UNIVERSITY OF PADUA

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Master Degree in Cognitive Neuroscience and Clinical Neuropsychology

Final Dissertation

Changes in Intramuscular Coherence in Tibialis Anterior Muscle following a Visuomotor Gait Task

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Index

1. Abstract

Humans preferred locomotor pattern is walking. Bipedal walking is a learned and demanding task that requires the coordination of the activity of several muscles and joints. Locomotion is rarely performed under ideal circumstances (flat surface, unobstructed path, etc.). It is more common for humans to find themselves having to change the walking gait's rhythmical nature to match the ever-changing characteristics of the environment. In order to do so, humans must evaluate the external circumstances, process all the incoming information, and select the appropriate motor plan without disrupting the ongoing gait pattern. Individuals predominantly regulate their locomotion based on visual information. The interaction between the visual and motor systems allows us to perform gait modifications and adaptations successfully and rapidly.

Coherence analysis is a non-invasive method that allows us to make inferences about the cortical and spinal involvement from electrophysiological measures.

This experiment aims at investigating whether there are differences in coherence before and after a visuomotor gait task performed on a treadmill in typically developed (TD) individuals and individuals with cerebral palsy (CP).

Participants (8 TD adults, *mean age* = 30.12 and 8 adults with CP, *mean age* = 26.37) performed a static contraction of the Tibialis Anterior (TA) muscle for 3 minutes followed by a Visuomotor gait task that required them to correctly place their foot on targets that appeared on the screen in front of them. Following the treadmill task, they were asked to repeat the TA muscle's static contraction.

Coherence analysis was used to quantify the coupling between two ends of the tibialis anterior muscle (proximal TA and distal TA) (intramuscular coherence).

Our results showed that there is an increase in coherence in the beta and gamma frequency bands following a visuomotor gait task and that this increase is significantly larger for TD adults. Moreover, coherence is higher for the reaching leg, meaning the leg that actively reaches the targets during the gait task. Lastly, our results showed that improvement in performance is correlated with a decrease in errors during the task.

2. Introduction

Locomotion is the most common form of movement in nature, it is fundamental for animals since it provides access to key resources for survival, such as food or shelter.

It can be defined as the process by which animals change their location from one position to another. Rose and Gamble in "Human Walking" in 2006 described locomotion as "a rhythmic displacement of the body parts that maintain the animal in constant forward progression" (Rose & Gamble, 2006). Walking is humans' preferred locomotion pattern. Bipedal plantigrade progression is a purely human and learned accomplishment that requires the coordination of several muscles and joints, resulting from the integration of the activity of spinal circuitries, sensory signals, and supraspinal motor commands. The human body can integrate and control the activity of many different segments in order to minimize energy expenditure while maximizing performance efficiency (Capaday, 2002).

Our unique pattern of walking has many distinguishable characteristics, and it can be divided into two main phases, a stance phase, and a swing phase. The so-called "gait cycle" (Fig. 1) begins with the stance phase by initial ground contact, which in typically developed individuals is determined by a heel strike. The stance phase lasts for the period during which the foot is on the ground. Furthermore, during the stance phase, there is a lengthening contraction of the ankle dorsiflexors muscle and subsequent lengthening contraction of the ankle plantar flexors which corresponds to heel rise. The controlled shift in space of the center of the body and the controlled fall with the opposite leg is immediately followed by the beginning of a new stance phase. (Kharb, Saini et al., 2011)

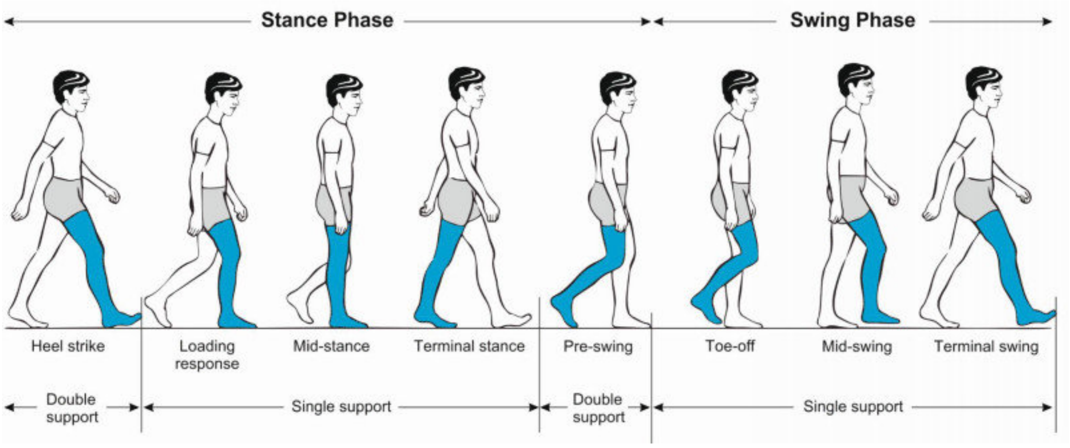

Figure 1: Phases of the gait cycle (picture from Pirker, Walter & Katzenschlager, Regina; 2016. Available via license Creative Common Attribution 4.0 International)

Moreover, humans have to integrate other body movements such as vertical displacement of the body, mediolateral pelvic tilt, horizontal pelvic rotation, flexion of the knee, lateral displacement of the torso, and rotation of the shoulder girdle (Inman, 1966).

The coordination between muscles, joints, and body segments is so well performed that humans' energy consumption can be easily compared to quadrupedal animals and it is more effective than bipedal walking in other primates (Abitbol, 1988).

A hallmark of healthy, safe, and independent control of walking is *automaticity*, which can be defined as "the ability of the nervous system to successfully control typical steady state walking with minimal use of attention-demanding executive control resources" (Clark, 2015).

Locomotion is rarely solely controlled by either automatic or executive control processes. *Automatic processing* is fast and is activated automatically in response to a particular input, whereas *executive control processing* is slow and serial and requires voluntary control and attention by the individual (Schneider and Shiffrin, 1977).

Shiffrin, Schneider, and colleagues proposed a framework that requires the two processes to balance each other in the control of walking, in order to perform efficiently and safely. Automatic processing can operate in highly demanding situations and requires less effort, whereas an executive control strategy is more suitable when obstacles and hazards need to be evaluated. However, assessing how these two processes are balanced in the central nervous system is not easy. Usually, researchers use paradigms that involve assessment during dual tasking. This method is based on the idea that during dual-task conditions a decrement in performance results from competition for executive resources, whereas during a single-task the performance does not decrease, and automatic processes are predominant. The dual-task paradigm, however, is complicated and there are too many confounding factors that can potentially influence the results.

A valid alternative for investigating this balance are neurophysiological measures. One of the most prominent is functional near-infrared spectroscopy (fNIRS) which provides continuous and non-invasive monitoring. Although this method seems to be promising there are drawbacks, for example, it only allows a superficial recording of the cortex with low spatial resolution. Other measures include electroencephalography and functional magnetic resonance.

From a neurophysiological point of view, specialized circuits in the central nervous system are involved in the coordination and automaticity of walking allowing a stable and flexible locomotor control strategy that does not entirely depend on attention

(Nielsen, 2003). The main locomotor circuits are located in the spinal cord, the brainstem, and the cerebellum. We know these circuits mainly from experiments conducted on animal models. The most well-known locomotor circuits are the Central Pattern Generator circuits of the spinal cord.

