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High-density EMG reveals atypical spatial activation of the gastrocnemius during walking in adolescents with Cerebral Palsy

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A B S T R A C T

Children with Cerebral Palsy (CP) exhibit less-selective, simplified muscle activation during gait due to injury of the developing brain. Abnormal motor unit recruitment, altered excitation-inhibition balance, and muscle morphological changes all affect the CP electromyogram. High-density surface electromyography (HDsEMG) has potential to reveal novel manifestations of CP neuromuscular pathology and functional deficits by assessing spatiotemporal details of myoelectric activity. We used HDsEMG to investigate spatial-EMG distribution and temporal-EMG complexity of gastrocnemius medialis (GM) muscle during treadmill walking in 11 adolescents with CP and 11 typically developed (TD) adolescents.

Our results reveal more-uniform spatial-EMG amplitude distribution across the GM in adolescents with CP, compared to distal emphasis in TD adolescents. More-uniform spatial-EMG was associated with stronger ankle co-contraction and spasticity. CP adolescents exhibited a non-significant trend towards elevated EMG-temporal complexity. Homogenous spatial distribution and disordered temporal evolution of myoelectric activity in CP suggests less-structured and desynchronized recruitment of GM motor units, in combination with muscle morphological changes. Using HDsEMG, we uncovered novel evidence of atypical spatiotemporal activation during gait in CP, opening paths towards deeper understanding of motor control deficits and better characterization of changes in muscular activation from interventions.

1. Introduction

Cerebral Palsy (CP) is the most-common lifelong physical disability, encompassing a group of movement disorders caused by non-progressive damage in the developing brain (McIntyre et al., 2022). The brain lesion generates a cascade of neuromusculoskeletal symptoms, such as smaller, stiffer, and weaker muscles, as well as motor control deficits (Graham et al., 2016; Mathewson and Lieber, 2015). As a consequence, children with CP adopt atypical gait patterns, which inhibit mobility, and can deteriorate over time, reducing community participation and quality of life (Bell et al., 2002; Lundh et al., 2018). Many gait deviations are marked by dysfunction of the ankle joint, with ankle musculature being especially affected in ambulatory CP (Eek and Beckung, 2008).

A variety of neuromuscular changes occur with CP. Muscular activation patterns of the locomotor muscles are altered during gait, such as elevated plantarflexor recruitment during early-stance related to walking on the forefoot (Romkes and Brunner, 2007). Individuals with CP also exhibit limitations in selective control of muscles and elevated co-contraction of agonists and antagonists (Mohammadyari Gharehbolagh et al., 2023), employing less-complex coordination patterns during walking (Steele et al., 2015). Additionally, evidence suggests CP generates imbalances in motor system excitation-inhibition, leading to over-activation of some motor circuits (Condliffe et al., 2016; Fogarty, 2023), with simultaneous loss of motor connections and subsequent weakness and under-activation of other circuits (Elder et al., 2003). Finally, abnormal neuromotor signaling during development, in combination with muscle disuse, contributes to degradation of muscle

Abbreviations: CP, Cerebral Palsy; TD, typically developed; EMG, electromyography; HDsEMG, High-Density surface electromyography; GM, Gastrocnemius Medialis; TA, Tibialis Anterior; SD, single-differential; CCA, canonical correlation analysis; SPM, statistical parametric mapping; RMS, root-mean square; MVIC, maximal voluntary isometric contraction.

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quality in CP, with intramuscular infiltration of non-contractile collagen and fat (Graham et al., 2016; Mathewson and Lieber, 2015).

Currently, there is a lack of comprehensive understanding of the links between neuromuscular symptoms and gross motor functional deficits in CP. Previous research on CP motor pathology has relied on bipolar electromyography; however, this method is limited by its local sampling that may not be sufficiently representative of the global muscle activation. Indeed, the electromyogram of a muscle is not uniform, and fiber arrangement and inhomogeneities in intramuscular structure often generate regional EMG variations (Vieira et al., 2017; Vieira and Botter, 2021). Further, the smallest level of motor control is not at the muscle level, but at the level of its motor units, whose territories do not extend to the entire muscle cross section (Carbonaro et al., 2022; Rohlén et al., 2023, Rohlén et al., 2020), opening the possibility for regional recruitment of muscle fibers (Hug et al., 2023; Vieira et al., 2011). Depending on muscle size and fiber arrangement, this may result in distinct spatial excitation patterns, often reported in literature (Nishikawa et al., 2022; Sanderson et al., 2019; Schlink et al., 2020a; Schlink et al., 2020b; Vieira et al., 2015).

High-density surface electromyography (HDsEMG) utilizes a grid of electrodes covering a large skin surface area over the muscle. HDsEMG may provide a more informative picture of muscular activation differences in CP due to the capability to evaluate the myoelectric signal in both temporal and spatial domains (Campanini et al., 2022). Studies using HDsEMG have revealed variations in spatial-EMG patterns with fatigue, exercise-induced muscle damage, and chronic pain (Gallina et al., 2011; Piitulainen et al., 2009; Sanderson et al., 2019), as well as in clinical conditions, such as knee osteoarthritis (Ogrezeanu et al., 2023), ankle instability (Mendez-Rebolledo et al., 2024), and scoliosis (Wang et al., 2022), indicating abnormal spatial activity may contribute to clinical symptoms. Additionally, HDsEMG has illuminated atypical spatial activation in neurological movement disorders, including Parkinson's (Nishikawa et al., 2017) and Stroke (Kallenberg and Hermens, 2011).

