers for diagnosing and prognosing the condition. Additionally, studying the neural oscillatory changes in different frequency bands can complement cortical findings and offer insights into the complete sensorimotor control pathway.

4.3. Investigating Spinal Activity during Motor Planning and Post-Motor Period

The primarily focus of this project was on the sustained isometric contraction period of the pinch-grip activity. However, intriguing findings from the ERSF results suggest distinct characteristics during other periods. This indicates the need for further evaluation of the frequency bands to understand the role of the spinal cord during motor planning and the post-motor recovery period. Bista et al. (Bista, 2024) demonstrated unique cortical activity during the pre-motor and post-motor phases of the pinch-grip activity. Recent fMRI studies

discussed in section I.3.1 of chapter I have also suggested that the spinal cord may exhibit distinct activity during motor planning and motor learning. These findings, combined with our preliminary observations, warrant an investigation into the electrophysiological properties of spinal activity during these periods. In the future, an analysis pipeline inspired by our results will be implemented to study the characteristics of spinal activity during motor-planning and post-motor recovery period.

4.4. Connectivity Measures with Brain and Muscle

The spinal cord is an integral of the sensorimotor communication and features a connection with both muscle and the brain. As discussed in the results section the coherence between brain and spinal cord activity (corticospinal coherence) was investigated in healthy participants. In the future, we aim to investigate the coherence between spinal cord activity and muscle activity (Spino-muscular coherence). This should further enhance our understanding of the role played by the spinal cord during sensorimotor communication. These coherence (corticospinal and spino-muscular) analyses will be further investigated in neurodegenerative conditions to further understand the neurophysiological changes in the sensorimotor pathways during degeneration that might help uncover novel biomarkers.

VIII. Bibliography

- Abdel-Aziz, K., Schneider, T., Solanky, B.S., Yiannakas, M.C., Altmann, D.R., Wheeler-Kingshott, C.A.M., Peters, A.L., Day, B.L., Thompson, A.J., Ciccarelli, O., 2015. Evidence for early neurodegeneration in the cervical cord of patients with primary progressive multiple sclerosis. *Brain* 138, 1568–1582. <https://doi.org/10.1093/brain/awv086>
- Adachi, Y., Oyama, D., Kawai, J., Kawabata, S., Uehara, G., 2013. Spinal cord evoked magnetic field measurement using a magnetospinography system equipped with a cryocooler, in: 2013 35th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC). Presented at the 2013 35th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC), IEEE, Osaka, pp. 4426–4429. <https://doi.org/10.1109/EMBC.2013.6610528>
- Agosta, F., Rocca, M.A., Valsasina, P., Sala, S., Caputo, D., Perini, M., Salvi, F., Prella, A., Filippi, M., 2009. A longitudinal diffusion tensor MRI study of the cervical cord

- and brain in amyotrophic lateral sclerosis patients. *J. Neurol. Neurosurg. Psychiatry* 80, 53–55. <https://doi.org/10.1136/jnnp.2008.154252>
- Ahn, S.-W., Kim, S.-H., Kim, J.-E., Kim, S.-M., Kim, S.H., Park, K.S., Sung, J.-J., Lee, K.-W., Hong, Y.-H., 2010. Reproducibility of the motor unit number index (MUNIX) in normal controls and amyotrophic lateral sclerosis patients. *Muscle Nerve* 42, 808–813. <https://doi.org/10.1002/mus.21765>
- Akaza, M., Kawabata, S., Ozaki, I., Miyano, Y., Watanabe, T., Adachi, Y., Sekihara, K., Sumi, Y., Yokota, T., 2020. Noninvasive measurement of sensory action currents in the cervical cord by magnetospinography. *Clin. Neurophysiol.* 132, 382–391. <https://doi.org/10.1016/j.clinph.2020.11.029>
- Alstermark, B., Isa, T., Pettersson, L. -G., Sasaki, S., 2007. The C3–C4 propriospinal system in the cat and monkey: a spinal pre-motoneuronal centre for voluntary motor control. *Acta Physiol.* 189, 123–140. <https://doi.org/10.1111/j.1748-1716.2006.01655.x>
- Alusi, S.H., Macerollo, A., MacKinnon, C.D., Rothwell, J.C., Bain, P.G., 2021. Tremor and Dysmetria in Multiple Sclerosis: A Neurophysiological Study. *Tremor Hyperkinetic Mov.* 11. <https://doi.org/10.5334/tohm.598>
- Andelova, M., Uher, T., Krasensky, J., Sobisek, L., Kusova, E., Srpova, B., Vodehnalova, K., Friedova, L., Motyl, J., Preiningerova, J.L., Kubala Havrdova, E., Horakova, D., Vaneckova, M., 2019. Additive Effect of Spinal Cord Volume, Diffuse and Focal Cord Pathology on Disability in Multiple Sclerosis. *Front. Neurol.* 10, 820. <https://doi.org/10.3389/fneur.2019.00820>
- Aoki, F., Fetz, E.E., Shupe, L., Lettich, E., Ojemann, G.A., 1999. Increased gamma-range activity in human sensorimotor cortex during performance of visuomotor tasks. *Clin. Neurophysiol.* 110, 524–537. [https://doi.org/10.1016/S1388-2457\(98\)00064-9](https://doi.org/10.1016/S1388-2457(98)00064-9)
- Arrambide, G., Rovira, A., Sastre-Garriga, J., Tur, C., Castilló, J., Río, J., Vidal-Jordana, A., Galán, I., Rodríguez-Acevedo, B., Midaglia, L., Nos, C., Mulero, P., Arévalo, M.J., Comabella, M., Huerga, E., Auger, C., Montalban, X., Tintore, M., 2018. Spinal cord lesions: A modest contributor to diagnosis in clinically isolated

- syndromes but a relevant prognostic factor. *Mult. Scler.* Houndmills Basingstoke Engl. 24, 301–312. <https://doi.org/10.1177/1352458517697830>
- Axente, M., Mirea, A., Sporea, C., Pădure, L., Drăgoi, C.M., Nicolae, A.C., Ion, D.A., 2022. Clinical and Electrophysiological Changes in Pediatric Spinal Muscular Atrophy after 2 Years of Nusinersen Treatment. *Pharmaceutics* 14, 2074. <https://doi.org/10.3390/pharmaceutics14102074>
- Backes, W.H., Mess, W.H., Wilmink, J.T., 2001. Functional MR Imaging of the Cervical Spinal Cord by Use of Median Nerve Stimulation and Fist Clenching. *Am. J. Neuroradiol.* 22, 1854–1859.
- Baker, S.N., 2007. Oscillatory interactions between sensorimotor cortex and the periphery. *Curr. Opin. Neurobiol., Motor systems / Neurobiology of behaviour* 17, 649–655. <https://doi.org/10.1016/j.conb.2008.01.007>
- Baker, S.N., Olivier, E., Lemon, R.N., 1997. Coherent oscillations in monkey motor cortex and hand muscle EMG show task-dependent modulation. *J. Physiol.* 501, 225–241.
- Ball, T., Demandt, E., Mutschler, I., Neitzel, E., Mehring, C., Vogt, K., Aertsen, A., Schulze-Bonhage, A., 2008. Movement related activity in the high gamma range of the human EEG. *NeuroImage* 41, 302–310. <https://doi.org/10.1016/j.neuroimage.2008.02.032>
- Ball, T., Schulze-Bonhage, A., Aertsen, A., Mehring, C., 2009. Differential representation of arm movement direction in relation to cortical anatomy and function. *J. Neural Eng.* 6, 016006. <https://doi.org/10.1088/1741-2560/6/1/016006>
- Bankim Subhash Chander, Matthias Deliano, Elena Azañón, Lars Büntjen, Max-Philipp Stenner, 2022. Non-invasive recording of high-frequency signals from the human spinal cord. *NeuroImage* 253, 119050–119050. <https://doi.org/10.1016/j.neuroimage.2022.119050>
- Bao, S.-C., Chen, C., Yuan, K., Yang, Y., Tong, R.K.-Y., 2021. Disrupted cortico-peripheral interactions in motor disorders. *Clin. Neurophysiol.* 132, 3136–3151. <https://doi.org/10.1016/j.clinph.2021.09.015>
- Barker, A.T., Jalinous, R., Freeston, I.L., 1985. NON-INVASIVE MAGNETIC STIMULATION OF HUMAN MOTOR CORTEX. *The Lancet*, Originally

published as Volume 1, Issue 8437 325, 1106–1107. [https://doi.org/10.1016/S0140-6736\(85\)92413-4](https://doi.org/10.1016/S0140-6736(85)92413-4)

- Bayram, M.B., Suviseshamuthu, E.S., Plow, E.B., Forrest, G.F., Yue, G.H., 2023. Aging-induced alterations in EEG spectral power associated with graded force motor tasks. *Exp. Brain Res.* 241, 905–915. <https://doi.org/10.1007/s00221-023-06572-2>
- Bede, P., Bokde, A.L.W., Byrne, S., Elamin, M., Fagan, A.J., Hardiman, O., 2012. Spinal cord markers in ALS: Diagnostic and biomarker considerations. *Amyotroph. Lateral Scler.* 13, 407–415. <https://doi.org/10.3109/17482968.2011.649760>
- Bede, P., Hardiman, O., 2014. Lessons of ALS imaging: Pitfalls and future directions — A critical review. *NeuroImage Clin.* 4, 436–443. <https://doi.org/10.1016/j.nicl.2014.02.011>
- Benjamini, Y., Hochberg, Y., 1995. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *J. R. Stat. Soc. Ser. B Methodol.* 57, 289–300. <https://doi.org/10.1111/j.2517-6161.1995.tb02031.x>
- Benjamini, Y., Krieger, A.M., Yekutieli, D., 2006. Adaptive linear step-up procedures that control the false discovery rate. *Biometrika* 93, 491–507. <https://doi.org/10.1093/biomet/93.3.491>
- Benmouna, K., Milants, C., Wang, F.C., 2018. Correlations between MUNIX and adapted multiple point stimulation MUNE methods. *Clin. Neurophysiol.* 129, 341–344. <https://doi.org/10.1016/j.clinph.2017.11.012>
- Berardelli, A., Inghilleri, M., Formisano, R., Accornero, N., Manfredi, M., 1987. Stimulation of motor tracts in motor neuron disease. *J. Neurol. Neurosurg. Psychiatry* 50, 732–737. <https://doi.org/10.1136/jnnp.50.6.732>
- Biberacher, V., Boucard, C.C., Schmidt, P., Engl, C., Buck, D., Berthele, A., Hoshi, M.-M., Zimmer, C., Hemmer, B., Mühlau, M., 2015. Atrophy and structural variability of the upper cervical cord in early multiple sclerosis. *Mult. Scler. Houndmills Basingstoke Engl.* 21, 875–884. <https://doi.org/10.1177/1352458514546514>
- Bischoff, C., Schoenle, P.W., Conrad, B., 1992. Increased F-wave duration in patients with spasticity. *Electromyogr. Clin. Neurophysiol.* 32, 449–453.

- Bista, S., 2024. Network Analysis in Amyotrophic Lateral Sclerosis during Voluntary Motor Task: Neurophysiological Biomarkers of Disrupted Cortical Connections (Thesis). Trinity College Dublin. School of Medicine. Discipline of Clinical Medicine.
- Bista, S., Coffey, A., Fasano, A., Buxo, T., Mitchell, M., Giglia, E.R., Dukic, S., Heverin, M., Muthuraman, M., Carson, R.G., Lowery, M., Hardiman, O., McManus, L., Nasserroleslami, B., 2023. Cortico-muscular coherence in primary lateral sclerosis reveals abnormal cortical engagement during motor function beyond primary motor areas. *Cereb. Cortex* 33, 8712–8723. <https://doi.org/10.1093/cercor/bhad152>
- Boekestein, W.A., Schelhaas, H.J., van Putten, M.J.A.M., Stegeman, D.F., Zwarts, M.J., van Dijk, J.P., 2012. Motor unit number index (MUNIX) versus motor unit number estimation (MUNE): A direct comparison in a longitudinal study of ALS patients. *Clin. Neurophysiol.* 123, 1644–1649. <https://doi.org/10.1016/j.clinph.2012.01.004>
- Bonacchi, R., Pagani, E., Meani, A., Cacciaguerra, L., Preziosa, P., De Meo, E., Filippi, M., Rocca, M.A., 2020. Clinical Relevance of Multiparametric MRI Assessment of Cervical Cord Damage in Multiple Sclerosis. *Radiology* 296, 605–615. <https://doi.org/10.1148/radiol.2020200430>
- Bonferroni, C., 1936. Teoria statistica delle classi e calcolo delle probabilita. *Pubblicazioni R Ist. Super. Sci. Econ. E Commerciali Firenze* 8, 3–62.
- Boonstra, T., 2013. The potential of corticomuscular and intermuscular coherence for research on human motor control. *Front. Hum. Neurosci.* 7.
- Bostock, H., 2016. Estimating motor unit numbers from a CMAP scan. *Muscle Nerve* 53, 889–896. <https://doi.org/10.1002/mus.24945>
- Bot, J.C.J., Barkhof, F., Polman, C.H., Lycklama à Nijeholt, G.J., de Groot, V., Bergers, E., Ader, H.J., Castelijns, J.A., 2004. Spinal cord abnormalities in recently diagnosed MS patients: added value of spinal MRI examination. *Neurology* 62, 226–233. <https://doi.org/10.1212/wnl.62.2.226>
- Bouwman, C.J.C., Wilmink, J.T., Mess, W.H., Backes, W.H., 2008. Spinal cord functional MRI at 3 T: Gradient echo echo-planar imaging versus turbo spin echo. *NeuroImage* 43, 288–296. <https://doi.org/10.1016/j.neuroimage.2008.07.024>