2.1 Central Pattern Generator

Animal studies conducted at the beginning of the 20th century provided evidence of the existence of spontaneous locomotor-like movements in animals with different spinal cord lesions when a limb was dropped from a flexed position (Flourens, 1824; Philippson, 1905). Moreover, evidence showed that decerebrated cats can perform basic stepping movements (Sherrington, 1910) and electrical input of the lumbar part of the spinal cord can elicit flexion and extension even in the absence of supraspinal input (Grillner, 1981). Following Graham Brown's preliminary work from 1914, evidence from the 1960s and onward showed that, even across different species, every rhythmic and stereotyped motor behavior (walking, flying, swimming, etc.) was extensively controlled by a neuronal network generally defined as Spinal Central Pattern Generator.

Results of studies conducted on patients affected by chronic spinal cord injury (SCI), showed spontaneous involuntary and rhythmic stepping-like movements in the lower extremities (Calencie, 1994 and 2006) suggesting the presence in humans of a spinal network designated to locomotion. Moreover, evidence from Dimitrijevic et al. 1998 showed that epidural electrical stimulation of the upper part of the lumbar section of the spinal cord (L1-L2) triggered stepping-like movements in patients with complete SCI.

Particularly, there is a spinal neuronal network composed of different sets of spinal interneurons, located in the lumbar section of the spinal cord, called the Central Pattern Generator (CPG). This network is responsible for generating and controlling basic locomotor rhythm by sending and receiving information through descending motor commands and sensory peripheral inputs.

Forssberg in 1985 proposed that the spinal pattern generator is already operational at birth, causing coordinated kicking movement in infants and the so-called “step reflex” or “walking reflex” that happens when infants stand upright.

With the development and maturation of the individual, the CPG becomes more fine-tuned and complex in order to facilitate and support adult locomotion (Dominici et al., 2011).

Changes in muscles and limb trajectories are coordinated by the integration of descending and peripheral inputs with activity produced by spinal networks. (Drew and Marigold 2015; Nielsen, 2003).

The idea that a rhythmic locomotor activity is based on a central locomotor drive suggests a synchronization that results from a common presynaptic drive to groups of motoneurons. Hansen and colleagues in 2001 analyzed coupling between paired recordings of motor activity from the same or different leg muscles while subjects were walking on a treadmill and showed that motor units discharged within a few milliseconds of each other. This temporal proximity in discharging is defined as *short-term synchrony* and is thought to be caused by a common synaptic drive originating from a common source in the central nervous system (Datta and Stephens, 1990). Datta and colleagues (1991) demonstrated that short-term synchronization is reduced in patients with lesions in the pyramidal tract. Subsequent evidence from Farmer et al. in 1993 on patients with hemiplegia showed that short-term synchronization is indeed supported by the pyramidal tract. Later studies used transcranial magnetic stimulation (TMS) to demonstrate that there is a decrease in electromyography (EMG) activity during gait following stimulation of the motor cortex (Petersen et al., 2001) confirming the involvement of the motor cortex in the activity of lower motoneurons.

Nowadays it is generally assumed that short-term synchronization and coherence in the beta frequency band (15-30 Hz) are supported by the contribution of the pyramidal tract.

2.2 Cortical control of locomotion

However, the CPG is not the only neuronal network in charge of the control of locomotion. The selection and execution of accurate movements during walking involve several cortical as well as subcortical structures.

Studies conducted on cats showed that cells in the Posterior Parietal Cortex (PPC) show substantial changes in discharge during gait modifications that require visual guidance. More specifically, the increase in cell discharge seems to represent the motor plan needed during that specific situation. Results showed that these modifications in discharge are independent of the limb (ipsilateral or contralateral to the recording site) and they began only 2-3 steps before the gait modification happened, even though the obstacles were already visible several steps before, suggesting that the discharge was not related to the properties or characteristics of the obstacle itself (Andujar et al., 2010).

Additional studies conducted on humans seem to support the hypothesis that the PPC contributes to motor planning in visually guided locomotion by determining the location of the hazard in relation to the state of the body at a given moment and evaluating gait modifications in order to safely avoid the obstacle (Andersen and Buneo, 2002; Drew and Marigold, 2015).

Differently from the PPC, cells in the motor cortex change their activation during the execution of the movement. When the gait is being modified, both motor cortical neurons and pyramidal tract neurons discharge, acting through spinal interneuronal networks that are part of the Central Pattern Generator, modulating the pattern and the rhythmicity of locomotion and providing the necessary changes in muscle activity to perform the chosen motor act.

Even though the PPC and the motor cortex play a critical role in the control of locomotion, they are part of a larger cortical and subcortical network.

The premotor cortex, the basal ganglia, the brainstem, and the cerebellum are strongly interconnected with the motor cortex and PPC and are involved in the voluntary control of locomotion. Moreover, the PPC and the motor cortex receive many inputs from sensorimotor areas which are strictly interconnected with the visual system through visual extrastriate areas.

Isolated electrical stimulation of brainstem regions showed to evoke walking-like behaviors in several vertebrates, mammals, and primates. Two main regions have been identified as the brainstem circuits of locomotor control, the mesencephalic locomotor region (MLR) and the subthalamic locomotor region (SLR).

The MLR seems to provide excitatory input to the spinal cord in order to initiate, modify and sustain the descending command for walking (Le Ray et al., 2011; Ryczko and Dubuc, 2013). The SLR instead seems to be relevant for scaling the locomotor output, by changing the speed or the rhythm (Narita et al., 2002).

Locomotor involvement of the cerebellum has been shown primarily in cats (Mori et al., 1998). Humans with cerebellar damage have demonstrated the central role of the cerebellum in the coordination of balance and control of walking. Patients with damage in this structure showed ataxic gait, impaired motor learning, compromised balance function, and difficulties in the planning of the movement.

Moreover, the descending excitatory output derived from cerebral motor pathways is crucial for the facilitation of both the brainstem and spinal circuits described above.

Transcranial magnetic stimulation and electroencephalography studies demonstrated that there is direct involvement of the motor cortex in driving the activation of the specific limb muscles required during walking (Petersen et al., 2001, 2013) suggesting that the automaticity of humans' locomotion depends on some ways, on cerebral circuits.

2.3 Visuomotor learning and adaptation and its neural basis

When performing a motor act individuals need to scale and adjust their movements or body parts in order to respond appropriately to the environment.

Motor adaptation refers to behavioral changes that involve adjusting a pre-existed and well-practiced action in response to a change in the body or the environment by selecting an alternative action or modifying the execution of the current action. Adaption is distinguishable from *de novo* motor learning, which consists in generating a new motor act.

Motor learning includes two main operations. The first one is defined as *skill acquisition*, which is the process through which individuals acquire the ability to select an appropriate motor act based on a particular context and execute the action with precision and accuracy. During the initial stages of motor learning, individuals learn the appropriate sequence of movements by trial and error and by being aware of sensory cues. The second one is *skill maintenance*, which is the ability to maintain and perform the learned motor skills throughout changing conditions. At first, movements can be slow, irregular, and not accurate, however, under normal circumstances, with practice they become more fluent and controlled and feedback becomes less important. Motor learning is fundamental in everyday life, it is present from very simple tasks to more complex and sophisticated movements. It takes time to learn a new motor skill, even though a performance improvement can be seen already from the first training session. Extended practice will make the skilled movement more consolidated and easily accessible in time.