The use of HDsEMG during movement has important clinical potential to uncover differences in spatial muscle activation in individuals with neuromotor disorders during activities relevant to everyday life, such as gait. Previous research has shown spatial activation in the gastrocnemius is amplified in the distal region during walking in healthy adults (Cronin et al., 2015; Schlink et al., 2020a; Schlink et al., 2020b), with activation shifting more-proximally following fatigue (Schlink et al., 2021). Intramuscular coherence has been shown to be altered during gait in individuals with incomplete spinal-cord injury, demonstrating the potential of dynamic HDsEMG to reveal novel abnormalities in activation following neuromotor disturbance (Zipser-Mohammadzada et al., 2022). However, no studies have used HDsEMG to compare spatial-EMG patterns between typically-developed individuals and those with CP during walking.

The temporal pattern of the myoelectric signal, as assessed using EMG complexity, can also be altered due to neurological disturbance. EMG complexity strongly relates to active motor unit properties, including firing rate and level of synchronization, as well as action potential conduction velocity (Mesin et al., 2016; Rampichini et al., 2020). Studies have displayed reductions in EMG complexity with fatigue, related to central and peripheral changes to neuromotor function and intramuscular physiology (Rampichini et al., 2020). EMG complexity has been shown to change in post-stroke individuals during target tracking and obstacle avoidance tasks, potentially related to neuromuscular coping strategies in paretic compared to non-paretic limbs (Ao et al., 2015; Chen et al., 2018). Individuals with Parkinson's Disease also exhibit altered EMG complexity (Flood et al., 2019), and complexity changes have been observed during spastic muscle contractions in post-stroke patients (Xie et al., 2020), indicating altered motor unit recruitment generating changes in EMG temporal properties. To our knowledge, no studies have utilized HDsEMG to evaluate EMG-temporal complexity during walking in adolescents with CP.

The aim of this study was to characterize differences in spatiotemporal EMG properties of the gastrocnemius medialis (GM) muscle between adolescents with CP and age-matched typically developed (TD) peers during treadmill walking using HDsEMG. We hypothesized a more spatially-homogenous EMG distribution and higher temporal-EMG complexity in adolescents with CP, based on lower levels of neuromuscular selectivity, abnormal excitation-inhibition balance, and degradations in muscle structural quality leading to less-distinct and more-disordered spatial and temporal activation patterns. Evaluating the spatiotemporal elements of the electromyogram of a key locomotor muscle in CP will expand our understanding of neuromuscular manifestations of CP, and could help in bridging knowledge on the links

Table 1
Participant Characteristics

Group	*Subject #*	*Age*	*Gender F/M*	*Weight (kg)*	*Unilateral / Bilateral*	*Affected / Dom. Leg*	*GMFCS*	*Treadmill Speed (m/s)*
TD	1	12	F	55	–	R	–	1.25
	2	13	F	58.5	–	R	–	1.06
	3	10	M	37.3	–	R	–	1.14
	4	14	F	59.1	–	R	–	1.11
	5	18	M	69	–	R	–	1.19
	6	15	M	65.1	–	R	–	1.17
	7	17	M	69.5	–	R	–	1.22
	8	9	M	29.5	–	R	–	1.17
	9	16	M	62	–	R	–	1.28
	10	16	M	68	–	R	–	1.17
	11	11	M	42.3	–	R	–	1.17
Group Avg	–	**13.7 ± 3.0**	**3 / 8**	**55.9 ± 13.7**	–	**11 / 0**	–	**1.18 ± 0.06**
CP	12	11	F	34.1	Bi	R	II	0.56
	13	14	M	66.2	Bi	R	II	0.75
	14	17	M	68.1	Bi	L	II	1.06
	15	18	M	69	Uni	R	I	1.00
	16	17	F	72	Uni	L	I	0.97
	17	15	M	53.1	Uni	R	I	1.22
	18	15	M	59.7	Uni	R	I	1.06
	19	10	M	28.7	Uni	L	II	0.92
	20	14	F	62.9	Uni	R	I	1.08
	21	14	F	62	Uni	L	I	1.17
	22	10	M	38.9	Uni	R	I	1.00
Group Avg	–	**14.1 ± 2.8**	**4 / 7**	**57.6 ± 14.8**	**8 / 3**	**7 / 4**	**7 / 4**	**0.98 ± 0.19**

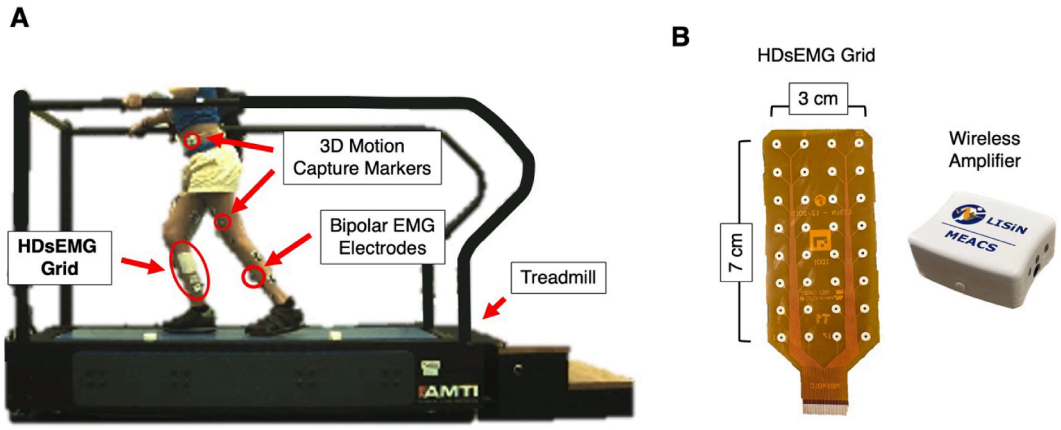

Fig. 1. Experimental Setup. A CP participant walking on the treadmill with the HDsEMG grid recording activity from the GM muscle. **B** The HDsEMG grid and wireless amplifier (34x30x15 mm).

between motor control changes and loss of mobility and function.