- Bradshaw, L.A., Wikswo, Jr., J.P., 2001. Spatial Filter Approach for Evaluation of the Surface Laplacian of the Electroencephalogram and Magnetoencephalogram. *Ann. Biomed. Eng.* 29, 202–213. <https://doi.org/10.1114/1.1352642>
- Branco, L.M.T., De Albuquerque, M., De Andrade, H.M.T., Bergo, F.P.G., Nucci, A., França, M.C., 2014. Spinal cord atrophy correlates with disease duration and severity in amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler. Front. Degener.* 15, 93–97. <https://doi.org/10.3109/21678421.2013.852589>
- Brown, P., 2003. Oscillatory nature of human basal ganglia activity: Relationship to the pathophysiology of Parkinson's disease. *Mov. Disord.* 18, 357–363. <https://doi.org/10.1002/mds.10358>
- Brown, P., 2000. Cortical drives to human muscle: the Piper and related rhythms. *Prog. Neurobiol.* 60, 97–108. [https://doi.org/10.1016/S0301-0082\(99\)00029-5](https://doi.org/10.1016/S0301-0082(99)00029-5)
- Brown, P., Williams, D., 2005. Basal ganglia local field potential activity: Character and functional significance in the human. *Clin. Neurophysiol.* 116, 2510–2519. <https://doi.org/10.1016/j.clinph.2005.05.009>
- Brown, W.F., Milner-Brown, H.S., 1976. Some electrical properties of motor units and their effects on the methods of estimating motor unit numbers. *J. Neurol. Neurosurg. Psychiatry* 39, 249–257. <https://doi.org/10.1136/jnnp.39.3.249>
- Bruel, A., Abadía, I., Collin, T., Sakr, I., Lorach, H., Luque, N.R., Ros, E., Ijspeert, A., 2024. The spinal cord facilitates cerebellar upper limb motor learning and control; inputs from neuromusculoskeletal simulation. *PLOS Comput. Biol.* 20, e1011008. <https://doi.org/10.1371/journal.pcbi.1011008>
- Buzsáki, G., Draguhn, A., 2004. Neuronal Oscillations in Cortical Networks. *Science* 304, 1926–1929. <https://doi.org/10.1126/science.1099745>
- Cao, B., Wei, Q., Ou, R., Zhang, L., Hou, Y., Chen, Y., Shang, H., 2019. Neurophysiological index is associated with the survival of patients with amyotrophic lateral sclerosis. *Clin. Neurophysiol.* 130, 1730–1733. <https://doi.org/10.1016/j.clinph.2019.05.012>
- Carvalhoes, C., de Barros, J.A., 2015. The surface Laplacian technique in EEG: Theory and methods. *Int. J. Psychophysiol.* 97, 174–188. <https://doi.org/10.1016/j.ijpsycho.2015.04.023>

- Carvalho, M. de, Scotto, M., Lopes, A., Swash, M., 2002. F-Waves and the corticospinal lesion in amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler. Other Motor Neuron Disord.* 3, 131–136. <https://doi.org/10.1080/146608202760834139>
- Casserly, C., Seyman, E.E., Alcaide-Leon, P., Guenette, M., Lyons, C., Sankar, S., Svendrovski, A., Baral, S., Oh, J., 2018. Spinal Cord Atrophy in Multiple Sclerosis: A Systematic Review and Meta-Analysis. *J. Neuroimaging Off. J. Am. Soc. Neuroimaging* 28, 556–586. <https://doi.org/10.1111/jon.12553>
- Chakarov, V., Naranjo, J.R., Schulte-Mönting, J., Omlor, W., Huethe, F., Kristeva, R., 2009. Beta-Range EEG-EMG Coherence With Isometric Compensation for Increasing Modulated Low-Level Forces. *J. Neurophysiol.* 102, 1115–1120. <https://doi.org/10.1152/jn.91095.2008>
- Chakraborty, P., Saha, S., Deco, G., Banerjee, A., Roy, D., 2023. Structural-and-dynamical similarity predicts compensatory brain areas driving the post-lesion functional recovery mechanism. *Cereb. Cortex Commun.* tgad012. <https://doi.org/10.1093/texcom/tgad012>
- Chatrian, G.E., Lettich, E., Nelson, P.L., 1985. Ten Percent Electrode System for Topographic Studies of Spontaneous and Evoked EEG Activities. *Am. J. EEG Technol.* 25, 83–92. <https://doi.org/10.1080/00029238.1985.11080163>
- Chen, Y., Haacke, E.M., Bernitsas, E., 2020. Imaging of the Spinal Cord in Multiple Sclerosis: Past, Present, Future. *Brain Sci.* 10, 857. <https://doi.org/10.3390/brainsci10110857>
- Chroni, E., Veltsista, D., Papapaulou, C., Trachani, E., 2017. Generation of Repeater F Waves in Healthy Subjects. *J. Clin. Neurophysiol.* 34, 236. <https://doi.org/10.1097/WNP.0000000000000360>
- Cioni, B., Meglio, M., 1986. Epidural Recordings of Electrical Events Produced in the Spinal Cord by Segmental, Ascending and Descending Volleys. *Stereotact. Funct. Neurosurg.* 49, 315–326. <https://doi.org/10.1159/000100161>
- Coffey, A., Bista, S., Fasano, A., Buxo, T., Mitchell, M., Giglia, E.R., Dukic, S., Fenech, M., Barry, M., Wade, A., Heverin, M., Muthuraman, M., Carson, R.G., Lowery, M., Hardiman, O., Nasserolelami, B., 2021. Altered supraspinal motor networks in

- survivors of poliomyelitis: A cortico-muscular coherence study. *Clin. Neurophysiol.* 132, 106–113. <https://doi.org/10.1016/j.clinph.2020.10.011>
- Cohen-Adad, J., 2017. Functional Magnetic Resonance Imaging of the Spinal Cord: Current Status and Future Developments. *Semin. Ultrasound CT MRI, Spinal Cord Imaging, Part 2* 38, 176–186. <https://doi.org/10.1053/j.sult.2016.07.007>
- Cohen-Adad, J., Mendili, M.-M.E., Morizot-Koutlidis, R., Lehericy, S., Meininger, V., Blanche, S., Rossignol, S., Benali, H., Pradat, P.-F., 2013. Involvement of spinal sensory pathway in ALS and specificity of cord atrophy to lower motor neuron degeneration. *Amyotroph. Lateral Scler. Front. Degener.* 14, 30–38. <https://doi.org/10.3109/17482968.2012.701308>
- Collorone, S., Cawley, N., Grussu, F., Prados, F., Tona, F., Calvi, A., Kanber, B., Schneider, T., Kipp, L., Zhang, H., Alexander, D.C., Thompson, A.J., Toosy, A., Wheeler-Kingshott, C.A.G., Ciccarelli, O., 2020. Reduced neurite density in the brain and cervical spinal cord in relapsing–remitting multiple sclerosis: A NODDI study. *Mult. Scler. J.* 26, 1647–1657. <https://doi.org/10.1177/1352458519885107>
- Conway, B.A., Halliday, D.M., Farmer, S.F., Shahani, U., Maas, P., Weir, A.I., Rosenberg, J.R., 1995. Synchronization between motor cortex and spinal motoneuronal pool during the performance of a maintained motor task in man. *J. Physiol.* 489, 917–924. <https://doi.org/10.1113/jphysiol.1995.sp021104>
- Cramer, G.D., Darby, S.A., Cramer, G.D., 2014. Clinical anatomy of the spine, spinal cord, and ANS, 3rd ed. ed. Elsevier, St. Louis, Mo.
- Crone, N.E., Miglioretti, D.L., Gordon, B., Lesser, R.P., 1998a. Functional mapping of human sensorimotor cortex with electrocorticographic spectral analysis. II. Event-related synchronization in the gamma band. *Brain* 121, 2301–2315. <https://doi.org/10.1093/brain/121.12.2301>
- Crone, N.E., Miglioretti, D.L., Gordon, B., Sieracki, J.M., Wilson, M.T., Uematsu, S., Lesser, R.P., 1998b. Functional mapping of human sensorimotor cortex with electrocorticographic spectral analysis. I. Alpha and beta event-related desynchronization. *Brain* 121, 2271–2299. <https://doi.org/10.1093/brain/121.12.2271>

- Crucchi, G., Aminoff, M.J., Curio, G., Guerit, J.-M., Kakigi, R., Mauguière, F., Rossini, P.M., Treede, R.-D., Garcia-Larrea, L., 2008. Recommendations for the clinical use of somatosensory-evoked potentials. *Clin. Neurophysiol.* 119, 1705–1719. <https://doi.org/10.1016/j.clinph.2008.03.016>
- Curio, G., 2000. Linking 600-Hz “Spikelike” EEG/MEG Wavelets (“ $\square$ -Bursts”) to Cellular Substrates. *J Clin Neurophysiol* 17.
- Curio, G., Mackert, B.M., Burghoff, M., Koetitz, R., Abraham-Fuchs, K., Härer, W., 1994. Localization of evoked neuromagnetic 600 Hz activity in the cerebral somatosensory system. *Electroencephalogr. Clin. Neurophysiol.* 91, 483–487. [https://doi.org/10.1016/0013-4694\(94\)90169-4](https://doi.org/10.1016/0013-4694(94)90169-4)
- Daams, M., Weiler, F., Steenwijk, M.D., Hahn, H.K., Geurts, J.J., Vrenken, H., van Schijndel, R.A., Balk, L.J., Tewarie, P.K., Tillema, J.-M., Killestein, J., Uitdehaag, B.M., Barkhof, F., 2014. Mean upper cervical cord area (MUCCA) measurement in long-standing multiple sclerosis: relation to brain findings and clinical disability. *Mult. Scler. Houndmills Basingstoke Engl.* 20, 1860–1865. <https://doi.org/10.1177/1352458514533399>
- Daube, J.R., 1985. Electrophysiologic Studies in the Diagnosis and Prognosis of Motor Neuron Diseases. *Neurol. Clin., Electrodiagnosis* 3, 473–493. [https://doi.org/10.1016/S0733-8619\(18\)31017-X](https://doi.org/10.1016/S0733-8619(18)31017-X)
- Davare, M., Duque, J., Vandermeeren, Y., Thonnard, J.-L., Olivier, E., 2007. Role of the Ipsilateral Primary Motor Cortex in Controlling the Timing of Hand Muscle Recruitment. *Cereb. Cortex* 17, 353–362. <https://doi.org/10.1093/cercor/bhj152>
- de Aguiar Neto, F.S., Rosa, J.L.G., 2019. Depression biomarkers using non-invasive EEG: A review. *Neurosci. Biobehav. Rev.* 105, 83–93. <https://doi.org/10.1016/j.neubiorev.2019.07.021>
- de Carvalho, M., Barkhaus, P.E., Nandedkar, S.D., Swash, M., 2018. Motor unit number estimation (MUNE): Where are we now? *Clin. Neurophysiol.* 129, 1507–1516. <https://doi.org/10.1016/j.clinph.2018.04.748>
- de Carvalho, M., Chio, A., Dengler, R., Hecht, M., Weber, M., Swash, M., 2005. Neurophysiological measures in amyotrophic lateral sclerosis: Markers of

- progression in clinical trials. *Amyotroph. Lateral Scler.* 6, 17–28.
<https://doi.org/10.1080/14660820410020600>
- de Carvalho, M., Scotto, M., Lopes, A., Swash, M., 2003. Clinical and neurophysiological evaluation of progression in amyotrophic lateral sclerosis. *Muscle Nerve* 28, 630–633. <https://doi.org/10.1002/mus.10469>
- de Carvalho, M., Swash, M., 2016. Lower motor neuron dysfunction in ALS. *Clin. Neurophysiol.* 127, 2670–2681. <https://doi.org/10.1016/j.clinph.2016.03.024>
- de Carvalho, M., Swash, M., 2010. Sensitivity of electrophysiological tests for upper and lower motor neuron dysfunction in ALS: A six-month longitudinal study. *Muscle Nerve* 41, 208–211. <https://doi.org/10.1002/mus.21495>
- de Carvalho, M., Swash, M., 2000. Nerve conduction studies in amyotrophic lateral sclerosis. *Muscle Nerve* 23, 344–352. [https://doi.org/10.1002/\(SICI\)1097-4598\(200003\)23:3<344::AID-MUS5>3.0.CO;2-N](https://doi.org/10.1002/(SICI)1097-4598(200003)23:3<344::AID-MUS5>3.0.CO;2-N)
- de Cheveigné, A., Wong, D.D.E., Di Liberto, G.M., Hjortkjær, J., Slaney, M., Lalor, E., 2018. Decoding the auditory brain with canonical component analysis. *NeuroImage* 172, 206–216. <https://doi.org/10.1016/j.neuroimage.2018.01.033>
- De Leener, B., Fonov, V.S., Collins, D.L., Callot, V., Stikov, N., Cohen-Adad, J., 2018. PAM50: Unbiased multimodal template of the brainstem and spinal cord aligned with the ICBM152 space. *NeuroImage* 165, 170–179. <https://doi.org/10.1016/j.neuroimage.2017.10.041>
- De Leener, B., Lévy, S., Dupont, S.M., Fonov, V.S., Stikov, N., Louis Collins, D., Callot, V., Cohen-Adad, J., 2017. SCT: Spinal Cord Toolbox, an open-source software for processing spinal cord MRI data. *NeuroImage* 145, 24–43. <https://doi.org/10.1016/j.neuroimage.2016.10.009>
- De Leener, B., Taso, M., Cohen-Adad, J., Callot, V., 2016. Segmentation of the human spinal cord. *Magn. Reson. Mater. Phys. Biol. Med.* 29, 125–153. <https://doi.org/10.1007/s10334-015-0507-2>
- Delorme, A., Mullen, T., Kothe, C., Akalin Acar, Z., Bigdely-Shamlo, N., Vankov, A., Makeig, S., 2011. EEGLAB, SIFT, NFT, BCILAB, and ERICA: New Tools for