Differences in motor learning exist between typically developed (TD) individuals and individuals with cerebral palsy (CP). Cerebral palsy is the commonest cause of severe physical disability in childhood. It can be defined as a syndrome comprising a heterogeneous group of motor disorders, including spasticity, paresis, incoordination, and dystonia. Eighty percent of children affected by CP have problems with locomotion, with foot drop and toe walking being the most frequent clinical problems (Fowler et al., 2010; Lorentzen et al., 2018). The main leading cause of these dysfunctions is a reduced force in the ankle dorsiflexor muscles, resulting from a motor cortex or a corticospinal tract lesion. (Petersen et al., 2013; Willerslev-Olsen et al., 2014a).

Studies demonstrated that motor learning and adaptation require more repetitions and time in children with CP compared to TD age-matched children (Valvano et al., 1998).

The need to maintain our skills in an ever-changing environment is so pervasive that the motor system possesses dedicated mechanisms and centers for adjusting our actions. Higher motor control centers modulate the gait pattern as a result of the information received by different sensory modalities. Above all, the visual system has a unique role in planning and guiding locomotion in different environments. The visual system can provide immediate and accurate information to guide gait changes according to environmental constraints (Patla, 1997).

Locomotor tasks that happen under environmental constraints require specific and precise movements in order to implement accommodation or avoidance strategies, such as adapting to different surfaces or avoiding an obstacle. Successful changes in locomotion rely on efficient visuomotor control which frequently acts during walking.

For example, when walking in a crowded street, individuals have to avoid collisions with other pedestrians, be aware of the traffic around them and of the surface they are walking on. Moreover, they must make step adjustments based on visual information about the surroundings while maintaining unperturbed locomotion (Matthis et al., 2016).

According to Patla (1997), the locomotor system has to control several attributes in order to perform efficiently. The locomotor system must set up the initial body posture, initiate and terminate locomotion, produce and maintain the necessary rhythmic activation for the limbs and trunk muscles, maintain stability through movement, alter the speed and guide locomotion through obstacles avoiding damage. Vision has a critical role in the control of many of these qualities, providing information about the external environment (exteroceptive function), controlling body parts' movements and position relative to the body itself (proprioceptive function), and relative to the external environment (exproprioceptive function) (Lee and Lishman, 1977).

In order to understand how visual information can influence and modulate locomotion, it is important to understand how the visual input is processed in the central nervous system.

Visual inputs from the retina are mainly processed by the primary visual cortex which receives afferents from the lateral geniculate nucleus, which is the structure that holds 90% of the incoming visual information. However, the retinal information is also sent to the superior colliculus and the pretectum, which respectively play a critical role in orienting saccadic eye movements in the visual field and controlling pupillary reflexes. Once the information is received by the primary visual cortex is sent to other cortical or subcortical visual centers for finer processing. Despite a well understanding of the neural mechanisms underlying both visual and motor systems, it is not yet fully understood how the visual information is translated into motor commands.

Several studies conducted on primates showed that there are many areas involved in visuomotor processing in animals. For example, the posterior parietal cortex seems to be involved in transforming eye-centered target locations into hand-centered target locations (Andersen and Buneo, 2002). Other studies showed the involvement of the parietal cortex and its projections to the dorsal and ventral premotor cortex (Calton et al., 2002). Moreover, neural recordings from basal ganglia, thalamus, and the cerebellum showed task-related activation during visually guided movements (Mushiake and Strick, 1993 and 1995).

Human neuropsychological data shows that individuals with parietal damage manifest different types of visuomotor deficits, such as gaze apraxia (difficulties in exploring space), optic ataxia (deficit in visually guided hand movements), and difficulties in programming and performing sequential movements (apraxia). Numerous studies support the hypothesis that visual inputs are transformed into motor commands through the activity of parietal and premotor cortical networks. Glickstein in 2000 suggested that the link between visual and motor cortical areas is provided by the dorsal stream of extrastriate visual areas, which heavily project to the pontine nuclei. In turn, pontine nuclei’s main relay point is the cerebellar cortex, which sends the information to the cerebellar nuclei that directly project to the descending motor tracts. The role of the cerebellum in visuomotor control is still not well understood, however, we know that it plays a critical role in movement coordination, by receiving information about ongoing movements from the spinal cord and planned movements from other motor centers.

2.4 Coherence

As previously mentioned, nowadays it is generally assumed that short-term synchronization reflects a common drive from the motor cortex and is supported by the contribution of the pyramidal tract.

Statistically, short-term synchronization can be exploited to reveal information on the temporal association between motor unit firing which in turn can be used to make inferences about the strength and the time course of the drive that arose from the CPG. (Kirkwood et al., 1982; Farmer et al., 1993).

Another analysis that can be performed on motor unit firing would be the so-called, coherence analysis. This approach can detect the frequency components by analyzing the coupling between motor unit firings.

Coherence can be observed in different frequency bands, such as alpha bands (5-15 Hz), beta bands (15-30 Hz), and gamma bands (30-60 Hz). Each frequency band is characteristic of specific functional activity in different circuits and has its own distinct origin.

Research conducted by Llinas and Volkind in 1973 and Llinas and Paré in 1995 suggested that coherence in the alpha range (5-15Hz) might be generated by the olivary-cerebellar system. Other studies have detected cortico-muscular coherence at this frequency, indicating the involvement of sensorimotor cortices (Raethjen et al., 2000; Mima and Hallett, 1999), whereas other researchers pointed out that oscillations in the alpha band may represent mechanical resonance problems. Despite these findings, there is still controversy regarding the origins of alpha-band coherence, especially since it is not always present between the cortex and muscles (Gwin and Ferris 2012; Hansen et al., 2002, Salenius et al., 1997).

In contrast, there is general agreement that coherence in the beta (15-30 Hz) and low gamma (30-45Hz) bands is driven by the primary motor cortex, reflecting the common corticospinal drive to the muscles (Grosse and Brown, 2002). Although the contribution of corticospinal activity to high gamma frequency band coherence (>45 Hz) cannot be completely ignored, it has been argued that intramuscular coherence within the high gamma frequency band reflects residual reticulospinal and propriospinal activity following spinal injury and other illnesses (Bravo-Esteban et al., 2017).

The coupling of motor units driven by corticospinal activity has been demonstrated by studies on corticomuscular coherence between magnetoencephalography (MEG) and electromyography (EMG) (Conway et al., 1995) and electroencephalography (EEG) and EMG (Petersen et al., 2013) suggesting that the motor cortex and the corticospinal tract directly contribute to the muscle activity.

Coherence in the beta band appears during tonic muscle contractions and is abolished during movement, whereas coherence in the gamma band is prominent during strong muscle contractions and slow movements.

Coherence may also be seen between different pools of motor neurons within the same muscle or between synergists muscles. EMG-EMG coherence can be defined as a non-invasive method to measure oscillatory drive to muscles during static or dynamic movements. It can quantify the common oscillatory drive to a pair of muscles (intermuscular coherence) or to two parts of the same muscle (intramuscular coherence). EMG-EMG coherence shares similarities with corticomuscular coherence and being a non-invasive method is frequently used as an indirect measure of corticospinal activity, allowing to avoid external neural perturbation.

Both coherence and synchrony have been shown to be dependent on intact central motor pathways including the corticospinal tract. Previous studies underlined the importance of the corticospinal drive to leg muscles during walking, pointing out that coherence at 15~50 Hz is reduced or absent in patients with impaired supraspinal control (Barthélemy et al., 2010; Hansen et al., 2005) cortical lesions (Farmer et al., 1993b) and cerebral palsy (Petersen et al., 2013).