2. Methods

2.1. Participants

Eleven adolescents with CP (14.1 ± 2.8 years old; 4 female) were recruited from the rehabilitation unit of the New Children's Hospital in Helsinki, Finland. Eleven TD adolescents (13.7 ± 3.0 years old; 3 female) were recruited as controls (Table 1). Bilateral and unilateral CP participants, as well as GMFCS levels I-II were included to capture a broad sample of ambulatory CP. This study was approved by the Helsinki University Hospital ethics committee (HUS/1074/2020). All protocols abided by the Declaration of Helsinki. Written informed consent from participants' parents, and verbal assent from participants were obtained before measurements.

2.2. Experimental Protocol

Participants walked on a treadmill for six-minutes while myoelectric activity from the GM was recorded using a 32-channel HDsEMG grid (8x4 electrode matrix, 10-mm interelectrode distance) Fig. 1. Signals were amplified (192 V/V), band-pass filtered (10–500 Hz), sampled at 2048 Hz, digitalized with a 16 bits A/D converter and transmitted through a Wi-Fi link to a personal computer for real-time visualization and storage (MEACS system, LISiN, Politecnico di Torino and ReC Bioengineering Laboratories s.r.l., Torino, Italy; Cerone et al., 2019). We focused on the GM because of its importance for propulsion during healthy gait, and because it is commonly affected by neuromotor symptoms in ambulatory CP. The grid was placed on the dominant leg of TD participants and more-affected leg of CP participants. The center of the grid was positioned approximately 1-cm above the midpoint of the GM, based on participants performing three heel-raises and identifying muscle boundaries and midpoint. Retroreflective markers were placed on bony landmarks according to the plug-in-gait lower body model, and tracked using three-dimensional motion capture (Vicon Motion Systems, Oxford, UK). Motion capture data was used for detecting relevant gait events (i.e., foot-strike and toe-off). EMG and kinematic signals were synchronized using a common trigger (Cerone et al., 2022). Treadmill speed was set at 0.9x participants' self-selected overground walking speed (Jung et al., 2016).

Prior to treadmill walking, participants performed maximal voluntary contraction and ankle spasticity testing using an isokinetic dynamometer (Con-Trex, CMV AG, Dübendorf, Switzerland). Metrics from dynamometer testing were utilized for secondary correlation testing, and detailed in the supplementary methods.

2.3. Data Processing

HDsEMG data from minute 4–5 of treadmill walking was analyzed. 32-channel monopolar data was converted to 28 single-differentials by subtracting channels longitudinally, then filtered with a 4th-order Butterworth filter between 20–450 Hz. Monopolar channels with baseline instability from bad electrode–skin contact were marked via visual inspection, and extrapolated as the average of adjacent channels.

The HDsEMG system has been shown to be robust against movement artifact, even in highly dynamic actions (Cerone et al., 2023). However, due to partly suboptimized reference-cable positioning, and novel experimental design in young clinical participants, we observed some residual movement artifact, especially at foot-strike. If movement artifact was observed, canonical correlation analysis (CCA) was used to clean signals (Al Harrach et al., 2017; Schlink et al., 2020a; Schlink et al., 2020b). In brief, decomposed canonical components from HDsEMG were correlated to isolated movement artifact (extracted based on occurrence within gait cycle). Components with highest Pearson's correlation to the artifact were removed, and the signal was reconstructed from remaining components. Cleaned signals were inserted back into the HDsEMG dataset over the gait cycle period where movement artifact was originally identified. CCA has been shown to effectively clean movement artifact from HDsEMG data (Al Harrach et al., 2017; Schlink et al., 2020a; Schlink et al., 2020b), and good signal quality was confirmed by visual analysis following CCA cleaning.

To evaluate proximal–distal EMG variations, we divided the GM area covered by the grid into proximal and distal regions based on the non-homogenous orientation of GM fibers relative to the skin along the muscle length. As the alignment between electrodes and fibers affects EMG outcomes (Vieira et al., 2017; Vieira and Botter, 2021), we computed proximal and distal outcomes separately, ensuring comparable electrode-fiber alignment within each region. The proximal region contained rows 1–4 over the part of the GM with in-depth pennate fiber orientation. Rows 5–7 were taken as the distal region over the curved part of the muscle transitioning to the aponeurosis, with fibers becoming more-parallel to the skin, enabling higher possibility to record propagating motor unit action potentials (MUAPs) from surface electrodes (see Fig. 6 in Vieira and Botter, 2021). The existence of propagating potentials was identified by visual analysis of the signals by an expert investigator. Row 4 divided non-propagating and propagating signal properties in both CP and TD groups.