- Advanced EEG Processing. *Comput. Intell. Neurosci.* 2011, 1–12.
<https://doi.org/10.1155/2011/130714>
- Dengler, R., de Carvalho, M., Shahrizaila, N., Nodera, H., Vucic, S., Grimm, A., Padua, L., Schreiber, S., Kneiser, M.K., Hobson-Webb, L.D., Boon, A.J., Smith, B.E., Litchy, W.J., Li, Y., Lenihan, M., Thompson, V.B., Stalberg, E., Sanders, D.B., Kincaid, J.C., 2020. AANEM - IFCN Glossary of Terms in Neuromuscular Electrodiagnostic Medicine and Ultrasound. *Muscle Nerve* 62, 10–12.
<https://doi.org/10.1002/mus.26868>
- Denker, M., Roux, S., Lindén, H., Diesmann, M., Riehle, A., Grün, S., 2011. The Local Field Potential Reflects Surplus Spike Synchrony. *Cereb. Cortex* 21, 2681–2695.
<https://doi.org/10.1093/cercor/bhr040>
- Desmedt, J.E., Cheron, G., 1983. Spinal and far-field components of human somatosensory evoked potentials to posterior tibial nerve stimulation analysed with oesophageal derivations and non-cephalic reference recording. *Electroencephalogr. Clin. Neurophysiol.* 56, 635–651. [https://doi.org/10.1016/0013-4694\(83\)90031-7](https://doi.org/10.1016/0013-4694(83)90031-7)
- Desmedt, J.E., Cheron, G., 1981. Prevertebral (oesophageal) recording of subcortical somatosensory evoked potentials in man: The spinal P13 component and the dual nature of the spinal generators. *Electroencephalogr. Clin. Neurophysiol.* 52, 257–275. [https://doi.org/10.1016/0013-4694\(81\)90055-9](https://doi.org/10.1016/0013-4694(81)90055-9)
- Desmedt, J.E., Huy, N.T., 1984. BIT-mapped colour imaging of the potential fields of propagated and segmental subcortical components of somatosensory evoked potentials in man. *Electroencephalogr. Clin. Neurophysiol.* 58, 481–497.
[https://doi.org/10.1016/0013-4694\(84\)90037-3](https://doi.org/10.1016/0013-4694(84)90037-3)
- Detre, J.A., Floyd, T.F., 2001. Functional MRI and Its Applications to the Clinical Neurosciences. *The Neuroscientist* 7, 64–79.
<https://doi.org/10.1177/107385840100700110>
- Di Sapia, A., Bertolotto, A., Melillo, F., Sperli, F., Malucchi, S., Troni, W., 2014. A new neurophysiological approach to assess central motor conduction damage to proximal and distal muscles of lower limbs. *Clin. Neurophysiol.* 125, 133–141.
<https://doi.org/10.1016/j.clinph.2013.06.018>

- Diedrichsen, J., Wiestler, T., Krakauer, J.W., 2013. Two Distinct Ipsilateral Cortical Representations for Individuated Finger Movements. *Cereb. Cortex* 23, 1362–1377. <https://doi.org/10.1093/cercor/bhs120>
- Doherty, T.J., Brown, W.F., 1993. The estimated numbers and relative sizes of thenar motor units as selected by multiple point stimulation in young and older adults. *Muscle Nerve* 16, 355–366. <https://doi.org/10.1002/mus.880160404>
- Dukic, S., McMackin, R., Costello, E., Metzger, M., Buxo, T., Fasano, A., Chipika, R., Pinto-Grau, M., Schuster, C., Hammond, M., Heverin, M., Coffey, A., Broderick, M., Iyer, P.M., Mohr, K., Gavin, B., McLaughlin, R., Pender, N., Bede, P., Muthuraman, M., van den Berg, L.H., Hardiman, O., Nasserouleslami, B., 2022. Resting-state EEG reveals four subphenotypes of amyotrophic lateral sclerosis. *Brain* 145, 621–631. <https://doi.org/10.1093/brain/awab322>
- El Mendili, M.M., Querin, G., Bede, P., Pradat, P.-F., 2019. Spinal Cord Imaging in Amyotrophic Lateral Sclerosis: Historical Concepts—Novel Techniques. *Front. Neurol.* 10.
- Emerson, R.G., Seyal, M., Pedley, T.A., 1984. SOMATOSENSORY EVOKED POTENTIALS FOLLOWING MEDIAN NERVE STIMULATION: I. THE CERVICAL COMPONENTS. *Brain* 107, 169–182. <https://doi.org/10.1093/brain/107.1.169>
- Engel, A.K., Fries, P., 2010. Beta-band oscillations — signalling the status quo? *Curr. Opin. Neurobiol., Cognitive neuroscience* 20, 156–165. <https://doi.org/10.1016/j.conb.2010.02.015>
- Ernst, M.J., Rast, F.M., Bauer, C.M., Marcar, V.L., Kool, J., 2013. Determination of thoracic and lumbar spinal processes by their percentage position between C7 and the PSIS level. *BMC Res. Notes* 6, 58. <https://doi.org/10.1186/1756-0500-6-58>
- Ernst, M.J., Sommer, B.B., Meichtry, A., Bauer, C.M., 2019. Intra-rater reliability of determining positions of cervical spinous processes and measuring their relative distances. *BMC Res. Notes* 12, 265. <https://doi.org/10.1186/s13104-019-4299-8>
- Ertekin, C., 1976. STUDIES ON the HUMAN EVOKED ELECTROSPINOGRAM. *Acta Neurol. Scand.* 53, 3–20. <https://doi.org/10.1111/j.1600-0404.1976.tb04321.x>

- Escorcio-Bezerra, M.L., Abrahao, A., de Castro, I., Chieia, M.A.T., de Azevedo, L.A., Pinheiro, D.S., de Oliveira Braga, N.I., de Oliveira, A.S.B., Manzano, G.M., 2016. MUNIX: Reproducibility and clinical correlations in Amyotrophic Lateral Sclerosis. *Clin. Neurophysiol.* 127, 2979–2984. <https://doi.org/10.1016/j.clinph.2016.06.011>
- Fachada, N., Rosa, A.C., 2018. micomp: A MATLAB/Octave toolbox for multivariate independent comparison of observations. *J. Open Source Softw.* 3, 430. <https://doi.org/10.21105/joss.00430>
- Farmer, S.F., Swash, M., Ingram, D.A., Stephens, J.A., 1993. Changes in motor unit synchronization following central nervous lesions in man. *J. Physiol.* 463, 83–105. <https://doi.org/10.1113/jphysiol.1993.sp019585>
- Fatehi, F., Grapperon, A.-M., Fathi, D., Delmont, E., Attarian, S., 2018. The utility of motor unit number index: A systematic review. *Neurophysiol. Clin.* 48, 251–259. <https://doi.org/10.1016/j.neucli.2018.09.001>
- Fathi, D., Mohammadi, B., Dengler, R., Bösel, S., Petri, S., Kollewé, K., 2016. Lower motor neuron involvement in ALS assessed by motor unit number index (MUNIX): Long-term changes and reproducibility. *Clin. Neurophysiol.* 127, 1984–1988. <https://doi.org/10.1016/j.clinph.2015.12.023>
- Fernández, V., 2021. The Use of Motor-Evoked Potentials in Clinical Trials in Multiple Sclerosis. *J. Clin. Neurophysiol.* 38, 166. <https://doi.org/10.1097/WNP.0000000000000734>
- Fierro, B., Raimondo, D., Modica, A., 1990. Analysis of F response in upper motoneurone lesions. *Acta Neurol. Scand.* 82, 329–334. <https://doi.org/10.1111/j.1600-0404.1990.tb03311.x>
- Finsterbusch, J., 2014. B0 Inhomogeneity and Shimming, in: Cohen-Adad, J., Wheeler-Kingshott, C.A.M. (Eds.), *Quantitative MRI of the Spinal Cord*. Academic Press, San Diego, pp. 68–90. <https://doi.org/10.1016/B978-0-12-396973-6.00006-X>
- Fisher, K.M., Zaaimi, B., Williams, T.L., Baker, S.N., Baker, M.R., 2012. Beta-band intermuscular coherence: a novel biomarker of upper motor neuron dysfunction in motor neuron disease. *Brain* 135, 2849–2864. <https://doi.org/10.1093/brain/aws150>

- Fujimoto, H., Kaneko, K., Taguchi, T., Ofuji, A., Yonemura, H., Kawai, S., 2001. Differential recording of upper and lower cervical N13 responses and their contribution to scalp recorded responses in median nerve somatosensory evoked potentials. *J. Neurol. Sci.* 187, 17–26. [https://doi.org/10.1016/S0022-510X\(01\)00509-3](https://doi.org/10.1016/S0022-510X(01)00509-3)
- Gass, A., Rocca, M.A., Agosta, F., Ciccarelli, O., Chard, D., Valsasina, P., Brooks, J.C.W., Bischof, A., Eisele, P., Kappos, L., Barkhof, F., Filippi, M., MAGNIMS Study Group, 2015. MRI monitoring of pathological changes in the spinal cord in patients with multiple sclerosis. *Lancet Neurol.* 14, 443–454. [https://doi.org/10.1016/S1474-4422\(14\)70294-7](https://doi.org/10.1016/S1474-4422(14)70294-7)
- Ghazni, N.F., Cahill, C.M., Stroman, P.W., 2010. Tactile Sensory and Pain Networks in the Human Spinal Cord and Brain Stem Mapped by Means of Functional MR Imaging. *Am. J. Neuroradiol.* 31, 661–667. <https://doi.org/10.3174/ajnr.A1909>
- Gilbertson, T., Lalo, E., Doyle, L., Lazzaro, V.D., Cioni, B., Brown, P., 2005. Existing Motor State Is Favored at the Expense of New Movement during 13–35 Hz Oscillatory Synchrony in the Human Corticospinal System. *J. Neurosci.* 25, 7771–7779. <https://doi.org/10.1523/JNEUROSCI.1762-05.2005>
- Gilmore, C.P., Bö, L., Owens, T., Lowe, J., Esiri, M.M., Evangelou, N., 2006. Spinal cord gray matter demyelination in multiple sclerosis—a novel pattern of residual plaque morphology. *Brain Pathol. Zurich Switz.* 16, 202–208. <https://doi.org/10.1111/j.1750-3639.2006.00018.x>
- Gobbelé, R., Buchner, H., Curio, G., 1998. High-frequency (600 Hz) SEP activities originating in the subcortical and cortical human somatosensory system. *Electroencephalogr. Clin. Neurophysiol.* 108, 182–189. [https://doi.org/10.1016/S0168-5597\(97\)00100-7](https://doi.org/10.1016/S0168-5597(97)00100-7)
- Gobbelé, R., Waberski, T.D., Dieckhöfer, A., Kawohl, W., Klostermann, F., Curio, G., Buchner, H., 2003. Patterns of Disturbed Impulse Propagation in Multiple Sclerosis Identified by Low and High Frequency Somatosensory Evoked Potential Components: *J. Clin. Neurophysiol.* 20, 283–290. <https://doi.org/10.1097/00004691-200307000-00008>

- Gunes, T., Sirin, N.G., Sahin, S., Kose, E., Isak, B., 2021. Use of CMAP, MScan fit-MUNE, and MUNIX in understanding neurodegeneration pattern of ALS and detection of early motor neuron loss in daily practice. *Neurosci. Lett.* 741, 135488. <https://doi.org/10.1016/j.neulet.2020.135488>
- H, J.H., 1958. Ten-twenty electrode system of the International Federation. *Electroencephalogr Clin Neurophysiol* 10, 371–375.
- Haines, D.E., Mihailoff, G.A., Yezierski, R.P., 2018. Fundamental Neuroscience for Basic and Clinical Applications, in: Haines, Duane E., Mihailoff, Gregory A. (Eds.), *Fundamental Neuroscience for Basic and Clinical Applications (Fifth Edition)*. Elsevier, pp. 138-151.e1. <https://doi.org/10.1016/B978-0-323-39632-5.00009-8>
- Hanajima, R., Chen, R., Ashby, P., Lozano, A.M., Hutchison, W.D., Davis, K.D., Dostrovsky, J.O., 2004. Very Fast Oscillations Evoked by Median Nerve Stimulation in the Human Thalamus and Subthalamic Nucleus. *J. Neurophysiol.* 92, 3171–3182. <https://doi.org/10.1152/jn.00363.2004>
- Hannaford, A., Pavey, N., van den Bos, M., Geevasinga, N., Menon, P., Shefner, J.M., Kiernan, M.C., Vucic, S., 2021. Diagnostic Utility of Gold Coast Criteria in Amyotrophic Lateral Sclerosis. *Ann. Neurol.* 89, 979–986. <https://doi.org/10.1002/ana.26045>
- Hashimoto, I., Mashiko, T., Imada, T., 1996. Somatic evoked high-frequency magnetic oscillations reflect activity of inhibitory interneurons in the human somatosensory cortex. *Electroencephalogr. Clin. Neurophysiol. Potentials Sect.* 100, 189–203. [https://doi.org/10.1016/0168-5597\(95\)00244-8](https://doi.org/10.1016/0168-5597(95)00244-8)
- Hermens, H.J., Freriks, B., Disselhorst-Klug, C., Rau, G., 2000. Development of recommendations for SEMG sensors and sensor placement procedures. *J. Electromyogr. Kinesiol.* 10, 361–374. [https://doi.org/10.1016/S1050-6411\(00\)00027-4](https://doi.org/10.1016/S1050-6411(00)00027-4)
- Hjorth, B., 1975. An on-line transformation of EEG scalp potentials into orthogonal source derivations. *Electroencephalogr. Clin. Neurophysiol.* 39, 526–530. [https://doi.org/10.1016/0013-4694\(75\)90056-5](https://doi.org/10.1016/0013-4694(75)90056-5)