2.5 Locomotor training induces cortical plasticity

Neuroplasticity is the ability of the brain to reorganize itself in response to different stimuli. Neuroplasticity can happen in different situations: it can be related to recovery after a brain injury or lesion or it can be a manifestation of normal brain development, which is the growth process that starts during conception and continues through the first three decades of life. Furthermore, the ability to learn new information or motor skills is also considered a manifestation of brain plasticity.

Numerous studies used non-invasive transcranial magnetic stimulation during a motor task to demonstrate an increase in excitability and a change in the cortical organization of movement representation in the primary motor cortex (Perez et al., 2004; Pascual-Leone, 1995). Nowadays it is generally believed that plastic changes in the motor cortex play an important role in motor learning and skill acquisition, more specifically motor learning has been demonstrated to be strictly correlated with both anatomical and physiological changes in the cortex. Learning seems to be an important driving factor for cortical plasticity.

Coherence analysis allows us to obtain information regarding the synaptic drive to motoneurons during voluntary movements.

Norton and Gorassini in 2006 showed that improvement in locomotor functioning in spinal cord injury subjects after treadmill training therapy was accompanied by increased coherence in the 24-40 Hz band. This increase in coherence is considered to reflect the plasticity of the corticospinal inputs (Barthelemy et al., 2011). Moreover, in a study conducted by Perez et al., in 2006 results showed that visuomotor skill training produces changes in the corticospinal drive to motor neurons, leading to increased EMG-EMG coherence within the Tibialis Anterior muscle.

Plastic changes induced by locomotor training are different between typically developed individuals and individuals with brain or spinal damage.

Cerebral palsy (CP) is a syndrome caused by abnormal development or damage to areas involved in the motor system, including the motor cortex and the corticospinal tract. Functional neuroimaging studies performed on CP patients showed that there are changes in brain pattern activation before and after training therapy. More specifically, fMRI images showed that the involvement of cortical areas decreases once there is a functional improvement in the trained movements. This might suggest that patients need to recruit additional areas when this function is impaired, whereas when it improves, fewer cortical areas are required to successfully perform the movement (Garvey et al., 2007). Another method to assess cortical plasticity after training is to perform a coherence analysis on electrophysiological measures. Petersen et al. in 2013 showed that children with hemiplegic cerebral palsy present impairment in EMG-EMG coherence in the tibialis anterior (TA) muscle, which relates to impaired control of the ankle dorsiflexors muscle. Moreover, children with CP showed a lack of age-related increase in beta and gamma band coherence, compared to typically developed (TD) children, whose increase in coherence was also accompanied by a reduction in step-to-step variability (less clumsiness and falls). Children with CP show impaired central drive to the TA muscle, which results in excessive foot drop and a gait pattern characterized by toe strikes (instead of heel) (Lorentzen et al., 2018). The TA muscle, being the prime dorsiflexor muscle of the ankle, has an essential role in the swing phase of gait. During the swing phase, EEG activity over the leg areas of the motor cortex is coherent with TA muscle EMG activity at frequencies that range between 24 and 40 Hz and 10 Hz (Petersen et al., 2013). This result supports the idea that TA EMG activity is synchronized with cortical activity, underlying a common oscillatory drive from the cortex to the muscle.

Following studies demonstrated that gait training in patients with cerebral palsy changes intramuscular EMG-EMG coherence in the beta and gamma frequency band for the tibialis anterior muscle (Willerslev-Olsen et al., 2015).

3. The objective of the present study

The present study aims at investigating differences in intramuscular coherence in different frequency bands between individuals with CP and TD individuals following a visuomotor gait task. As was previously discovered the central drive to motor neurons can be influenced by training and exercise, and it can be easily and non-invasively measured with coherence analysis. In this study, we measured EMG-EMG coherence from two separate electrodes positioned on the two extremities of the Tibialis Anterior muscle (proximal and distal) of both legs. The training involved five rounds of a visuomotor gait task performed on a treadmill, in which participants were instructed to adjust their gait according to visual targets projected onto a screen placed in front of them. Before and after the training they were asked to perform a static contraction of the Tibialis Anterior muscle for 3 minutes, which was later used for measuring intramuscular coherence.

Previous research suggested that improvement in performance and changes in coherence can be already visible from the first training session (Perez et al., 2006).

The aim is to investigate whether intramuscular coherence changes occur even after a short training session (5 rounds or 3 minutes each) and whether these changes are correlated to a reduction of error rate during the task.

4. Methods

4.1 Participants

22 individuals participated in this study. 11 subjects with CP (8 adults, mean age = 30.12 ± 6.13 years; 2 children, mean age = 6.5 ± 0.5 years) and 11 subjects without CP (8 adults, mean age = 26.37 ± 4 years; 4 children, mean age = 9.25 ± 1 year).

Four adults from the CP group had unilateral cerebral palsy (three were primarily affected on the left side, whereas one was primarily affected on the right side), and four adults had bilateral cerebral palsy (two were most affected on the right side and two on the left side). Whereas one child had unilateral CP and the other one had bilateral CP (both primarily affected on the right side). All individuals affected by CP showed difficulties to some extent in gait function.

CP's severity was classified into levels according to the Gross Motor Function Classification System (GMFCS). Regarding the adults, four participants were classified with GMFCS level II, one participant with GMFCS level III, and three participants with GMFCS level I. One child was classified with GMFCS level I and the other one with GMFCS level III.

Before all experiments, participants received oral and written information about the research project and gave their consent to participate. Children's consent was given by their parents or tutor.

Children were excluded from the analysis of the data because of the low number of participants.

4.2 Gross Motor Function Classification System (GMFCS)

Individuals with Cerebral Palsy were assessed with the Gross Motor Function Classification System (GMFCS).

The GMFCS is a clinical assessment divided into 5 levels that mainly describes self-initiated movements, giving particular emphasis to truncal control in sitting and walking. The distinction in levels is based on functional abilities, the need for assistive measures (walkers, wheelchairs, or canes), and quality of movement. The focus for the gross motor function is on what the individual can do as usual and everyday performance, rather than what they are more capable of doing.

The original version of the GMFCS is dated 1997, nowadays clinicians use an expanded and revised version (GMFCS – E&R) that includes an age band for individuals between 12 to 18 years. The different levels of the GMFCS classification can be divided as follows:

- GMFCS Level I: children can walk indoors and outdoors without needing support. They can perform gross motor skills like running and jumping but speed, coordination, and balance are limited.
- GMFCS Level II: children can walk in most settings by holding onto a railing. However, they can experience fatigue and difficulty while walking long distances and/or on uneven terrains.
- GMFCS Level III: children need to use hand-held mobility devices indoors, and they need support and assistance for long distances.
- GMFCS Level IV: children need to use assistive mobility devices (wheelchairs with or without power) that require physical assistance in most settings.

- GMFCS Level V: children are always transported with a manual wheelchair and all areas of motor functioning are impaired. Moreover, their ability to maintain postural control, head and neck positioning is severely restricted.

4.3 Neurological examination

All participants with CP underwent a neurological examination, which was performed before the beginning of the experiment.

With a goniometer, the passive range of motion (ROM) of the knee and the ankle was measured by slowly moving the joint into full extension and flexion.