To quantify EMG-spatial distribution, we computed the modified entropy across the HDsEMG grid (Farina et al., 2008). First, EMG amplitude was estimated as the root-mean square (RMS). Modified entropy, or spatial entropy, was then computed as:

$$Entropy = \sum_{i=1}^{28} p^2(i) log_2(p^2(i))$$

Where $p^2(i)$ represents the square of the EMG-RMS value of each channel *i*, normalized to the sum of squares of all channels. Spatial entropy describes the spatial-EMG amplitude distribution, with greater entropy indicating greater spatial uniformity of EMG amplitude across the grid, while lower entropy indicates more-heterogeneous distribution and spatially-localized EMG amplitude. Spatial entropy is commonly used for comparing EMG-spatial distribution between two conditions or populations (Arvanitidis et al., 2021; Schlink et al., 2021).

To capture the temporal complexity of the EMG signal, we computed Higuchi's Fractal Dimension (HFD), which quantifies the space-filling propensity of a signal, with values bounded between 1 and 2 and a higher-HFD signifying a more-complex and convoluted signal (Higuchi, 1988; Santuz and Akay, 2020). The fractal dimension of the EMG has been shown to strongly relate to motor unit properties, especially the firing rate and level of synchronization (Mesin et al., 2016, Mesin et al., 2009), making it a compelling tool to evaluate the temporal-EMG pattern between CP and TD individuals. To supplement HFD, the median spectral frequency (MDF) of EMG was also taken to describe the frequency content of the signal. HFD and MDF were averaged over the proximal GM region to represent signal complexity and spectral properties in the in-depth pennate part of the muscle.

Foot-strike and foot-off events were identified from the anteroposterior velocity of the toe-marker, which has shown to be a reliable method to classify gait events during treadmill walking (Visscher et al., 2021). The EMG linear envelope was generated by rectifying and low-pass filtering the 28 single-differential channels using a 2nd-order Butterworth filter at 10 Hz, time-normalizing to the gait cycle, and averaging across channels. Considering the GM muscle is primarily active during stance-phase, our primary spatiotemporal EMG comparisons between CP and TD were computed over the stance-phase of gait from foot-strike to toe-off. Additionally, we computed a spatial entropy envelope to track evolution of spatial-EMG changes across the gait cycle as EMG-spatial entropy time-normalized to the gait cycle. HDsEMG videos displaying spatial activation changes throughout gait were also generated and presented in supplementary materials. At least 30 strides were taken from the one-minute walking period for each participant.

2.4. Statistical analysis

Shapiro-Wilk and Kolmogorov-Smirnov with Lilliefors correction tests were used to assess normality. Outcomes needed to pass both tests to be considered normal, and analyzed using parametric tests; while outcomes found to have non-normal distribution were analyzed using non-parametric tests. We compared EMG-spatial entropy, EMG-temporal complexity (HFD), and median frequency (MDF) computed over stance-phase between CP and TD groups using independent samples t-tests or Mann-Whitney U-tests. To evaluate proximal–distal differences in EMG amplitude and temporal complexity, we compared EMG-RMS and HFD from proximal-distal regions within-groups using paired t-tests or Wilcoxon signed-ranks tests. Statistical parametric mapping (SPM) was performed to identify periods of significant difference between CP and TD EMG and spatial entropy envelopes (Pataky et al., 2016). Alpha was set at $p = 0.05$ and Bonferroni corrections were performed to account for multiple comparisons for each group of tests (i.e., three independent-samples t-tests performed – p-value corrected for three tests, corrected p-value: 0.017, etc.). P-values presented in Results are re-corrected to significance level $p = 0.05$ for clarity. Effect-size was computed using Cohen's D (Cohen, 2013). A supplementary correlation analysis was performed to evaluate relationships between EMG-spatial entropy and EMG-temporal complexity to other neuromuscular (spasticity and co-contraction) and functional (ankle movement pattern during gait and plantarflexor strength) outcomes across the two groups (supplementary methods).

3. Results

3.1. Between-Group comparisons

Comparing EMG-spatial distribution between CP and TD groups, we found significantly increased EMG-spatial entropy during stance-phase in the CP group (CP: 4.73 ± 0.02, TD: 4.53 ± 0.11; d = 3.10, $p < 0.001$), indicating more-homogenous EMG-amplitude distribution across the GM in adolescents with CP (Fig. 2A). EMG-temporal complexity (HFD) exhibited an elevated trend in the CP group (CP: 1.44 ± 0.07, TD: 1.37 ± 0.06; d = 1.09, p = 0.088), but this difference was not significant. MDF also appeared higher in CP adolescents, but not significantly (CP: 115 ± 30 Hz, TD: 90 ± 23 Hz; d = 0.93, p = 0.212). The CP group exhibited elevated GM activity during early-stance, pre-swing, and late-swing (2.9–20.3 %, 52.6–68.6 %, 84.3–98.6 %; $p < 0.001$), based on EMG envelopes (Fig. 2D).

3.2. Within-Group comparisons between Proximal-Distal GM activity

In the TD cohort, the distal GM region exhibited 29.5 ± 14.7% higher EMG-RMS compared to the proximal region (d = 0.54, p = 0.008). EMG signal complexity (HFD) was significantly greater in the proximal compared to the distal region in TD (d = -0.81, p = 0.019).