- Hochman, S., 2007. Spinal cord. *Curr. Biol.* 17, R950–R955.
<https://doi.org/10.1016/j.cub.2007.10.014>
- Hoshino, Y., Kawabata, S., Adachi, Y., Watanabe, T., Sekihara, K., Sasaki, T., Hashimoto, J., Fujita, K., Nimura, A., Okawa, A., 2022. Magnetoneurography as a novel functional imaging technique for the ulnar nerve at the elbow. *Clin. Neurophysiol.* 138, 153–162. <https://doi.org/10.1016/j.clinph.2022.03.017>
- Hotelling, H., 1936. Relations Between Two Sets of Variates. *Biometrika* 321–377.
- Hu, N., Wang, J., Liu, M., 2021. Split hand in amyotrophic lateral sclerosis: A systematic review and meta-analysis. *J. Clin. Neurosci.* 90, 293–301.
<https://doi.org/10.1016/j.jocn.2021.06.015>
- Huynh, W., Simon, N.G., Grosskreutz, J., Turner, M.R., Vucic, S., Kiernan, M.C., 2016. Assessment of the upper motor neuron in amyotrophic lateral sclerosis. *Clin. Neurophysiol.* 127, 2643–2660. <https://doi.org/10.1016/j.clinph.2016.04.025>
- Ihara, A., Hirata, M., Yanagihara, K., Ninomiya, H., Imai, K., Ishii, R., Osaki, Y., Sakihara, K., Izumi, H., Imaoka, H., Kato, A., Yoshimine, T., Yorifuji, S., 2003. Neuromagnetic gamma-band activity in the primary and secondary somatosensory areas. *NeuroReport* 14, 273.
- Ikuta, F., Zimmerman, H.M., 1976. Distribution of plaques in seventy autopsy cases of multiple sclerosis in the United States. *Neurology* 26, 26–28.
https://doi.org/10.1212/wnl.26.6_part_2.26
- Inghilleri, M., Iacovelli, E., 2011. Clinical neurophysiology in ALS. *Arch. Ital. Biol.* 149, 57–63. <https://doi.org/10.4449/aib.v149i1.1264>
- Ingram, D.A., Thompson, A.J., Swash, M., 1988. Central motor conduction in multiple sclerosis: evaluation of abnormalities revealed by transcutaneous magnetic stimulation of the brain. *J. Neurol. Neurosurg. Psychiatry* 51, 487–494.
- Inoue, K., Hashimoto, I., Nakamura, S., 2001. High-frequency oscillations in human posterior tibial somatosensory evoked potentials are enhanced in patients with Parkinson's disease and multiple system atrophy. *Neurosci. Lett.* 297, 89–92.
[https://doi.org/10.1016/S0304-3940\(00\)01678-5](https://doi.org/10.1016/S0304-3940(00)01678-5)

- Inoue, K., Hashimoto, I., Shirai, T., Kawakami, H., Miyachi, T., Mimori, Y., Matsumoto, M., 2004. Disinhibition of the somatosensory cortex in cervical dystonia—decreased amplitudes of high-frequency oscillations. *Clin. Neurophysiol.* 115, 1624–1630. <https://doi.org/10.1016/j.clinph.2004.02.006>
- Insola, A., Padua, L., Mazzone, P., Valeriani, M., 2010. Effect of movement on SEPs generated by dorsal column nuclei. *Clin. Neurophysiol.* 121, 921–929. <https://doi.org/10.1016/j.clinph.2010.01.006>
- Insola, A., Padua, L., Mazzone, P., Valeriani, M., 2008. Unmasking of presynaptic and postsynaptic high-frequency oscillations in epidural cervical somatosensory evoked potentials during voluntary movement. *Clin. Neurophysiol.* 119, 237–245. <https://doi.org/10.1016/j.clinph.2007.09.132>
- Isa, T., Ohki, Y., Seki, K., Alstermark, B., 2006. Properties of Propriospinal Neurons in the C₃–C₄ Segments Mediating Disynaptic Pyramidal Excitation to Forelimb Motoneurons in the Macaque Monkey. *J. Neurophysiol.* 95, 3674–3685. <https://doi.org/10.1152/jn.00103.2005>
- Issa, N.P., Frank, S., Roos, R.P., Soliven, B., Towle, V.L., Rezanian, K., 2017. Intermuscular coherence in amyotrophic lateral sclerosis: A preliminary assessment: IMC in ALS. *Muscle Nerve* 55, 862–868. <https://doi.org/10.1002/mus.25426>
- Iyer, P.M., Mohr, K., Broderick, M., Gavin, B., Burke, T., Bede, P., Pinto-Grau, M., Pender, N.P., McLaughlin, R., Vajda, A., Heverin, M., Lalor, E.C., Hardiman, O., Nasserolsami, B., 2017. Mismatch Negativity as an Indicator of Cognitive Sub-Domain Dysfunction in Amyotrophic Lateral Sclerosis. *Front. Neurol.* 8, 395. <https://doi.org/10.3389/fneur.2017.00395>
- Jacobsen, A.B., Bostock, H., Fuglsang-Frederiksen, A., Duez, L., Beniczky, S., Møller, A.T., Blicher, J.U., Tankisi, H., 2017. Reproducibility, and sensitivity to motor unit loss in amyotrophic lateral sclerosis, of a novel MUNE method: MScanFit MUNE. *Clin. Neurophysiol.* 128, 1380–1388. <https://doi.org/10.1016/j.clinph.2017.03.045>
- Jeanmonod, D., Sindou, M., Mauguie`re, F., 1989. Three transverse dipolar generators in the human cervical and lumbo-sacral dorsal horn: evidence from direct intraoperative recordings on the spinal cord surface. *Electroencephalogr. Clin.*

Neurophysiol. Potentials Sect. 74, 236–240. [https://doi.org/10.1016/0013-4694\(89\)90010-2](https://doi.org/10.1016/0013-4694(89)90010-2)

Jeanmonod, D., Sindou, M., Mauguière, F., 1991. The human cervical and lumbo-sacral evoked electrospinogram. Data from intra-operative spinal cord surface recordings. *Electroencephalogr. Clin. Neurophysiol. Potentials Sect.* 80, 477–489. [https://doi.org/10.1016/0168-5597\(91\)90129-L](https://doi.org/10.1016/0168-5597(91)90129-L)

Jerath, N., Kimura, J., 2019. Chapter 15 - F wave, A wave, H reflex, and blink reflex, in: Levin, K.H., Chauvel, P. (Eds.), *Handbook of Clinical Neurology, Clinical Neurophysiology: Basis and Technical Aspects*. Elsevier, pp. 225–239. <https://doi.org/10.1016/B978-0-444-64032-1.00015-1>

Jones, S.M., Streletz, L.J., Raab, V.E., Knobler, R.L., Lublin, F.D., 1991. Lower Extremity Motor Evoked Potentials in Multiple Sclerosis. *Arch. Neurol.* 48, 944–948. <https://doi.org/10.1001/archneur.1991.00530210070026>

Joundi, R.A., Jenkinson, N., Brittain, J.-S., Aziz, T.Z., Brown, P., 2012. Driving Oscillatory Activity in the Human Cortex Enhances Motor Performance. *Curr. Biol.* 22, 403–407. <https://doi.org/10.1016/j.cub.2012.01.024>

Jurcak, V., Tsuzuki, D., Dan, I., 2007. 10/20, 10/10, and 10/5 systems revisited: Their validity as relative head-surface-based positioning systems. *NeuroImage* 34, 1600–1611. <https://doi.org/10.1016/j.neuroimage.2006.09.024>

Kale, N., Agaoglu, J., Onder, G., Tanik, O., 2009. Correlation between disability and transcranial magnetic stimulation abnormalities in patients with multiple sclerosis. *J. Clin. Neurosci.* 16, 1439–1442. <https://doi.org/10.1016/j.jocn.2009.03.009>

Kandel, E.R., Koester, J., Mack, S., Siegelbaum, S. (Eds.), 2021. *Principles of neural science*, Sixth edition. ed. McGraw Hill, New York.

Kaneko, K., Kawai, S., Taguchi, T., Fuchigami, Y., Ito, T., Morita, H., 1998. Correlation between spinal cord compression and abnormal patterns of median nerve somatosensory evoked potentials in compressive cervical myelopathy: Comparison of surface and epidurally recorded responses. *J. Neurol. Sci.* 158, 193–202. [https://doi.org/10.1016/S0022-510X\(98\)00119-1](https://doi.org/10.1016/S0022-510X(98)00119-1)

- Katayama, T., Suppa, A., Rothwell, J.C., 2010. Somatosensory evoked potentials and high frequency oscillations are differently modulated by theta burst stimulation over primary somatosensory cortex in humans. *Clin. Neurophysiol.* 121, 2097–2103. <https://doi.org/10.1016/j.clinph.2010.05.014>
- Keegan, J.J., Garrett, F.D., 1948. The segmental distribution of the cutaneous nerves in the limbs of man. *Anat. Rec.* 102, 409–437. <https://doi.org/10.1002/ar.1091020403>
- Kent-Braun, J.A., Walker, C.H., Weiner, M.W., Miller, R.G., 1998. Functional significance of upper and lower motor neuron impairment in amyotrophic lateral sclerosis. *Muscle Nerve* 21, 762–768. [https://doi.org/10.1002/\(SICI\)1097-4598\(199806\)21:6<762::AID-MUS8>3.0.CO;2-5](https://doi.org/10.1002/(SICI)1097-4598(199806)21:6<762::AID-MUS8>3.0.CO;2-5)
- Khanna, P., Carmenta, J.M., 2015. Neural oscillations: beta band activity across motor networks. *Curr. Opin. Neurobiol., Large-Scale Recording Technology* (32) 32, 60–67. <https://doi.org/10.1016/j.conb.2014.11.010>
- Kilavik, B.E., Zaepffel, M., Brovelli, A., MacKay, W.A., Riehle, A., 2013. The ups and downs of beta oscillations in sensorimotor cortex. *Exp. Neurol., Special Issue: Neuronal oscillations in movement disorders* 245, 15–26. <https://doi.org/10.1016/j.expneurol.2012.09.014>
- Kilner, J.M., Baker, S.N., Salenius, S., Jousmäki, V., Hari, R., Lemon, R.N., 1999. Task-dependent modulation of 15-30 Hz coherence between rectified EMGs from human hand and forearm muscles. *J. Physiol.* 516, 559–570. <https://doi.org/10.1111/j.1469-7793.1999.0559v.x>
- Kim, S.-G., Jin, T., Fukuda, M., 2010. Spatial Resolution of fMRI Techniques, in: Ulmer, S., Jansen, O. (Eds.), *fMRI: Basics and Clinical Applications*. Springer, Berlin, Heidelberg, pp. 15–21. https://doi.org/10.1007/978-3-540-68132-8_3
- Kinany, N., Khatibi, A., Lungu, O., Finsterbusch, J., Büchel, C., Marchand-Pauvert, V., Van De Ville, D., Vahdat, S., Doyon, J., 2023. Decoding cerebro-spinal signatures of human behavior: Application to motor sequence learning. *NeuroImage* 275, 120174. <https://doi.org/10.1016/j.neuroimage.2023.120174>

- Kinany, N., Pirondini, E., Martuzzi, R., Mattera, L., Micera, S., Van de Ville, D., 2019. Functional imaging of rostrocaudal spinal activity during upper limb motor tasks. *NeuroImage* 200, 590–600. <https://doi.org/10.1016/j.neuroimage.2019.05.036>
- Kinany, Nawal, Pirondini, E., Micera, S., Van De Ville, D., 2023. Spinal Cord fMRI: A New Window into the Central Nervous System. *The Neuroscientist* 29, 715–731. <https://doi.org/10.1177/10738584221101827>
- Klostermann, F., Gobbele, R., Buchner, H., Curio, G., 2002. Intrathalamic non-propagating generators of high-frequency (1000 Hz) somatosensory evoked potential (SEP) bursts recorded subcortically in man. *Clin. Neurophysiol.* 113, 1001–1005. [https://doi.org/10.1016/S1388-2457\(02\)00119-0](https://doi.org/10.1016/S1388-2457(02)00119-0)
- Klostermann, F., Nikulin, V.V., Kühn, A.A., Marzinzik, F., Wahl, M., Pogosyan, A., Kupsch, A., Schneider, G.-H., Brown, P., Curio, G., 2007. Task-related differential dynamics of EEG alpha- and beta-band synchronization in cortico-basal motor structures. *Eur. J. Neurosci.* 25, 1604–1615. <https://doi.org/10.1111/j.1460-9568.2007.05417.x>
- Kopell, N., Ermentrout, G.B., Whittington, M.A., Traub, R.D., 2000. Gamma rhythms and beta rhythms have different synchronization properties. *Proc. Natl. Acad. Sci.* 97, 1867–1872. <https://doi.org/10.1073/pnas.97.4.1867>
- Kreiter, D., Postma, A.A., Hupperts, R., Gerlach, O., 2024. Hallmarks of spinal cord pathology in multiple sclerosis. *J. Neurol. Sci.* 456, 122846. <https://doi.org/10.1016/j.jns.2023.122846>
- Kruskal, W.H., Wallis, W.A., 1952. Use of Ranks in One-Criterion Variance Analysis. *J. Am. Stat. Assoc.* 47, 583–621. <https://doi.org/10.1080/01621459.1952.10483441>
- Landelle, C., Lungu, O., Vahdat, S., Kavounoudias, A., Marchand-Pauvert, V., De Leener, B., Doyon, J., 2021. Investigating the human spinal sensorimotor pathways through functional magnetic resonance imaging. *NeuroImage* 245, 118684. <https://doi.org/10.1016/j.neuroimage.2021.118684>
- Lawrence, J.M., Stroman, P.W., Kollias, S.S., 2008. Functional magnetic resonance imaging of the human spinal cord during vibration stimulation of different

dermatomes. *Neuroradiology* 50, 273–280. <https://doi.org/10.1007/s00234-007-0338-6>

Lewelt, A., Krosschell, K.J., Scott, C., Sakonju, A., Kissel, J.T., Crawford, T.O., Acsadi, G., D’anjou, G., Elsheikh, B., Reyna, S.P., Schroth, M.K., Maczulski, J.A., Stoddard, G.J., Elovic, E., Swoboda, K.J., 2010. Compound muscle action potential and motor function in children with spinal muscular atrophy. *Muscle Nerve* 42, 703–708. <https://doi.org/10.1002/mus.21838>

Little, S., Brown, P., 2014. The functional role of beta oscillations in Parkinson’s disease. *Parkinsonism Relat. Disord., Proceedings of XX World Congress on Parkinson’s Disease and Related Disorders* 20, S44–S48. [https://doi.org/10.1016/S1353-8020\(13\)70013-0](https://doi.org/10.1016/S1353-8020(13)70013-0)

Lotte, F., Congedo, M., Lécuyer, A., Lamarche, F., Arnaldi, B., 2007. A review of classification algorithms for EEG-based brain–computer interfaces. *J. Neural Eng.* 4, R1. <https://doi.org/10.1088/1741-2560/4/2/R01>

Madi, S., Flanders, A.E., Vinitski, S., Herbison, G.J., Nissanov, J., 2001. Functional MR Imaging of the Human Cervical Spinal Cord. *Am. J. Neuroradiol.* 22, 1768–1774.