Both the knee and the ankle were examined to evaluate the presence of catch and/or clonus. In order to evaluate muscle stiffness, the Modified Ashworth Scale (MAS) was used (Bohannon and Smith, 1987).

Furthermore, Patella and Achilles' tendon reflexes were assessed with a reflex hammer using a 0-2 ordinal grading scale.

Lastly, a 0-5 ordinal grading scale was used to assess voluntary muscle strength in the ankle plantarflexion and dorsiflexion and knee flexion and extension.

5. Experimental procedure

5.1 Static contraction of the Tibialis Anterior muscle

The current study was divided into three main sessions.

First, each participant was asked to perform ankle dorsiflexion for both the right and left leg to measure the Maximum Voluntary Contraction (MVC). They were asked to perform three attempts, this allowed us to select the best performance out of three. Moreover, the MVC was used to calculate the 10% MVC level.

Each participant performed dorsiflexion of both legs at a 10% MVC level for 3 minutes, while electromyographic activity was measured for the proximal section of the Tibialis Anterior (prox TA) and the distal section of the Tibialis Anterior (dist TA). The distance between the two portions of the TA muscle differed between participants since we had to consider differences between muscle length in children and adults (distance for adults = 7-10 cm, distance for children = 5-8 cm).

Subjects were seated in a chair (Fig. 2), which was adjusted according to the height of the participant, with the foot firmly fastened to a force pedal that could measure the force exerted on it. The leg was positioned at 90° flexion at the hip joint, 115° at the knee joint, and 120° dorsiflexion of the ankle joint.

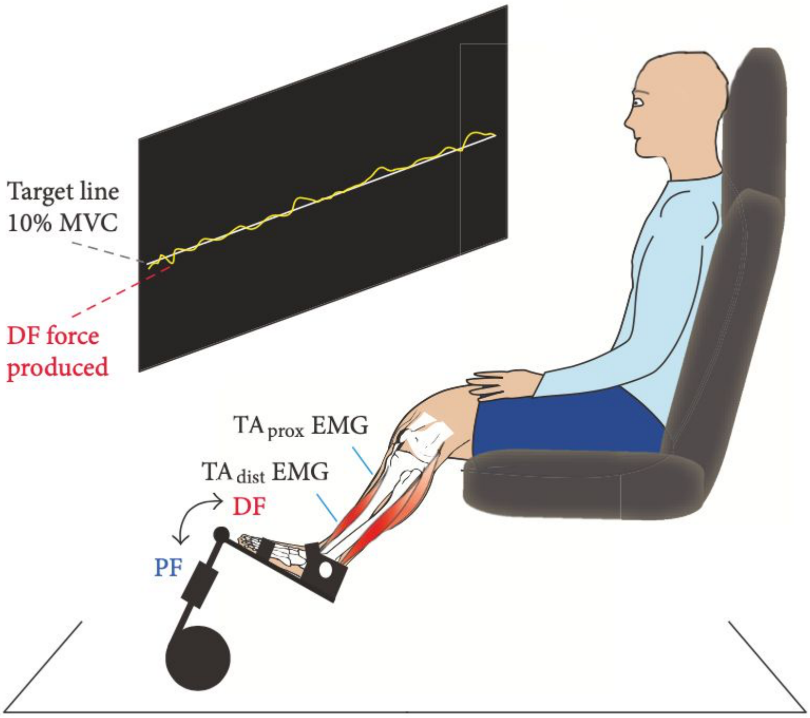

Figure 2: Visual representation of the setup for the static dorsiflexion of the Tibialis Anterior muscle

First, subjects performed three maximal dorsiflexion contractions separated by 20 s of rest to measure maximal voluntary dorsiflexion strength. Online visual feedback of force produced was projected onto a screen placed in front of the subject as a moving blue trace on a white background. During MVC, verbal encouragement was provided to ensure maximal efforts. MVC was determined as peak force production across the three trials.

Subsequently, subjects performed a static contraction, where they were asked to maintain a force level of 10% MVC during dorsiflexion for three minutes. The target force level was displayed on the screen as a horizontal line, and subjects were instructed to follow this line as precisely as possible with the moving blue trace depicting real-time force production. While participants were performing static dorsiflexion, EMG activity was recorded.

After this first part of the experiment, participants performed 5 trials of a Visuomotor gait task (described below) and then they repeated the static contraction of both legs for 3 minutes each.

5.2 VisuoMotorGait Task

Following the static contraction of both legs, participants were asked to perform a Visuomotor Gait task (Fig. 3). The task was performed on a treadmill and the walking speed was adjusted depending on the preferred speed of each participant. Subjects were familiarized with the treadmill and the task by an initial 1-minute familiarity round, in which targets appeared where the foot was dropped.

During the actual learning task, participants were asked to maintain their gaze on a screen positioned in front of them, where a visual representation of their feet (green for the right foot and red for the left foot) was presented. While walking, targets appeared on the upper part of the screen and moved downwards matching the speed of the treadmill, and subjects had to position their foot on them, without disrupting locomotion. Targets were designed to appear either laterally (right or left side) at a maximal 25% of stride length, or at shorter or longer (maximal at 60% of stride length) distances from the original foot position. Targets were designed as bullseyes in order to visually stimulate the participant in driving as precisely as possible the foot towards the center of the target. The outer ring was red and 10 cm radius, the middle ring was yellow, and 6.5 cm radius and the bullseye was black and had 2 cm radius. Depending on the accuracy of the foot position on the target, different points were given to the participants: 1 point for the outer ring, 2 points for the middle one and 3 points when participants hit the bullseye. Each hit corresponded to a specific tone that worked as a positive feedback for the participant.

On the screen, the score and a loading bar were visible during the entirety of the task.

Subjects were asked to perform 5 trials, each one lasting 3 minutes.

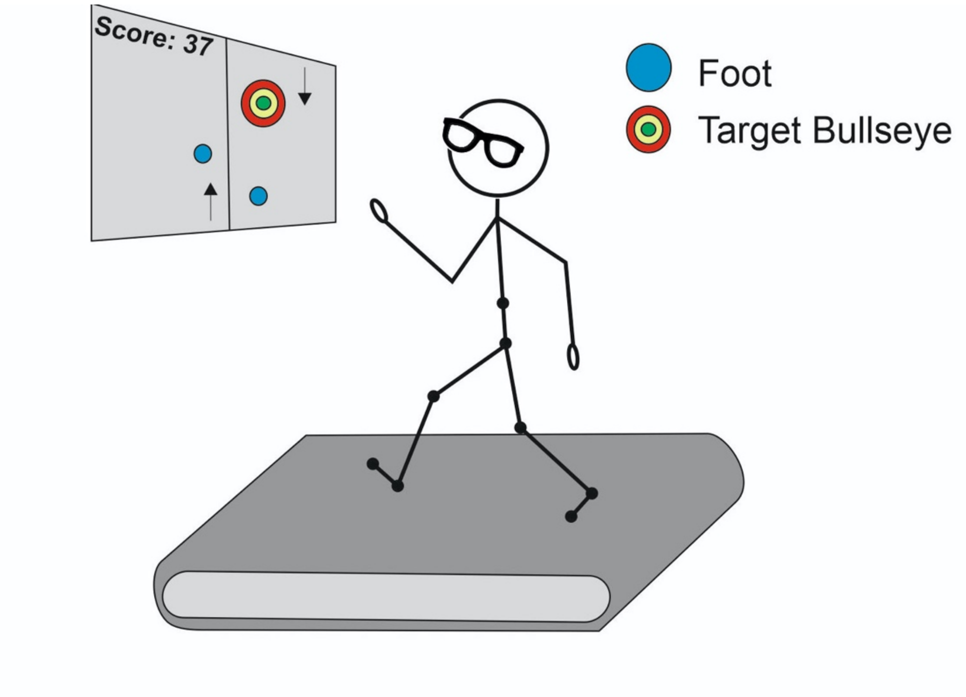

Figure 3: Visual representation of the Visuomotor Gait task

5.3 Motion Capture

A motion capture system with 9 cameras (Qualysis, Sweden) was used to record the 3D position of reflective markers placed on different anatomical landmarks on the subject. 11 markers were positioned on the hip, the lateral joint space (knee), lateral malleolus (ankle), calcaneus (heel), fifth metatarsal head (toe), and sacrum. The sampling rate for the kinematics was 120 Hz.