In the CP group, EMG-RMS was 9.5 ± 10.8% higher in the distal compared to the proximal region, but this difference was not significant (p = 0.114). There were no significant proximal–distal differences in signal complexity in CP adolescents.

3.3. Evolution of GM spatial activation throughout the gait cycle

There were statistically significant differences in spatial entropy envelopes between CP and TD groups for the majority of single-support phase (14.8 – 17.6%, 19.5 – 47.0%; $p < 0.01$), with adolescents with CP exhibiting higher spatial entropy when the GM muscle was most strongly active (Fig. 3).

4. Discussion

This study was the first to use HDsEMG to compare spatiotemporal activation of the GM muscle between adolescents with CP and age-matched TD controls during treadmill walking. The novel finding was a more spatially-homogeneous EMG-amplitude distribution across the GM in adolescents with CP, compared to distal emphasis in TD participants. Altered motor unit activations and/or territories in combination with muscle morphological changes in CP may generate more-uniform spatial activation patterns during gait. Temporal-EMG complexity and spectral frequency also both displayed elevated trends in CP, pointing towards more-disordered neuromuscular recruitment. Significant differences in EMG-spatial distribution between CP and TD groups were found especially during the mid-stance phase of gait, when the GM muscle is most active, signifying highly contrasting spatial activation strategies between the groups.

4.1. Spatiotemporal activation of GM muscle in typically developed adolescents

TD adolescents displayed 30% higher EMG amplitude in the distal compared to proximal GM region during gait (Fig. 2A). Previous research in healthy adults has shown similar results, with increased EMG amplitude in the distal part of both lateral (Cronin et al., 2015), and medial (Schlink et al., 2020a; Schlink et al., 2020b) gastrocnemii muscles during walking and running. We observed contrasting differences in EMG-temporal complexity, with significantly higher EMG complexity in the proximal compared to the distal region in TD adolescents (Fig. 2B).

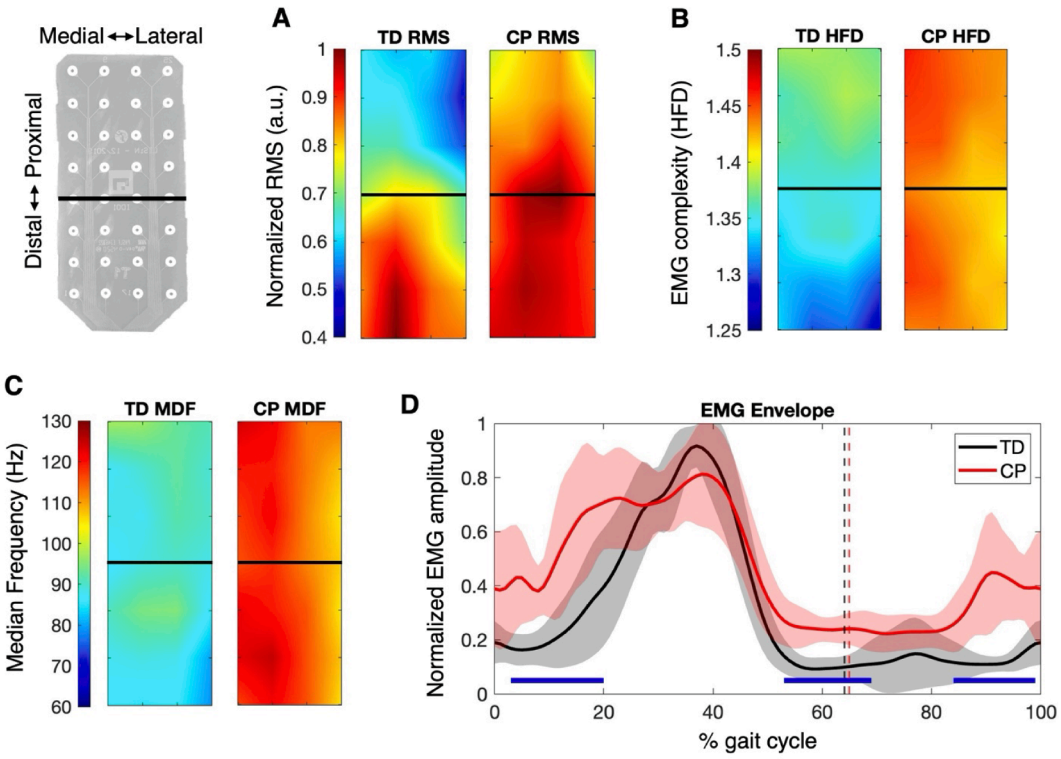

Fig. 2. Between-Group Differences. **A–C** Group average heatmaps of EMG amplitude (normalized RMS [**A**]), EMG-temporal complexity (HFD [**B**]), and median frequency (MDF [**C**]) from the stance-phase of gait. Heatmaps present the 28 single-differential channels interpolated by a factor of 8 for visualization. RMS maps were normalized to the peak value across the grid. Black horizontal lines represent the split between proximal and distal GM regions. **D** Group average EMG linear envelopes. Shading represents ± 1 standard deviation from the mean. Blue horizontal bars display periods of significant difference between CP and TD curves identified from SPM analysis. Vertical dashed lines indicate group average instances of foot-off, and separation of stance and swing phases.