Maieron, M., Iannetti, G.D., Bodurka, J., Tracey, I., Bandettini, P.A., Porro, C.A., 2007. Functional Responses in the Human Spinal Cord during Willed Motor Actions: Evidence for Side- and Rate-Dependent Activity. *J. Neurosci.* 27, 4182–4190. <https://doi.org/10.1523/JNEUROSCI.3910-06.2007>

Mann, H.B., Whitney, D.R., 1947. On a Test of Whether one of Two Random Variables is Stochastically Larger than the Other. *Ann. Math. Stat.* 18, 50–60.

Mardell, L.C., O’Neill, G.C., Tierney, T.M., Timms, R.C., Zich, C., Barnes, G.R., Bestmann, S., 2022. Concurrent spinal and brain imaging with optically pumped magnetometers (preprint). *Neuroscience*. <https://doi.org/10.1101/2022.05.12.491623>

Mauguière, F., 2003a. Chapter 5 Somatosensory evoked responses, in: Hallett, M. (Ed.), *Handbook of Clinical Neurophysiology, Handbook of Clinical Neurophysiology*. Elsevier, pp. 45–75. [https://doi.org/10.1016/S1567-4231\(09\)70153-4](https://doi.org/10.1016/S1567-4231(09)70153-4)

- Mauguière, F., 2003b. Chapter 5 Somatosensory evoked responses, in: Hallett, M. (Ed.), *Handbook of Clinical Neurophysiology*, Handbook of Clinical Neurophysiology. Elsevier, pp. 45–75. [https://doi.org/10.1016/S1567-4231\(09\)70153-4](https://doi.org/10.1016/S1567-4231(09)70153-4)
- Mayhew, S.D., Porcaro, C., Tecchio, F., Bagshaw, A.P., 2017. fMRI characterisation of widespread brain networks relevant for behavioural variability in fine hand motor control with and without visual feedback. *NeuroImage* 148, 330–342. <https://doi.org/10.1016/j.neuroimage.2017.01.017>
- McComas, A.J., Fawcett, P.R.W., Campbell, M.J., Sica, R.E.P., 1971. Electrophysiological estimation of the number of motor units within a human muscle. *J. Neurol. Neurosurg. Psychiatry* 34, 121–131. <https://doi.org/10.1136/jnnp.34.2.121>
- McGavern, D.B., Murray, P.D., Rivera-Quiñones, C., Schmelzer, J.D., Low, P.A., Rodriguez, M., 2000. Axonal loss results in spinal cord atrophy, electrophysiological abnormalities and neurological deficits following demyelination in a chronic inflammatory model of multiple sclerosis. *Brain J. Neurol.* 123 Pt 3, 519–531. <https://doi.org/10.1093/brain/123.3.519>
- McMackin, R., Bede, P., Pender, N., Hardiman, O., Nasserroleslami, B., 2019. Neurophysiological markers of network dysfunction in neurodegenerative diseases. *NeuroImage Clin.* 22, 101706. <https://doi.org/10.1016/j.nicl.2019.101706>
- Mendili, M.-M.E., Cohen-Adad, J., Pelegrini-Issac, M., Rossignol, S., Morizot-Koutlidis, R., Marchand-Pauvert, V., Iglesias, C., Sangari, S., Katz, R., Lehericy, S., Benali, H., Pradat, P.-F., 2014. Multi-Parametric Spinal Cord MRI as Potential Progression Marker in Amyotrophic Lateral Sclerosis. *PLOS ONE* 9, e95516. <https://doi.org/10.1371/journal.pone.0095516>
- Menon, P., Kiernan, M.C., Yiannikas, C., Stroud, J., Vucic, S., 2013. Split-hand index for the diagnosis of amyotrophic lateral sclerosis. *Clin. Neurophysiol.* 124, 410–416. <https://doi.org/10.1016/j.clinph.2012.07.025>
- Metzger, M., Dukic, S., McMackin, R., Giglia, E., Mitchell, M., Bista, S., Costello, E., Peelo, C., Tadjine, Y., Sirenko, V., Plaitano, S., Coffey, A., McManus, L., Farnell Sharp, A., Mehra, P., Heverin, M., Bede, P., Muthuraman, M., Pender, N., Hardiman, O., Nasserroleslami, B., 2023. Functional network dynamics revealed by

- EEG microstates reflect cognitive decline in amyotrophic lateral sclerosis. *Hum. Brain Mapp.* 45, e26536. <https://doi.org/10.1002/hbm.26536>
- Miller, K.J., Leuthardt, E.C., Schalk, G., Rao, R.P.N., Anderson, N.R., Moran, D.W., Miller, J.W., Ojemann, J.G., 2007. Spectral Changes in Cortical Surface Potentials during Motor Movement. *J. Neurosci.* 27, 2424–2432. <https://doi.org/10.1523/JNEUROSCI.3886-06.2007>
- Mima, T., Toma, K., Koshy, B., Hallett, M., 2001. Coherence Between Cortical and Muscular Activities After Subcortical Stroke. *Stroke* 32, 2597–2601. <https://doi.org/10.1161/hs1101.098764>
- Moccia, M., Ruggieri, S., Ianniello, A., Toosy, A., Pozzilli, C., Ciccarelli, O., 2019. Advances in spinal cord imaging in multiple sclerosis. *Ther. Adv. Neurol. Disord.* 12, 1756286419840593. <https://doi.org/10.1177/1756286419840593>
- Moreno-López, Y., Olivares-Moreno, R., Cordero-Erausquin, M., Rojas-Piloni, G., 2016. Sensorimotor Integration by Corticospinal System. *Front. Neuroanat.* 10. <https://doi.org/10.3389/fnana.2016.00024>
- Muthukumaraswamy, S.D., 2010. Functional Properties of Human Primary Motor Cortex Gamma Oscillations. *J. Neurophysiol.* 104, 2873–2885. <https://doi.org/10.1152/jn.00607.2010>
- Nair, G., Carew, J.D., Usher, S., Lu, D., Hu, X.P., Benatar, M., 2010. Diffusion tensor imaging reveals regional differences in the cervical spinal cord in amyotrophic lateral sclerosis. *NeuroImage* 53, 576–583. <https://doi.org/10.1016/j.neuroimage.2010.06.060>
- Nakanishi, M., Wang, Y., Chen, X., Wang, Y.-T., Gao, X., Jung, T.-P., 2018. Enhancing Detection of SSVEPs for a High-Speed Brain Speller Using Task-Related Component Analysis. *IEEE Trans. Biomed. Eng.* 65, 104–112. <https://doi.org/10.1109/TBME.2017.2694818>
- Nandedkar, S.D., Barkhaus, P.E., Stålberg, E.V., 2015. Cumulative motor index: An index to study progression of amyotrophic lateral sclerosis. *J. Clin. Neurophysiol.* 32, 79–85. <https://doi.org/10.1097/WNP.0000000000000116>

- Nandedkar, S.D., Barkhaus, P.E., Stålberg, E.V., 2011. Reproducibility of MUNIX in patients with amyotrophic lateral sclerosis. *Muscle Nerve* 44, 919–922. <https://doi.org/10.1002/mus.22204>
- Nandedkar, S.D., Barkhaus, P.E., Stålberg, E.V., 2010. Motor unit number index (MUNIX): principle, method, and findings in healthy subjects and in patients with motor neuron disease. *Muscle Nerve* 42, 798–807. <https://doi.org/10.1002/mus.21824>
- Nandedkar, S.D., Nandedkar, D.S., Barkhaus, P.E., Stalberg, E.V., 2004. Motor unit number index (MUNIX). *IEEE Trans. Biomed. Eng.* 51, 2209–2211. <https://doi.org/10.1109/TBME.2004.834281>
- Nasserolelami, B., Dukic, S., Buxo, T., Coffey, A., McMackin, R., Muthuraman, M., Hardiman, O., Lowery, M.M., Lalor, E.C., 2019. Non-Parametric Rank Statistics for Spectral Power and Coherence (preprint). *Bioinformatics*. <https://doi.org/10.1101/818906>
- Nasserolelami, B., Lakany, H., Conway, B.A., 2014. EEG signatures of arm isometric exertions in preparation, planning and execution. *NeuroImage* 90, 1–14. <https://doi.org/10.1016/j.neuroimage.2013.12.011>
- Neal, P.J., Katirji, B., 2008. Performance standards of the nerve conduction study technologist. *Am. J. Electroneurodiagnostic Technol.* 48, 72–78.
- Nestor, D., Nelson, R., 1990. Performing motor and sensory neuronal conduction studies in adult humans.
- Neuwirth, C., Barkhaus, P.E., Burkhardt, C., Castro, J., Czell, D., de Carvalho, M., Nandedkar, S., Stålberg, E., Weber, M., 2017. Motor Unit Number Index (MUNIX) detects motor neuron loss in pre-symptomatic muscles in Amyotrophic Lateral Sclerosis. *Clin. Neurophysiol.* 128, 495–500. <https://doi.org/10.1016/j.clinph.2016.11.026>
- Neuwirth, C., Nandedkar, S., Stålberg, E., Weber, M., 2010. Motor unit number index (MUNIX): A novel neurophysiological technique to follow disease progression in amyotrophic lateral sclerosis. *Muscle Nerve* 42, 379–384. <https://doi.org/10.1002/mus.21707>

- Ng, M.-C., Wu, E.X., Lau, H.-F., Hu, Y., Lam, E.Y., Luk, K.D., 2008. Cervical spinal cord BOLD fMRI study: Modulation of functional activation by dexterity of dominant and non-dominant hands. *NeuroImage* 39, 825–831. <https://doi.org/10.1016/j.neuroimage.2007.09.026>
- Nielsen, J.B., 2004. Sensorimotor integration at spinal level as a basis for muscle coordination during voluntary movement in humans. *J. Appl. Physiol.* 96, 1961–1967. <https://doi.org/10.1152/japplphysiol.01073.2003>
- Nierula, B., Stephani, T., Kaptan, M., Mouraux, A., Maess, B., Villringer, A., Curio, G., Nikulin, V.V., Eippert, F., 2022. Non-invasive multi-channel electrophysiology of the human spinal cord – assessing somatosensory processing from periphery to cortex (preprint). *Neuroscience*. <https://doi.org/10.1101/2022.12.05.519148>
- Nijeholt, G.J., Bergers, E., Kamphorst, W., Bot, J., Nicolay, K., Castelijns, J.A., van Waesberghe, J.H., Ravid, R., Polman, C.H., Barkhof, F., 2001. Post-mortem high-resolution MRI of the spinal cord in multiple sclerosis: a correlative study with conventional MRI, histopathology and clinical phenotype. *Brain J. Neurol.* 124, 154–166. <https://doi.org/10.1093/brain/124.1.154>
- Nowak, M., Zich, C., Stagg, C.J., 2018. Motor Cortical Gamma Oscillations: What Have We Learnt and Where Are We Headed? *Curr. Behav. Neurosci. Rep.* 5, 136–142. <https://doi.org/10.1007/s40473-018-0151-z>
- Nudo, R.J., Masterton, R.B., 1986. Stimulation-induced [¹⁴C]2-deoxyglucose labeling of synaptic activity in the central auditory system. *J. Comp. Neurol.* 245, 553–565. <https://doi.org/10.1002/cne.902450410>
- Oguz Akarsu, E., Sirin, N.G., Kocasoy Orhan, E., Erbas, B., Dede, H.O., Baslo, M.B., Idrisoglu, H.A., Oge, A.E., 2020. Repeater F-waves in amyotrophic lateral sclerosis: Electrophysiologic indicators of upper or lower motor neuron involvement? *Clin. Neurophysiol.* 131, 96–105. <https://doi.org/10.1016/j.clinph.2019.09.030>
- Oja, H., 2010. *Multivariate Nonparametric Methods with R: An approach based on spatial signs and ranks*. Springer.
- Oja, H., Randles, R.H., 2004. Multivariate Nonparametric Tests. *Stat. Sci.* 19, 598–605. <https://doi.org/10.1214/088342304000000558>