5.4 Electrophysiological recordings

During the visuomotor task, electromyographical activity from 12 muscles was recorded. Electrodes were placed on both legs on the soleus, the gastrocnemius medialis, the tibialis anterior (proximal and distal section), the vastus lateralis, and the biceps femoris. EMG was recorded using the Delsys Trigno Wireless system (USA). The skin was carefully prepared for the sensors by gentle abrasion of the skin and, if necessary, shaving of the area. The sampling rate for the EMG Delsys system was 1926 Hz.

5.5 Coherence Analysis

Data from the two electrodes placed on both proximal and distal Tibialis Anterior muscles during static contraction was used for the coherence analysis.

The coherence analysis was performed on MATLAB R2021b (MathWorks Inc., Natick, MA, USA).

The frequency domain analysis was performed using the method described in detail by Halliday et al., in 1995.

As described by Halliday and Farmer in 2010 in order to maximize the information related to the timing of the motor unit action potentials (MUAP) and suppress the information generated by the MUAP shape, a full wave rectification of the normalized EMG signals can be used (Myers et al., 2003; Halliday and Farmer, 2010). Moreover, the two rectified signals from the TA were normalized to have unit variance (Halliday and Rosenberg, 2000). Rectified and normalized EMG is presumed to be a realization of stationary zero mean time series denoted by x and y. The auto spectra $f_{xx}(\lambda)$, $f_{yy}(\lambda)$ from the two EMGs, and their cross-spectra $f_{xy}(\lambda)$ were estimated by averaging Fourier transformation from non-overlapping segments of data taken from each signal. Time and frequency domain measures were estimated from the auto and cross-spectra. Coherence is defined and estimated from the squared magnitude of the cross-spectrum normalized by the product of the two spectra (Halliday et al., 1995). The equation is:

$$|R_{xy}(\lambda)|^2 = \frac{|f_{xy}(\lambda)|^2}{f_{xx}(\lambda)\, f_{yy}(\lambda)}$$

Moreover, we estimated three functions characterizing the signals' correlation structure: coherence $|R_{xy}(\lambda)|^2$, which is defined over the range [0, 1] with 0 in case of independency and 1 in case of perfect linear relationship; phase $\varphi_{xy}(\lambda)$ which is important in order to define timing relations between the EMG signals and it is defined over the range $[-\pi, +\pi]$; and cumulant density $q_{xy}(u)$ which provides measures of statistical dependence between random processes, providing unbounded representation in the time domain of the EMG-EMG correlation structure analogous to the motor unit cross-correlogram (Halliday et al., 1995).

Regarding our data, coherence analysis provided a measure of the fraction of the activity in one surface EMG signal at any given frequency that could be predicted by the activity in the second surface EMG signal.

Coherence estimates quantify both the strength and the range of frequencies of the common synaptic inputs across the TA motor neuron pool.

Estimates of pooled coherence provide a frequency domain measure that can be helpful to describe the correlation structure across different normalized data sets by providing a normative measure of linear association in the 0 to 1 range (Amjad et al., 1997). Pooled cumulant density estimates can provide a time domain measure of this correlation. Whereas pooled spectra are a measure of normalized average spectrum for the EMG signals (Amjad et al., 1997).

Moreover, Amjad et al., in 1997 also described the interpretation of the pooled estimates of coherence, cumulant, spectrum and phase as similar to those obtained for individual inferences, but in pooled estimates, the inference relates to the entire population.

In this study, the method used to calculate pooled coherence estimates was to pool the individual coherence estimates (Halliday and Rosenberg, 2000; Farmer et al., 2007). The equation for the pooled coherence across k records at a specific λ frequency is:

$$\left|\frac{\sum_{i=1}^{k} L_i\, R_{xy}^{i}\,(\lambda)}{\sum_{i=1}^{k} L_i}\right|$$

Estimates from this pooled coherence equation provide as result a single parameter describing the correlation structure as a function of the frequency within the range of records (k) coming from a single population. Moreover, the pooled phase is calculated as the phase angle of the pooled cross-spectral estimate and the pooled cumulant density is estimated by the inverse Fourier transformation of the pooled cross-spectral estimate.

The pooled coherence framework contains a χ^2 extended difference of coherence test. The χ^2 test is based on the hypothesis that the population has equal coherence values. A further pooled spectral analysis is performed on the two sets of pooled spectra data from each population. Significant values of the χ^2 variate would indicate that the hypothesis does not provide a significant interpretation of the data.

The χ^2 test is applied to each frequency separately and the results of the χ^2 difference test can provide a metric of the pooled coherence difference at each frequency between the two populations.

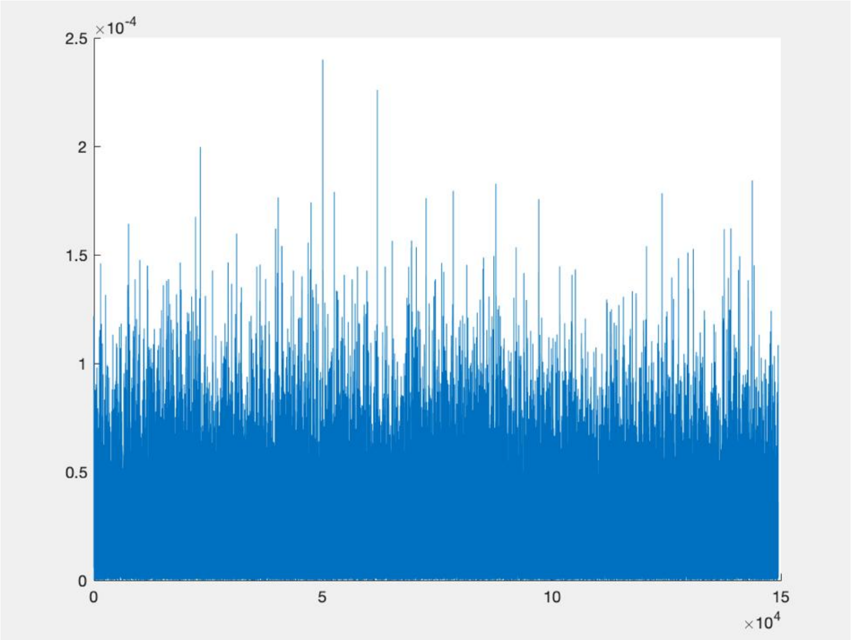

Figure 4A: representation of raw EMG data from a single participant for the proximal section of the Tibialis Anterior muscle