The pennate architecture of the GM means more active motor units can be recorded from a smaller area on the skin surface, but action potential propagation is less emphasized in the EMG signal compared to muscles with more-parallel fascicle orientation (Mesin et al., 2011). Due to the curved shape of the GM muscle, fascicle orientation in the distal region becomes more-parallel to the skin, making recording of action potential propagation more-likely (Vieira and Botter, 2021). Considering single-differential channels act as spatial filters enhancing propagating components of the signal (Merletti and Muceli, 2019), distal emphasis of EMG amplitude in TD individuals may be largely explained by higher likelihood for propagating potentials in the lower-part of the GM due to muscle anatomy. Higher EMG complexity in the proximal GM may instead be related to greater numbers of motor units represented in the surface EMG signal in the proximal-pennate region, generating a more-complex signal.

Neurophysiological factors in proximal–distal EMG differences in TD adolescents also may play a role. For instance, some evidence points towards small and spatially-distinct motor unit territories within the human GM, as well as proximal–distal segmentation of motor unit populations (Vieira et al., 2015, 2011), although larger territories have also been described in literature (Héroux et al., 2015). Thus, proximal–distal differences in EMG amplitude and complexity in the GM of TD participants could indicate selective recruitment of regionally distinct motor unit populations, based on functional demands of walking. Further research combining musculoskeletal imaging and HDsEMG is needed to elucidate the contribution of structural and neurophysiological parameters to the spatial-EMG pattern of the GM muscle during gait in TD adolescents.

4.2. More-Uniform Spatial-EMG distribution across GM muscle in adolescents with CP

In-line with our primary hypothesis, CP adolescents showed significantly greater EMG-spatial entropy across the GM muscle during gait, indicating more-uniform spatial spread of myoelectric amplitude (Fig. 2A). Further, no significant proximal–distal differences in EMG-RMS nor signal complexity were observed in the CP group. When examining the evolution of spatial activation across the gait cycle, the CP group showed significantly higher EMG-spatial entropy especially over mid-stance, when GM is most active, suggesting high spatial entropy is a differentiating feature of the GM activation strategy of CP adolescents during walking.

Evidence clearly suggests motor control alterations in individuals with CP, with deficits in selectivity of muscle and movement control (Noble et al., 2019), elevated co-contraction of agonist and antagonist muscles during movement (Mohammadyari Gharehbolagh et al., 2023), and adoption of less-complex inter-muscular coordination patterns for walking (Steele et al., 2015). The more-uniform spatial-EMG pattern we observed in the CP group reveals for the first time more-disordered and less-distinct spatial activation across the surface of a key locomotor muscle in adolescents with CP. Contrasted with the relatively consistent spatial activation pattern observed in TD adolescents (singular EMG burst during mid-late stance emphasized in the distal GM region), the CP adolescents exhibited varied spatial activation patterns between individuals that often included multiple activation bursts across different regions of the GM muscle (see supplementary HDsEMG videos for demonstration). At the group-level, these varied spatial patterns manifested as more-uniform and less-distinct spatial activation patterns in the CP adolescents.

Abnormal spatial activation could arise from fundamental neuromuscular disturbances that underlie deficits in motor control selectivity