- Oostenveld, R., Fries, P., Maris, E., Schoffelen, J.-M., 2011. FieldTrip: Open Source Software for Advanced Analysis of MEG, EEG, and Invasive Electrophysiological Data. *Comput. Intell. Neurosci.* 2011, 1–9. <https://doi.org/10.1155/2011/156869>
- Oostenveld, R., Praamstra, P., 2001. The 10% electrode system for high-resolution EEG and ERP measurements. *Clin. Neurophysiol.*
- Ozaki, I., Hashimoto, I., 2011. Exploring the physiology and function of high-frequency oscillations (HFOs) from the somatosensory cortex. *Clin. Neurophysiol.* 122, 1908–1923. <https://doi.org/10.1016/j.clinph.2011.05.023>
- Paquin, M.-È., Mendili, M.M.E., Gros, C., Dupont, S.M., Cohen-Adad, J., Pradat, P.-F., 2018. Spinal Cord Gray Matter Atrophy in Amyotrophic Lateral Sclerosis. *Am. J. Neuroradiol.* 39, 184–192. <https://doi.org/10.3174/ajnr.A5427>
- Perez, M.A., Cohen, L.G., 2008. Mechanisms Underlying Functional Changes in the Primary Motor Cortex Ipsilateral to an Active Hand. *J. Neurosci.* 28, 5631–5640. <https://doi.org/10.1523/JNEUROSCI.0093-08.2008>
- Perrin, F., Pernier, J., Bertrand, O., Echallier, J.F., 1989. Spherical splines for scalp potential and current density mapping. *Electroencephalogr. Clin. Neurophysiol.* 72, 184–187. [https://doi.org/10.1016/0013-4694\(89\)90180-6](https://doi.org/10.1016/0013-4694(89)90180-6)
- Petrova, N., Carassiti, D., Altmann, D.R., Baker, D., Schmierer, K., 2018. Axonal loss in the multiple sclerosis spinal cord revisited. *Brain Pathol. Zurich Switz.* 28, 334–348. <https://doi.org/10.1111/bpa.12516>
- Pfurtscheller, G., Graitmann, B., Huggins, J.E., Levine, S.P., Schuh, L.A., 2003. Spatiotemporal patterns of beta desynchronization and gamma synchronization in corticographic data during self-paced movement. *Clin. Neurophysiol.* 114, 1226–1236. [https://doi.org/10.1016/S1388-2457\(03\)00067-1](https://doi.org/10.1016/S1388-2457(03)00067-1)
- Pfurtscheller, G., Stancák, A., Neuper, C., 1996. Post-movement beta synchronization. A correlate of an idling motor area? *Electroencephalogr. Clin. Neurophysiol.* 98, 281–293. [https://doi.org/10.1016/0013-4694\(95\)00258-8](https://doi.org/10.1016/0013-4694(95)00258-8)
- Piché, M., Cohen-Adad, J., Nejad, M.K., Perlberg, V., Xie, G., Beaudoin, G., Benali, H., Rainville, P., 2009. Characterization of cardiac-related noise in fMRI of the cervical

- spinal cord. Magn. Reson. Imaging 27, 300–310. <https://doi.org/10.1016/j.mri.2008.07.019>
- Pierrot-Deseilligny, E., 2002. Propriospinal transmission of part of the corticospinal excitation in humans. *Muscle Nerve* 26, 155–172. <https://doi.org/10.1002/mus.1240>
- Pierrot-Deseilligny, E., Burke, D., 2012. *The Circuitry of the Human Spinal Cord: Spinal and Corticospinal Mechanisms of Movement*, 1st ed. Cambridge University Press. <https://doi.org/10.1017/CBO9781139026727>
- Polat, K., Güneş, S., 2007. Classification of epileptiform EEG using a hybrid system based on decision tree classifier and fast Fourier transform. *Appl. Math. Comput.* 187, 1017–1026. <https://doi.org/10.1016/j.amc.2006.09.022>
- Porcaro, C., Mayhew, S.D., Bagshaw, A.P., 2021. Role of the Ipsilateral Primary Motor Cortex in the Visuo-Motor Network During Fine Contractions and Accurate Performance. *Int. J. Neural Syst.* 31, 2150011. <https://doi.org/10.1142/S0129065721500118>
- Prestor, B., Gnidovec, B., Golob, P., 1997. Long sensory tracts (cuneate fascicle) in cervical somatosensory evoked potential after median nerve stimulation. *Electroencephalogr. Clin. Neurophysiol.* 104, 470–479. [https://doi.org/10.1016/s0168-5597\(97\)00040-3](https://doi.org/10.1016/s0168-5597(97)00040-3)
- Prestor, B., Golob, P., 1999. Intra-operative spinal cord neuromonitoring in patients operated on for intramedullary tumors and syringomyelia. *Neurol. Res.* 21, 125–129. <https://doi.org/10.1080/01616412.1999.11740908>
- Proudfoot, M., van Ede, F., Quinn, A., Colclough, G.L., Wu, J., Talbot, K., Benatar, M., Woolrich, M.W., Nobre, A.C., Turner, M.R., 2018. Impaired corticomuscular and interhemispheric cortical beta oscillation coupling in amyotrophic lateral sclerosis. *Clin. Neurophysiol.* 129, 1479–1489. <https://doi.org/10.1016/j.clinph.2018.03.019>
- Rea, P., 2015. *Essential clinical anatomy of the nervous system*. Elsevier/AP, Academic Press is an imprint of Elsevier, Amsterdam ; Boston.
- Restuccia, D., Mauguière, F., 1991. The contribution of median nerve SEPs in the functional assessment of the cervical spinal cord in syringomyelia. A study of 24 patients. *Brain* 114, 361–379. <https://doi.org/10.1093/brain/114.1.361>

- Rexed, B., 1954. A cytoarchitectonic atlas of the spinal cord in the cat. *J. Comp. Neurol.* 100, 297–379. <https://doi.org/10.1002/cne.901000205>
- Rexed, B., 1952. The cytoarchitectonic organization of the spinal cord in the cat. *J. Comp. Neurol.* 96, 415–495. <https://doi.org/10.1002/cne.900960303>
- Rosenfeld, J., Swash, M., 2006. What's in a name? *Neurology* 66, 624–625. <https://doi.org/10.1212/01.wnl.0000205597.62054.db>
- Rossi, F.H., Franco, M.C., Estevez, A.G., Rossi, F.H., Franco, M.C., Estevez, A.G., 2013. Pathophysiology of Amyotrophic Lateral Sclerosis, in: *Current Advances in Amyotrophic Lateral Sclerosis*. IntechOpen. <https://doi.org/10.5772/56562>
- Sakaki, K., Hoshino, Y., Kawabata, S., Adachi, Y., Watanabe, T., Sekihara, K., Sekihara, K., Ishii, S., Tomori, M., Tomizawa, S., Enomoto, M., Okawa, A., 2020. Evaluation of neural activity by magnetospinography with 3D sensors. *Clin. Neurophysiol.* 131, 1252–1266. <https://doi.org/10.1016/j.clinph.2020.02.025>
- Salenius, S., Salmelin, R., Neuper, C., Pfurtscheller, G., Hari, R., 1996. Human cortical 40 Hz rhythm is closely related to EMG rhythmicity. *Neurosci. Lett.* 213, 75–78. [https://doi.org/10.1016/0304-3940\(96\)12796-8](https://doi.org/10.1016/0304-3940(96)12796-8)
- Schabrun, S.M., Ridding, M.C., Galea, M.P., Hodges, P.W., Chipchase, L.S., 2012. Primary sensory and motor cortex excitability are co-modulated in response to peripheral electrical nerve stimulation. *PLOS ONE* 7, 1–7. <https://doi.org/10.1371/journal.pone.0051298>
- Shibuya, K., Kuboyama, N., Tanaka, J., 2014. Changes in ipsilateral motor cortex activity during a unilateral isometric finger task are dependent on the muscle contraction force. *Physiol. Meas.* 35, 417. <https://doi.org/10.1088/0967-3334/35/3/417>
- Shimoji, K., Higashi, H., Kano, T., 1971. Epidural recording of spinal electrogram in man. *Electroencephalogr. Clin. Neurophysiol.* 30, 236–239. [https://doi.org/10.1016/0013-4694\(71\)90059-9](https://doi.org/10.1016/0013-4694(71)90059-9)
- Shin-Yi Lin, C., Howells, J., Rutkove, S., Nandedkar, S., Neuwirth, C., Noto, Y., Shahrizaila, N., Whittaker, R.G., Bostock, H., Burke, D., Tankisi, H., 2024. Neurophysiological and imaging biomarkers of lower motor neuron dysfunction in

- motor neuron diseases/amyotrophic lateral sclerosis: IFCN handbook chapter. Clin. Neurophysiol. 162, 91–120. <https://doi.org/10.1016/j.clinph.2024.03.015>
- Šoda, J., Pavelin, S., Vujović, I., Rogić Vidaković, M., 2023. Assessment of Motor Evoked Potentials in Multiple Sclerosis. Sensors 23, 497. <https://doi.org/10.3390/s23010497>
- Stachowski, N.J., Dougherty, K.J., 2021. Spinal Inhibitory Interneurons: Gatekeepers of Sensorimotor Pathways. Int. J. Mol. Sci. 22, 2667. <https://doi.org/10.3390/ijms22052667>
- Stinear, C.M., Walker, K.S., Byblow, W.D., 2001. Symmetric facilitation between motor cortices during contraction of ipsilateral hand muscles. Exp. Brain Res. 139, 101–105. <https://doi.org/10.1007/s002210100758>
- Stouffer, S.A., Suchman, E.A., Devinney, L.C., Star, S.A., Williams Jr., R.M., 1949. The American soldier: Adjustment during army life. (Studies in social psychology in World War II), Vol. 1, The American soldier: Adjustment during army life. (Studies in social psychology in World War II), Vol. 1. Princeton Univ. Press, Oxford, England.
- Stroman, P.W., Ryner, L.N., 2001. Functional MRI of motor and sensory activation in the human spinal cord. Magn. Reson. Imaging 19, 27–32. [https://doi.org/10.1016/S0730-725X\(01\)00226-0](https://doi.org/10.1016/S0730-725X(01)00226-0)
- Student, 1908. The Probable Error of a Mean. Biometrika 6, 1–25. <https://doi.org/10.2307/2331554>
- Sue, C.M., Yiannikas, C., Clouston, P.D., Lim, C.L., Graham, S., 1997. Transcranial cortical stimulation in disorders of the central motor pathways. J. Clin. Neurosci. 4, 19–25. [https://doi.org/10.1016/S0967-5868\(97\)90005-9](https://doi.org/10.1016/S0967-5868(97)90005-9)
- Sumiya, S., Kawabata, S., Hoshino, Y., Adachi, Y., Sekihara, K., Tomizawa, S., Tomori, M., Ishii, S., Sakaki, K., Ukegawa, D., Ushio, S., Watanabe, T., Okawa, A., 2017. Magnetospinography visualizes electrophysiological activity in the cervical spinal cord. Sci. Rep. 7, 2192–2192. <https://doi.org/10.1038/s41598-017-02406-8>

- Swann, N.C., de Hemptinne, C., Aron, A.R., Ostrem, J.L., Knight, R.T., Starr, P.A., 2015. Elevated synchrony in Parkinson disease detected with electroencephalography. *Ann. Neurol.* 78, 742–750. <https://doi.org/10.1002/ana.24507>
- Szurhaj, W., Bourriez, J.-L., Kahane, P., Chauvel, P., Mauguière, F., Derambure, P., 2005. Intracerebral study of gamma rhythm reactivity in the sensorimotor cortex. *Eur. J. Neurosci.* 21, 1223–1235. <https://doi.org/10.1111/j.1460-9568.2005.03966.x>
- Szurhaj, W., Labyt, E., Bourriez, J.-L., Kahane, P., Chauvel, P., Mauguière, F., Derambure, P., 2006. Relationship between intracerebral gamma oscillations and slow potentials in the human sensorimotor cortex. *Eur. J. Neurosci.* 24, 947–954. <https://doi.org/10.1111/j.1460-9568.2006.04876.x>
- Tanaka, H., Katura, T., Sato, H., 2013. Task-related component analysis for functional neuroimaging and application to near-infrared spectroscopy data. *NeuroImage* 64, 308–327. <https://doi.org/10.1016/j.neuroimage.2012.08.044>
- The MathWorks, I., 2023. MATLAB and Computer Vision System Toolbox.
- Thompson, A.J., Banwell, B.L., Barkhof, F., Carroll, W.M., Coetzee, T., Comi, G., Correale, J., Fazekas, F., Filippi, M., Freedman, M.S., Fujihara, K., Galetta, S.L., Hartung, H.P., Kappos, L., Lublin, F.D., Marrie, R.A., Miller, A.E., Miller, D.H., Montalban, X., Mowry, E.M., Sorensen, P.S., Tintoré, M., Traboulsee, A.L., Trojano, M., Uitdehaag, B.M.J., Vukusic, S., Waubant, E., Weinshenker, B.G., Reingold, S.C., Cohen, J.A., 2018. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. *Lancet Neurol.* 17, 162–173. [https://doi.org/10.1016/S1474-4422\(17\)30470-2](https://doi.org/10.1016/S1474-4422(17)30470-2)
- Tzagarakis, C., Ince, N.F., Leuthold, A.C., Pellizzer, G., 2010. Beta-Band Activity during Motor Planning Reflects Response Uncertainty. *J. Neurosci.* 30, 11270–11277. <https://doi.org/10.1523/JNEUROSCI.6026-09.2010>
- Ulloa, J.L., 2022. The Control of Movements via Motor Gamma Oscillations. *Front. Hum. Neurosci.* 15, 787157. <https://doi.org/10.3389/fnhum.2021.787157>
- Urasaki, E., Wada, S.-I., Kadoya, C., Tokimura, T., Yokota, A., Matsuoka, S., Fukumura, A., Hamada, S., 1990. Skin and epidural recording of spinal somatosensory evoked potentials following median nerve stimulation: correlation between the absence of