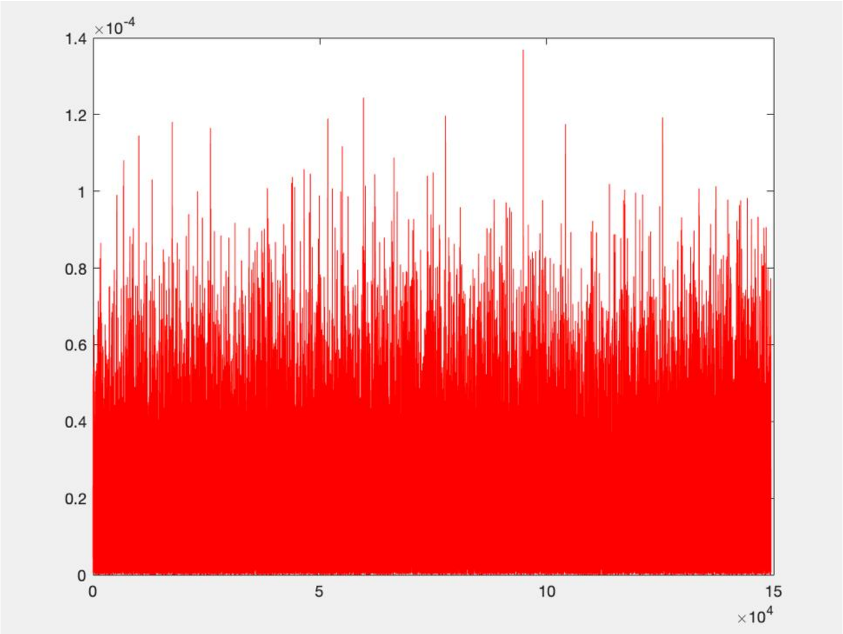

Figure 4B: representation of raw EMG data from a single participant for the distal section of the Tibialis Anterior muscle

Figure 4A-B shows an example of raw EMG-EMG data from a single participant before the coherence analysis. As seen in the figure raw EMG signal is present for both proximal (Fig. 4A) and distal (Fig. 4B) sections of the Tibialis Anterior muscle.

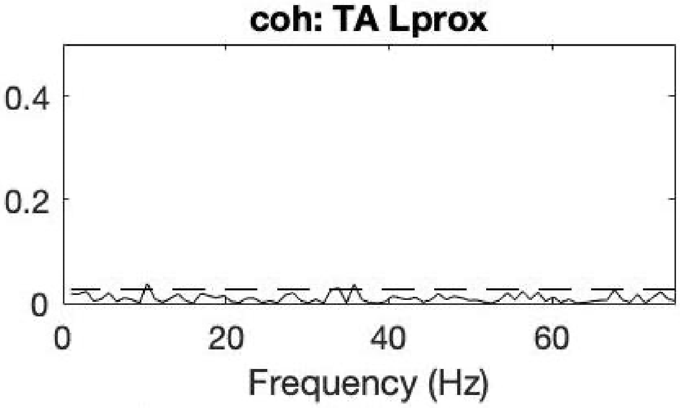

Figure 5A: Example of EMG-EMG coherence from a single TD participant before training

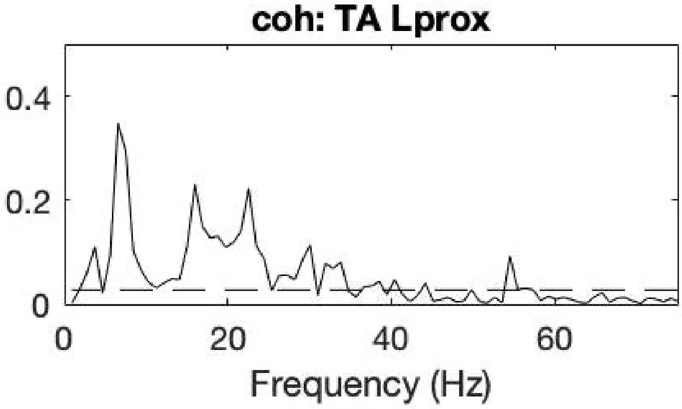

Figure 5B: Example of EMG-EMG coherence from a single TD participant after training

Figure 5 A-B shows an example of EMG-EMG coherence from a single TD participant. As seen in the figure there is a change between the two graphs, showing a significant coupling for broad frequencies from 15 to 35 Hz (Fig. 5B) which reflects EMG-EMG coherence after treadmill training.

Moreover, in Figure 6 A-B, we can see that the cumulant density function for the same participant before training (Fig. 6A) was characterized by a broad signal, whereas, after the treadmill training (Fig. 6B), we can see a short-lasting peak on either side of the zero, which indicates short-term synchronization.

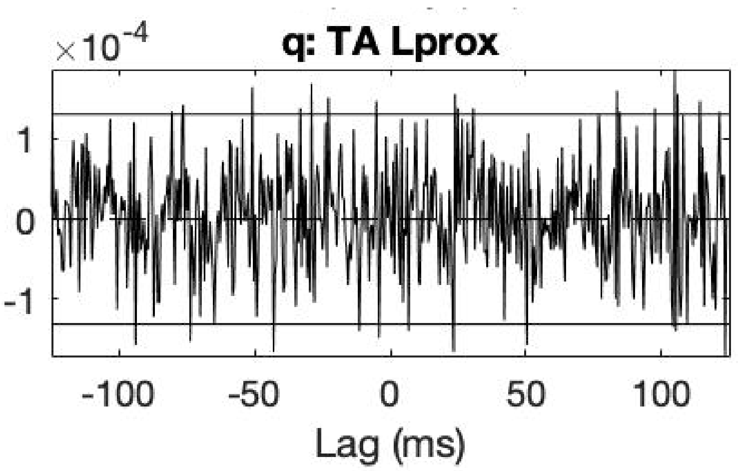

Figure 6A: Example of cumulant density function for a single TD participant before training

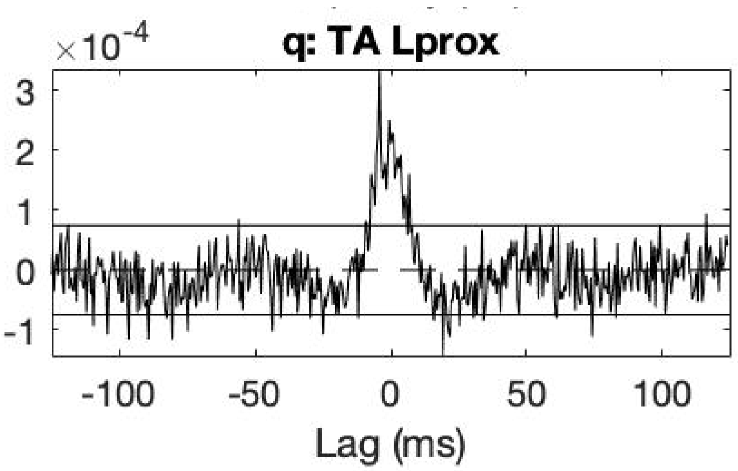

Figure 6B: Example of cumulant density function for a single TD participant after training

5.6 Statistics

In addition to pooled statistics, we also performed a two-way repeated measures (RM) ANOVA analysis on the results produced by the coherence analysis to verify whether there would be any differences between the two groups (CP and TD) in the two conditions (before and after training).

More specifically we performed a two-way RM ANOVA to compare several conditions: right and left leg before and after training, reaching and standing leg before and after training and we also looked at whether there would be a difference in coherence when comparing the two legs (reaching vs standing).