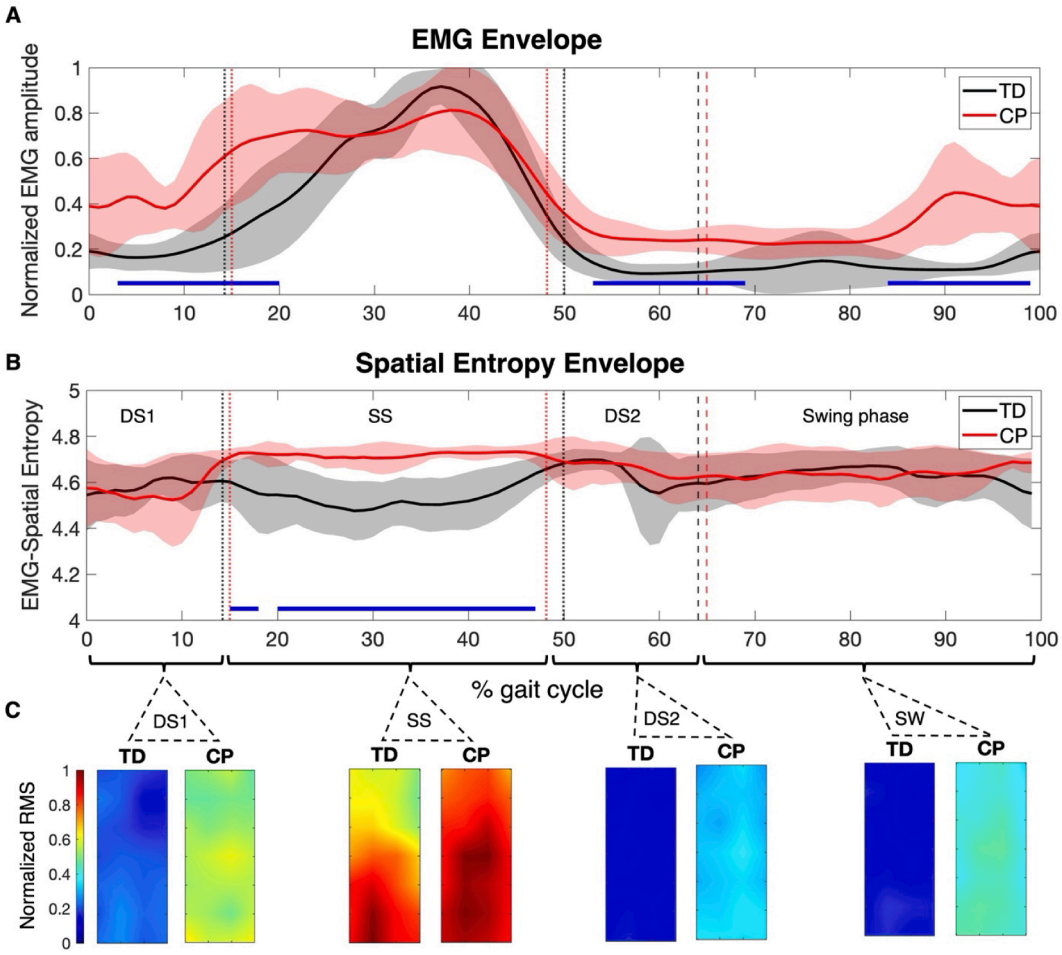

Fig. 3. Evolution of GM Spatial Activity Across the Gait Cycle. A EMG Linear envelopes from TD and CP groups. **B** Spatial entropy envelopes displaying changes in spatial-EMG distribution over the gait cycle. Gait events separating relevant gait subphases (double-support 1 [DS1], single-support [SS], double-support 2 [DS2], and swing-phase [SW]) are marked as vertical dotted/dashed lines (in order: opposite foot-off, opposite foot-strike, same foot-off). Blue horizontal lines above the x-axis indicate periods of significant difference between TD and CP groups from statistical parametric mapping (SPM). **C** EMG-RMS heatmaps are displayed from the four gait subphases to visualize changes in spatial-EMG amplitude distribution during walking.

in CP. Indeed, we observed moderate-strong associations between elevated EMG-spatial entropy and stronger ankle muscle co-contraction during gait and higher levels of plantarflexor spasticity in the CP group (supplementary results). Both co-contraction and spasticity in CP have been linked to excitation-inhibition imbalance and impaired neuromuscular inhibition caused by the initial brain lesion (Condliffe et al., 2016; Fogarty, 2023). Inhibitory synaptic connections are crucial for healthy motor control, enabling selective control of muscles and blocking undesirable overactivation. Damage or malformation to inhibitory connections reduces the precision of activation in CP, and may generate phenotypic symptoms, including diminished selective motor control, elevated co-contraction, and hyper-sensitive reflexes (Mohammadyari Gharehbolagh et al., 2023; Valadão et al., 2022). Indeed, atypical homogenous GM spatial activation in CP adolescents may reflect impaired selective inhibition of motor units during gait leading to altered intra-muscular coordination.

Disturbed neuromuscular development in CP generates a cascade of diverse consequences affecting neuromusculoskeletal structure and function (Graham et al., 2016). Research has consistently reported diminished morphological muscle quality in CP, and loss of contractile tissue replaced by build-up of collagen and fat (Handsfield et al., 2022; Mathewson and Lieber, 2015). Build-up of non-contractile tissue in the muscle may increase diffusion of the intramuscular potential, and diminish EMG amplitude and spatial selectivity, resulting in more-homogenous spatial activation recorded by HDsEMG. However, subcutaneous fat, known to reduce EMG amplitude and spatial sensitivity, is not altered in ambulatory and higher-functioning individuals with CP (Whitney et al., 2020). Further, there was no significant difference in body-mass index (BMI) between our CP and TD groups (CP: 20.6 ± 3.8 kg/m^2, TD: 19.3 ± 1.7 kg/m^2; $p = 0.321$), suggesting body composition likely did not strongly contribute to observed differences in spatial activation between CP and TD adolescents.

Alterations in GM gross-muscle morphology could also contribute to spatial activation differences in CP. Changes in pennation angle towards a sharper angle could align the fascicles in a more-parallel orientation relative to the skin throughout a longer length of the muscle, causing propagating and non-propagating regions to become less distinct, and generating a more-uniform spatial spread of EMG amplitude. Previous research on pennation angles in CP report mixed results; however, the majority of studies indicate small or insignificant differences in pennation angles with CP (D'Souza et al., 2019; Handsfield et al., 2022; Shortland et al., 2002). Mixed results reinforce the heterogeneity of the CP population, but indicate no generalized changes in pennation angle caused by CP. Recent research, however, indicates greater GM muscle-belly lengthening during gait in CP, compared to relatively isometric muscle behavior in TD adolescents (Cenni et al., 2024). More muscle-belly lengthening could stretch the fascicles and produce a sharper pennation angle during dynamic walking, potentially contributing to more-uniform spatial-EMG distribution.

Thus, alterations in both underlying neurophysiology and motor unit

properties in combination with gross changes in muscle morphology and function likely contribute to the more-homogenous and disordered spatial activation pattern observed in CP adolescents. Future research is needed to uncover anatomical and neuronal mechanisms generating greater EMG-spatial entropy in CP, and changes in spatial-EMG patterns in response to different treatments.

4.3. Trend towards elevated Temporal-EMG complexity in CP

Our results showed trends towards higher temporal-EMG complexity and median frequency in the adolescents with CP compared to TD controls, partly supporting our hypothesis of a more-complex EMG signal in the CP group. Although differences in group-average values were present, high inter-individual variability likely contributed to the lack of statistical significance of these results.