- spinal N13 and impaired pain sense. *J. Neurol.* 237, 410–415.
<https://doi.org/10.1007/BF00314731>
- Ushio, S., Hoshino, Y., Kawabata, S., Adachi, Y., Sekihara, K., Sumiya, S., Ukegawa, D., Sakaki, K., Taishi Watanabe, Watanabe, T., Hasegawa, Y., Okawa, A., 2019. Visualization of the electrical activity of the cauda equina using a magnetospinography system in healthy subjects. *Clin. Neurophysiol.* 130, 1–11.
<https://doi.org/10.1016/j.clinph.2018.11.001>
- Vahdat, S., Lungu, O., Cohen-Adad, J., Marchand-Pauvert, V., Benali, H., Doyon, J., 2015. Simultaneous Brain–Cervical Cord fMRI Reveals Intrinsic Spinal Cord Plasticity during Motor Sequence Learning. *PLOS Biol.* 13, e1002186.
<https://doi.org/10.1371/journal.pbio.1002186>
- Valsasina, P., Agosta, F., Benedetti, B., Caputo, D., Perini, M., Salvi, F., Prella, A., Filippi, M., 2007. Diffusion anisotropy of the cervical cord is strictly associated with disability in amyotrophic lateral sclerosis. *J. Neurol. Neurosurg. Psychiatry* 78, 480–484. <https://doi.org/10.1136/jnnp.2006.100032>
- Verber, N.S., Shephard, S.R., Sassani, M., McDonough, H.E., Moore, S.A., Alix, J.J.P., Wilkinson, I.D., Jenkins, T.M., Shaw, P.J., 2019. Biomarkers in Motor Neuron Disease: A State of the Art Review. *Front. Neurol.* 10.
<https://doi.org/10.3389/fneur.2019.00291>
- Waldman, A.D., Catania, C., Pisa, M., Jenkinson, M., Lenardo, M., Luca, G.C.D., 2022. Histopathological Characterization of Staged Lesion Topography in the Multiple Sclerosis Spinal Cord. <https://doi.org/10.1101/2022.06.14.22276413>
- Waldman, A.D., Catania, C., Pisa, M., Jenkinson, M., Lenardo, M.J., DeLuca, G.C., 2024. The prevalence and topography of spinal cord demyelination in multiple sclerosis: a retrospective study. <https://doi.org/10.1101/2022.06.14.22276413>
- Wang, F., Zhang, L., Yue, L., Zeng, Y., Zhao, Q., Gong, Q., Zhang, J., Liu, D., Luo, X., Xia, X., Wan, L., Hu, L., 2021. A novel method to simultaneously record spinal cord electrophysiology and electroencephalography signals. *NeuroImage* 232, 117892.
<https://doi.org/10.1016/j.neuroimage.2021.117892>

- Wang, F.-C., Delwaide, P.J., 1995. Number and relative size of thenar motor units estimated by an adapted multiple point stimulation method. *Muscle Nerve* 18, 969–979. <https://doi.org/10.1002/mus.880180908>
- Watanabe, T., Kawabata, S., Hoshino, Y., Ushio, S., Sasaki, T., Miyano, Y., Ozaki, I., Adachi, Y., Sekihara, K., Okawa, A., 2019. Novel functional imaging technique for the brachial plexus based on magnetoneurography. *Clin. Neurophysiol.* 130, 2114–2123. <https://doi.org/10.1016/j.clinph.2019.08.006>
- Weber, K.A., Chen, Y., Paliwal, M., Law, C.S., Hopkins, B.S., Mackey, S., Dhaher, Y., Parrish, T.B., Smith, Z.A., 2020. Assessing the spatial distribution of cervical spinal cord activity during tactile stimulation of the upper extremity in humans with functional magnetic resonance imaging. *NeuroImage* 217, 116905. <https://doi.org/10.1016/j.neuroimage.2020.116905>
- Weber, K.A., Chen, Y., Wang, X., Kahnt, T., Parrish, T.B., 2016. Lateralization of cervical spinal cord activity during an isometric upper extremity motor task with functional magnetic resonance imaging. *NeuroImage* 125, 233–243. <https://doi.org/10.1016/j.neuroimage.2015.10.014>
- Westfall, J., Kenny, D.A., Judd, C.M., 2014. Statistical power and optimal design in experiments in which samples of participants respond to samples of stimuli. *J. Exp. Psychol. Gen.* 143, 2020–2045. <https://doi.org/10.1037/xge0000014>
- Wolpert, D.M., Diedrichsen, J., Flanagan, J.R., 2011. Principles of sensorimotor learning. *Nat. Rev. Neurosci.* 12, 739–751. <https://doi.org/10.1038/nrn3112>
- Yacoub, E., Wald, L.L., 2018. Pushing the spatio-temporal limits of MRI and fMRI. *NeuroImage*, Pushing the spatio-temporal limits of MRI and fMRI 164, 1–3. <https://doi.org/10.1016/j.neuroimage.2017.11.034>
- Yoshizawa, T., Nose, T., Moore, G.J., Sillerud, L.O., 1996. Functional Magnetic Resonance Imaging of Motor Activation in the Human Cervical Spinal Cord. *NeuroImage* 4, 174–182. <https://doi.org/10.1006/nimg.1996.0068>
- Zanette, G., Tinazzi, M., Manganotti, P., Bonato, C., Polo, A., 1995. Two distinct cervical N13 potentials are evoked by ulnar nerve stimulation. *Electroencephalogr. Clin.*

Neurophysiol. Potentials Sect. 96, 114–120. [https://doi.org/10.1016/0168-5597\(94\)00212-W](https://doi.org/10.1016/0168-5597(94)00212-W)

Zewdie, E., Kirton, A., 2016. Chapter 1 - TMS Basics: Single and Paired Pulse Neurophysiology, in: Kirton, Adam, Gilbert, D.L. (Eds.), Pediatric Brain Stimulation. Academic Press, Oxford, pp. 3–22. <https://doi.org/10.1016/B978-0-12-802001-2.00001-1>

Zhang, W., Tan, C., Sun, F., Wu, H., Zhang, B., 2018. A Review of EEG-Based Brain-Computer Interface Systems Design. Brain Sci. Adv. 4, 156–167. <https://doi.org/10.26599/BSA.2018.9050010>

IX. Appendix

1. Appendix- Chapter IV

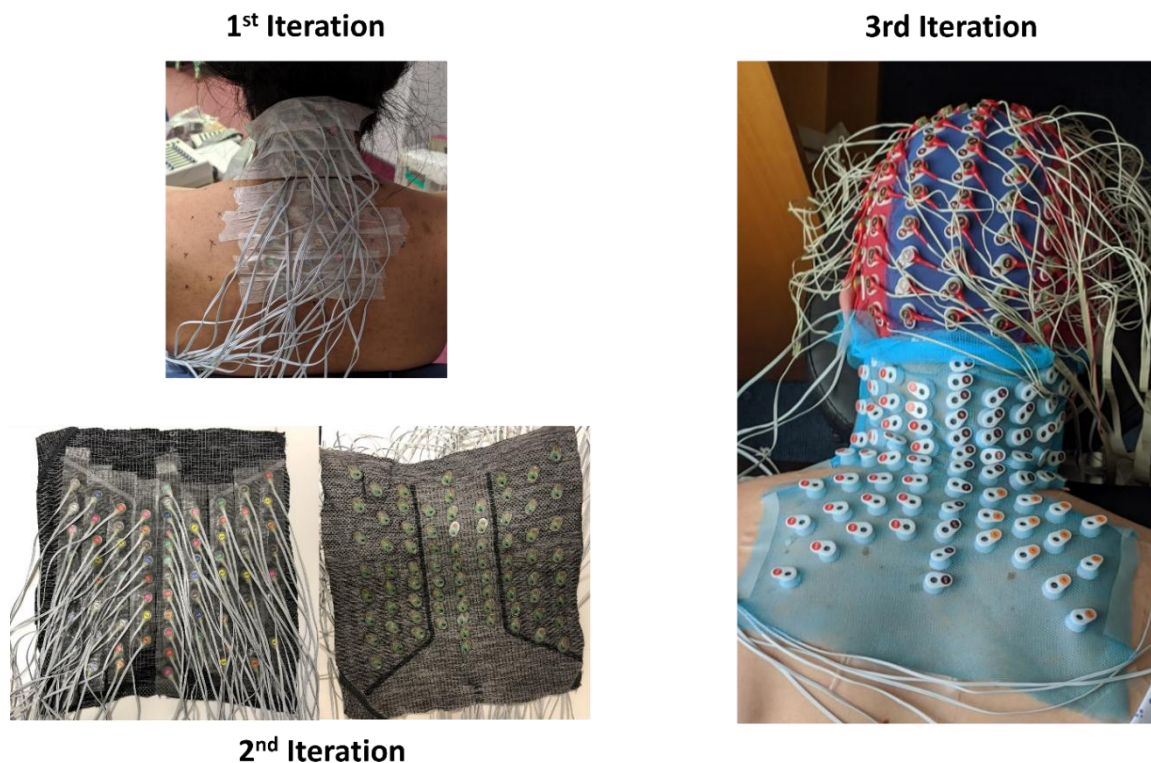


Figure 31: The previous iterations of the electrode patch development. The electrode patch went through multiple iterations before the final version shown in the section IV.2.4 was realized. 1st Iteration: In the first iteration the electrode locations were marked over the skin of the participant, then flat-type electrodes were placed at the marked site and were firmly stuck to the back using adhesive tape. In the second iteration, the electrode patch was carved out of a breathable cloth. The electrode locations were cut-out in the cloth as per the designed electrode placement system. The flattype electrodes were held in the cut-out position using adhesive tape. Before placing the electrode patch on the participant adhesive pads were put on the flat-type electrodes, after which the electrode patch was placed on the participants. The electrodes which weren't held

tightly was reattached for better connection. In the third iteration of the electrode patch was developed using breathable netted nylon sheet and pin-type electrodes. The pin-electrode holders were embedded in the netted fibre as per the designed electrode placement location. The nylon electrode patch was placed over the skin of the participant and tape was used to attach it to the surface.

2. Appendix- Chapter V

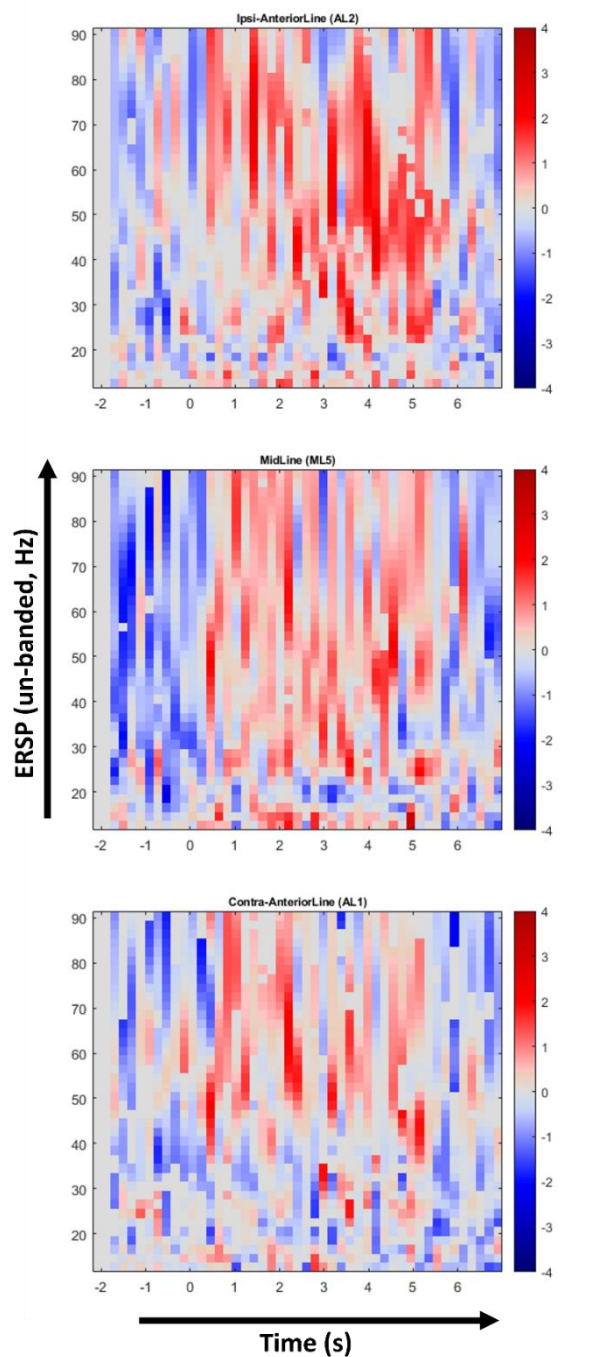


Figure 32: The group-average unbanding ERSPs for ipsi-antrolateral (AL2), midline (ML6), and contra-lateral (AL1) ESG electrodes at the lower cervical cord are shown in the figure. The figure showcases the gERSPs in the pre-motor (-2s to 0s), execution (0s to 0.75s), sustained (0.75s to 4.75s), termination (4.75s to 5.5s), and post-motor period of the motor task (5s – 7s).