Tao et al showed muscle activity complexity between and across muscles during gait, measured using multivariate multi-scale entropy analysis, varied in children with CP compared to a TD control group (Tao et al., 2015). At some scales, muscle activity complexity was significantly higher in CP, with authors interpreting over-activation and spasticity generating higher inter-muscular complexity in CP. Conversely, muscle activity complexity was reduced in CP at different scales and amongst different muscles, representing under-activation or loss of muscle-couplings during gait (Tao et al., 2015). These findings showcase the multifaceted effects of CP on neuromuscular activation, and how positive (i.e., overactivation and spasticity) and negative (i.e., weakness and paralysis) features of the motor disorder generate differential impacts on EMG patterns. We observed trends towards elevated EMG-temporal complexity and median frequency in the GM muscle of CP adolescents during gait, potentially displaying predominance of overactivation as well as spasticity generating a more-complex and disordered EMG signal. More research is needed to characterize EMG-temporal complexity in CP across different clinical classifications and locomotor muscles.

Research indicates a shift within CP muscle from slow-to-fast fiber types and fast-twitch fiber predominance (Deschrevel et al., 2023; Pontén and Stål, 2007). Similar slow-to-fast fiber type shift is seen in other muscle-wasting disorders characterized by disuse and/or denervation, such as spinal-cord injury (Ciciliot et al., 2013). Research in adults with CP also shows lower numbers of motor units controlling the hand musculature, but increased motor unit size (Marciniak et al., 2015). Larger motor units may be accompanied by larger motor unit territories, as motor unit territories have been shown to be enlarged with diabetic peripheral neuropathy (Favretto et al., 2023), and following spinal-cord injury (Thomas et al., 1997). Increase in motor unit size and territory may be generated by neuropathic loss of connection to some motor units followed by reinnervation and axonal branching to orphaned fibers (Favretto et al., 2023; Garg et al., 2017). Dominance of large motor units and predominantly fast-twitch fibers in CP could lead to higher discharge rates, lower levels of synchronization, and increased amplitude per motor unit firing, producing a more-complex and higher-frequency EMG signal. Additionally, larger motor units with enlarged territories could generate a more-homogenic EMG-spatial distribution, as well as a more temporally-complex EMG signal, as observed in our CP cohort. However, further study into motor unit size and territory change in CP is required.

5. Limitations

The lack of assessment of muscle anatomical variables was a limitation. Without structural information, we cannot say whether features such as pennation angle, muscle size, fat infiltration, and other non-neural factors contributed to our results. Future studies should employ combined HDsEMG-ultrasonography to examine relationships between muscle structure and HDsEMG signal characteristics in individuals with CP (Botter et al., 2013).

The small size of our sample was a further limitation. As no similar studies using HDsEMG during gait in CP have been performed, we could not perform statistical power analysis. However, our sample size is similar to other electromyographic studies with CP participants (Conner et al., 2020; Romkes and Brunner, 2007; Valadão et al., 2022).

Another important limitation was the focus on solely the GM muscle. We chose the GM due to its importance in propulsive power during gait, and that it is so often affected by neuromuscular symptoms in ambulatory CP. However, examination of other locomotor muscles, such as the tibialis anterior, using HDsEMG during gait would expand our understanding of manifestations of CP motor control disturbances across electromyograms, and their links with mobility and function.

6. Conclusion

We used HDsEMG to compare spatiotemporal characteristics of the electromyogram of the GM muscle between adolescents with CP and age-matched TD adolescents during treadmill walking. In-line with our primary hypothesis, CP adolescents exhibited higher EMG-spatial entropy, signifying more-uniform and less-precise EMG-spatial distribution across the GM muscle surface, compared to distal EMG emphasis in TD adolescents. We also observed trends towards higher EMG-temporal complexity and frequency in CP adolescents, implying potentially more-disordered GM muscle recruitment during gait in CP. Less-selective motor unit activations, abnormal anatomical distribution of motor units, and morphological changes in the muscle could all contribute to atypical myoelectric activation of the GM in both space and time during gait in CP. Further, positive associations between EMG-spatial entropy and EMG-temporal complexity with greater ankle co-contraction and spasticity in the CP group may point towards common underlying mechanisms, such as imbalances in motor system excitation-inhibition. Our findings demonstrate the clinical potential of HDsEMG to reveal novel features of motor pathology in CP, meriting further research into causes of altered spatiotemporal activation, as well as how spatiotemporal EMG features in CP change in response to different treatments.

7. Ethics approval and consent to participate.

The present study was approved by the Ethical Committee of the Helsinki University Hospital (HUS/1074/2020). Written informed consent was secured by parents or guardians and verbal assent by all study participants was obtained prior to beginning measurements. All study participants provided consent for publication of data and images.

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Author contributions

MT: conceptualization, performing experiments, analysis, and writing. MP: conceptualization, performing experiments, review, and editing. AG: conceptualization, review, editing, and interpretation. IV: conceptualization, review, editing, and supervision. AB: conceptualization, review, interpretation, editing. JPK: funding, conceptualization, performing experiments, review, editing, and supervision. HP: conceptualization, interpretation, review, editing, and supervision. All authors approved final manuscript.

CRediT authorship contribution statement

Maxwell Thurston: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Mika Peltoniemi:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Alessandra Giangrande:** Writing – review & editing, Validation, Methodology, Investigation, Data curation, Conceptualization. **Ivan Vujaklija:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Conceptualization. **Alberto Botter:** Writing – review & editing, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Juha-Pekka Kulmala:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Harri Piitulainen:** Writing – review & editing, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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