3. ESG power spectrums

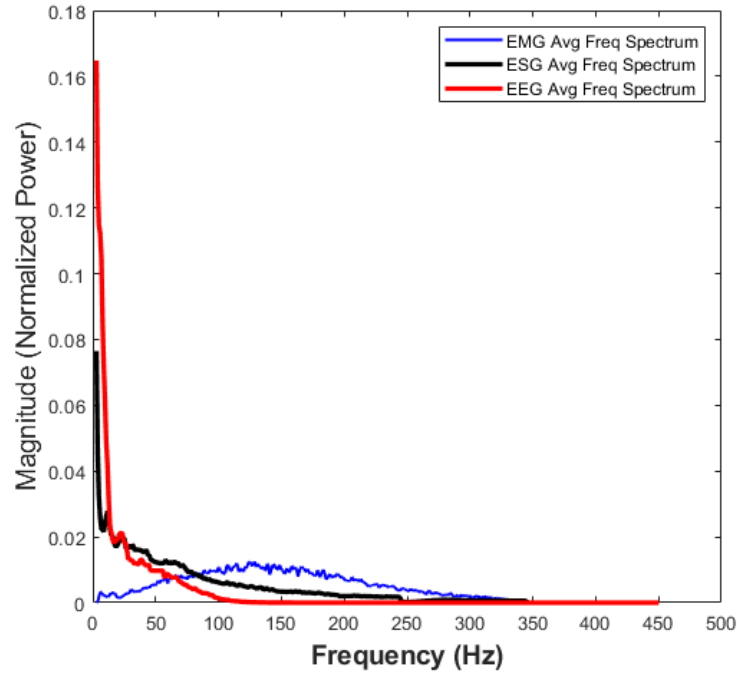


Figure 33: Average Power spectrum of ESG signal at ML6 electrode across 10 healthy participants is plotted alongside the average EEG power spectrum at C3 electrode location and average bi-EMG power spectrum (ABP) during sustained contraction. The plotted power spectrum is normalized w.r.t. the total power; i.e. area under the curve for all three spectra equals 1. The figure shows differences between ESG, EEG, and EMG spectral power distribution.

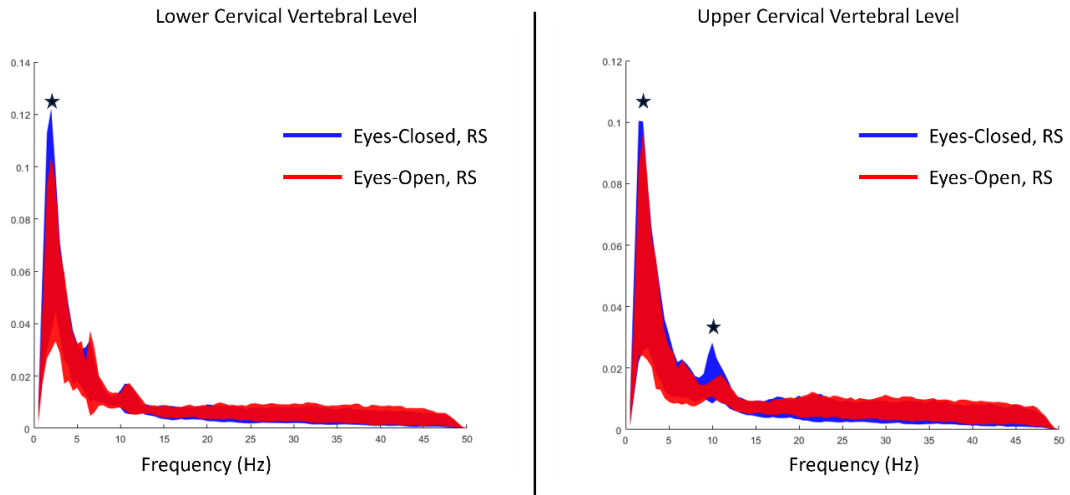


Figure 34: The power spectra of ESG ($n=10$) at the lower cervical level (left) and upper cervical level (right) during the eyes-open (red, EO) and eyes-closed (blue, EC) resting state paradigm. A significant difference in spectral power between EO and EC paradigm was observed in the delta frequency band for both cervical levels. However, only the upper cervical level demonstrated a significant difference in spectral power in the alpha frequency-band between EO and EC.

4. Consent Forms

4.1. Patient Consent Form



INFORMED PATIENT CONSENT FORM

Study Title: Developing novel non-invasive electrophysiological biomarkers of dysfunction in spinal and cortical pathways in Motor Neuron Disease and sensorimotor impairments: ESG/ Spinal Cord Mapping Aspect	
There are four sections in this form. Section 1 contains statements of understanding and asks you to tick each if you understand. Please ask any questions you may have when reading each of the statements. Section 2 asks for your informed consent. Please select either 'yes' or 'no' to indicate your choice. Section 3 asks for your informed future consent. Please select either 'yes' or 'no' to indicate your choice. Section 4 asks for your informed consent on storage and future use of the data. Please select either 'yes' or 'no' to indicate your choice. Thank you for participating.	
1. General Understanding	Tick
I have read and understood the Patient 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.	
I understand that taking part in this study is entirely voluntary. I understand that not taking part will have no negative impact on me.	
I understand that I can leave this study at any time without giving a reason. I understand that leaving this study will not affect my medical care now or in the future.	
I understand that I will not be paid for taking part in this study or receive any benefits from any products developed as a result of this research study or from any profits that may arise from the future use of data or products derived from it.	
I understand that in the case of incidental findings from this research study, that may be an indirect sign of a previously unknown disease, I will be asked for permission to discuss the findings with Prof. Hardiman, my GP or other appropriate doctors.	
I know how to contact the research team if I need to.	
By ticking each box above and choosing my options below and signing this document I agree to participate in this study as described in the Participant Information Leaflet.	
2. Consent	



I consent to take part in this research study having been fully informed of the risks.	<table><tr><td>Yes</td><td>No</td></tr><tr><td>☐</td><td>☐</td></tr></table>	Yes	No	☐	☐
Yes	No				
☐	☐				
I consent to the use of information about me (personal data) being used for this research study as described in the participant information leaflet.	<table><tr><td>Yes</td><td>No</td></tr><tr><td>☐</td><td>☐</td></tr></table>	Yes	No	☐	☐
Yes	No				
☐	☐				
I consent to information I have previously provided to the Academic Unit of Neurology being shared for this research project. I have been assured that information about me will be kept private and confidential.	<table><tr><td>Yes</td><td>No</td></tr><tr><td>☐</td><td>☐</td></tr></table>	Yes	No	☐	☐
Yes	No				
☐	☐				
3. Future Contact					
I give permission for my contact details to be stored by the research team for contacting me in relation to this study?	<table><tr><td>Yes</td><td>No</td></tr><tr><td>☐</td><td>☐</td></tr></table>	Yes	No	☐	☐
Yes	No				
☐	☐				
I consent to be re-contacted by researchers about possible future research to for which I may be eligible.	<table><tr><td>Yes</td><td>No</td></tr><tr><td>☐</td><td>☐</td></tr></table>	Yes	No	☐	☐
Yes	No				
☐	☐				
4. Storage and Future Use of Information					
I give permission for data to be stored and used for <u>possible future research related ALS</u> by academic research institutions (research institutes, hospitals, not-for-profit organisations) without further consent being required but only if the research is approved by a Research Ethics Committee.	<table><tr><td>Yes</td><td>No</td></tr><tr><td>☐</td><td>☐</td></tr></table>	Yes	No	☐	☐
Yes	No				
☐	☐				
I give permission for data to be used for <u>possible future research related to ALS</u> by researchers working for commercial/pharmaceutical companies but only if the research is approved by a Research Ethics Committee	<table><tr><td>Yes</td><td>No</td></tr><tr><td>☐</td><td>☐</td></tr></table>	Yes	No	☐	☐
Yes	No				
☐	☐				
I agree that data I provide can be shared with researchers working on <u>neurological research</u> related to ALS outside of the EEA subject to a GDPR compliant legal agreement.	<table><tr><td>Yes</td><td>No</td></tr><tr><td>☐</td><td>☐</td></tr></table>	Yes	No	☐	☐
Yes	No				
☐	☐				

_____	_____	_____
Participant Name (Block Capitals)	Participant Signature	Date

To be completed by the Principal Investigator or nominee.

I, the undersigned, have taken the time to fully explain to the above participant the nature and purpose of this study in a way that they could understand.



Trinity College Dublin
Coláiste na Tríonóide, Baile Átha Cliath
The University of Dublin

I have explained the risks involved. I have invited them to ask questions on any aspect of the study that concerned them.

I have given a copy of the participant information leaflet and consent form to the participant with contact details of the study team.

Researcher name _____

Title and qualifications _____

Signature _____

Date _____

**Copies to be created and retained: 1 for Participant, 1 for PI and research record.*

4.2. Control Consent Form



INFORMED CONTROL CONSENT FORM

Study Title: Developing novel non-invasive electrophysiological biomarkers of dysfunction in spinal and cortical pathways in Motor Neuron Disease and sensorimotor impairments: ESG/ Spinal Cord Mapping Aspect	
There are four sections in this form. Section 1 contains statements of understanding and asks you to tick each if you understand. Please ask any questions you may have when reading each of the statements. Section 2 asks for your informed consent. Please select either 'yes' or 'no' to indicate your choice. Section 3 asks for your informed future consent. Please select either 'yes' or 'no' to indicate your choice. Section 4 asks for your informed consent on storage and future use of the data. Please select either 'yes' or 'no' to indicate your choice. Thank you for participating.	
1. General Understanding	Tick
I have read and understood the Control 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.	
I understand that taking part in this study is entirely voluntary. I understand that not taking part will have no negative impact on me.	
I understand that I can leave this study at any time without giving a reason. I understand that leaving this study will not affect my medical care now or in the future.	
I understand that I will not be paid for taking part in this study or receive any benefits from any products developed as a result of this research study or from any profits that may arise from the future use of data or products derived from it.	
I understand that in the case of incidental findings from this research study, that may be an indirect sign of a previously unknown disease, I will be asked for permission to discuss the findings with Prof. Hardiman, my GP or other appropriate doctors.	
I know how to contact the research team if I need to.	
By ticking each box above and choosing my options below and signing this document I agree to participate in this study as described in the Participant Information Leaflet.	
2. Consent	

I consent to take part in this research study having been fully informed of the risks.	<table border="1"> <tr> <td>Yes</td> <td>No</td> </tr> <tr> <td>☐</td> <td>☐</td> </tr> </table>	Yes	No	☐	☐
Yes	No				
☐	☐				
I consent to the use of information about me (personal data) being used for this research study as described in the participant information leaflet.	<table border="1"> <tr> <td>Yes</td> <td>No</td> </tr> <tr> <td>☐</td> <td>☐</td> </tr> </table>	Yes	No	☐	☐
Yes	No				
☐	☐				
I consent to information I have previously provided to the Academic Unit of Neurology being shared for this research project. I have been assured that information about me will be kept private and confidential.	<table border="1"> <tr> <td>Yes</td> <td>No</td> </tr> <tr> <td>☐</td> <td>☐</td> </tr> </table>	Yes	No	☐	☐
Yes	No				
☐	☐				
3. Future Contact					
I give permission for my contact details to be stored by the research team for contacting me in relation to this study?	<table border="1"> <tr> <td>Yes</td> <td>No</td> </tr> <tr> <td>☐</td> <td>☐</td> </tr> </table>	Yes	No	☐	☐
Yes	No				
☐	☐				
I consent to be re-contacted by researchers about possible future research to for which I may be eligible.	<table border="1"> <tr> <td>Yes</td> <td>No</td> </tr> <tr> <td>☐</td> <td>☐</td> </tr> </table>	Yes	No	☐	☐
Yes	No				
☐	☐				
4. Storage and Future Use of Information					
I give permission for data to be stored and used for <u>possible future research related ALS</u> by academic research institutions (research institutes, hospitals, not-for-profit organisations) without further consent being required but only if the research is approved by a Research Ethics Committee.	<table border="1"> <tr> <td>Yes</td> <td>No</td> </tr> <tr> <td>☐</td> <td>☐</td> </tr> </table>	Yes	No	☐	☐
Yes	No				
☐	☐				
I give permission for data to be used for <u>possible future research related to ALS</u> by researchers working for commercial/pharmaceutical companies but only if the research is approved by a Research Ethics Committee	<table border="1"> <tr> <td>Yes</td> <td>No</td> </tr> <tr> <td>☐</td> <td>☐</td> </tr> </table>	Yes	No	☐	☐
Yes	No				
☐	☐				
I agree that data I provide can be shared with researchers working on <u>neurological research</u> related to ALS outside of the EEA subject to a GDPR compliant legal agreement.	<table border="1"> <tr> <td>Yes</td> <td>No</td> </tr> <tr> <td>☐</td> <td>☐</td> </tr> </table>	Yes	No	☐	☐
Yes	No				
☐	☐				

Participant Name (Block Capitals)	Participant Signature	Date

To be completed by the Principal Investigator or nominee.

I, the undersigned, have taken the time to fully explain to the above participant the nature and purpose of this study in a way that they could understand.



Trinity College Dublin
Coláiste na Tríonóide, Baile Átha Cliath
The University of Dublin

I have explained the risks involved. I have invited them to ask questions on any aspect of the study that concerned them.

I have given a copy of the participant information leaflet and consent form to the participant with contact details of the study team.

Researcher name _____

Title and qualifications _____

Signature _____

Date _____

**Copies to be created and retained: 1 for Participant, 1 for PI and research record.*

5. Edinburgh Handedness Inventory

Edinburgh Handedness Inventory¹

Participant ID: _____

Please indicate with a one (1) your preference in using your left or right hand in the following tasks.

Where the preference is so strong you would never use the other hand, unless absolutely forced to, put a two (2).

If you are indifferent, put a one in each column (1|1).

Some of the activities require both hands. In these cases, the part of the task or object for which hand preference is wanted is indicated in parentheses.

Task / Object	Left Hand	Right Hand
1. Writing		
2. Drawing		
3. Throwing		
4. Scissors		
5. Toothbrush		
6. Knife (without fork)		
7. Spoon		
8. Broom (upper hand)		
9. Striking a Match (match)		
10. Opening a Box (lid)		
Stop here.		
Total checks:	LH =	RH =
Cumulative Total	CT = LH + RH =	
Difference	D = RH - LH =	
Result	R = (D / CT)x100 =	
Interpretation: (Left Handed: R < -40) (Ambidextrous: -40 > R < +40) (Right Handed: R > +40)		

¹Oldfield, R. C. (1971). The assessment and analysis of handedness: The Edinburgh inventory. *Neuropsychologia*, 9, 97-113.