Moreover, we performed a correlation analysis (Spearman rank correlation) on the coherence results and the change in performance, calculated considering the error rate from the first to last trial of the Visuomotor gait task.

6. Results

6.1 Changes in coherence after treadmill training in right vs left leg

The two-way repeated measure ANOVA analysis performed on the coherence analysis results from the static contraction before (pre) and after (post) the treadmill training between the two groups (TD and CP) showed a significantly larger change in coherence in both beta ($p<0.05$) and low gamma ($p<0.05$) frequency bands following the training for TD participants when compared with CP participants.

6.2 Changes in coherence after treadmill training in reaching vs standing leg

The analysis performed on the comparison between the two groups (CP and TD) for the reaching and standing leg showed a much larger increase in coherence for the reaching leg in the TD group in both beta ($p<0.05$) and gamma ($p<0.05$) frequency bands (Fig. 7A). Coherence in the alpha and high gamma frequency bands did not show any significant difference before and after training. The analysis performed on the standing leg did not show any statistically significant difference (Fig. 7B).

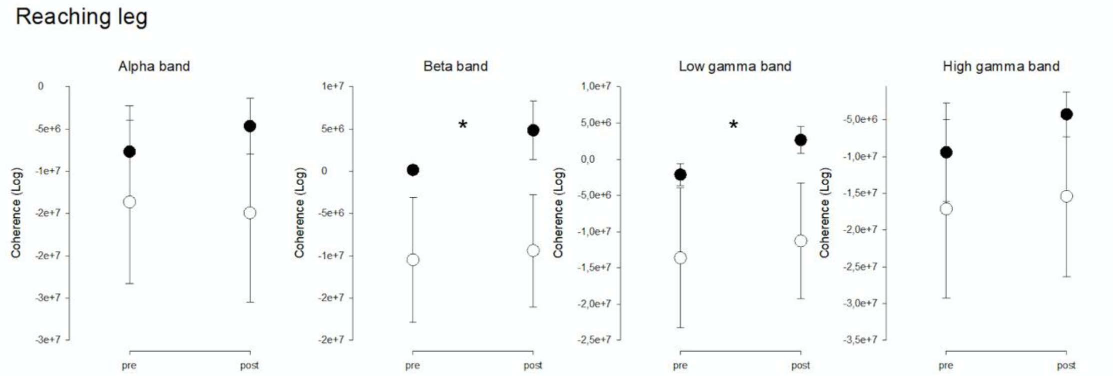

Figure 7A: Differences in coherence for the Reaching leg before and after training for all frequency bands. Full circles indicate TD participants, empty circles indicate CP participants.

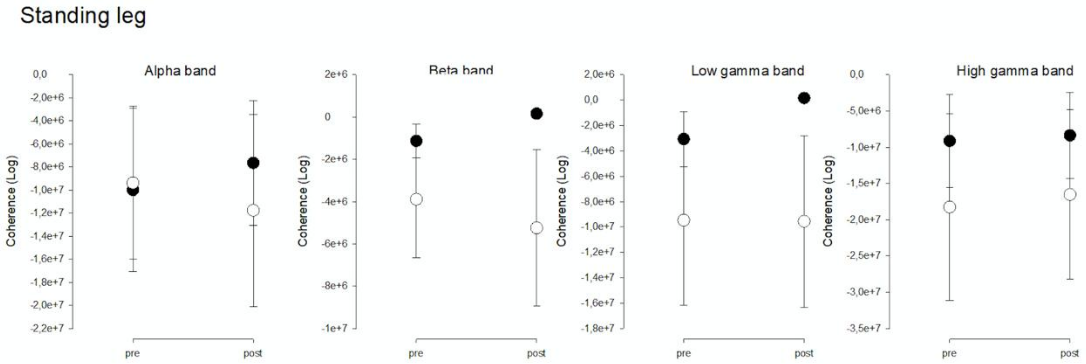

Figure 7B: Differences in coherence for the Standing leg for all frequency bands. Full circles indicate TD participants, empty circles indicate CP participants.

6.3 Changes in coherence between the two legs

Furthermore, we also compared the reaching vs. standing leg to see whether there would be any differences in coherence between the two legs in both groups. The analysis did not produce any significant results, most probably due to the low number of subjects in the study.

6.4 Correlation between the increase in coherence and increase in performance (decrease of error rate)

Lastly, Spearman rank correlation analysis showed that there is a significant correlation between the increase in low gamma coherence and improvement in performance for TD adults ($p < 0.05$), whereas the adults with CP did not show a positive correlation.

7. Discussion

The purpose of this study was to investigate whether there would be differences in intramuscular coherence in the Tibialis Anterior muscle before and after treadmill training that involved a visuomotor gait task. This study demonstrated that intramuscular coherence in the Tibialis Anterior muscle increases after visuomotor gait training.

Differently from previous studies, we aimed at investigating intramuscular coherence differences before and after short-lasting treadmill training, whereas previous research found in the literature mostly analyzed either long-lasting differences produced by intensive training (Willerslev-Olsen et al., 2015) or differences in coherence during static contraction without any gait training (Spedden et al., 2018) or during walking (Petersen et al., 2013; Spedden et al., 2019).

As was previously mentioned, there is general agreement in the literature that both beta and gamma band coherence reflect the activity of the corticospinal tract. Our result is in line with previous studies that showed that the TA muscle activity and the cortical activity are synchronized, pointing to a shared oscillatory drive from the cortex to the muscle. More specifically, several studies showed that both beta and gamma band intramuscular coherence reflect corticospinal activity during both static contraction and walking (Barthelemy et al., 2010; Farmer et al., 1993; Hansen et al., 2005, Mima et al., 2000; Nielsen et al., 2008, Petersen et al., 2013). This coherence in the 15-45 Hz frequency band is greatly reduced after stroke (Nielsen et al., 2008; Petersen et al., 2013), spinal cord injury (Barthelemy et al., 2010; Hansen et al., 2005), and cortical lesion (Farmer et al., 1993b).

In our study we found that coherence increased significantly in the beta (15-30 Hz) and gamma (30-45 Hz) frequency bands, supporting previous results that showed that EEG activity over the leg areas of the motor cortex is coherent with EMG activity in the TA muscle at frequencies that range between 25 and 40 Hz (Petersen et al., 2013). Coherence in the alpha and high gamma frequency bands (respectively 5-15 Hz and >45Hz) did not show any significant difference before and after the training, supporting the idea that changes in these frequency bands are not related to the corticospinal activity (Llinas and Volkind, 1973; Llinas and Paré, 1995).

Moreover, results from the statistical analysis conducted on coherence data before and after the treadmill training demonstrated that an increase in coherence after training is present in both TD and CP adults, even though the magnitude of the change is significantly different between the two groups, being much larger for TD adults.

This result supports previous evidence that showed how training induces changes in coherence in beta and gamma frequency bands in individuals affected by CP (Willerslev-Olsen et al., 2015).

Additionally, our results showed that the increase in beta and gamma frequency bands is much larger for the reaching leg, meaning the leg that is actively involved in the task, for TD adults. This result is in line with the idea that increases in coherence in the trained leg are most likely reflecting changes in the corticospinal drive to motoneurons as part of the motor learning process. As was previously mentioned, the motor cortex is highly involved in the process of learning a new motor task, especially in the early stages of acquisition (Müllbacher et al., 2001).

Furthermore, other studies conducted with neuroimaging techniques and transcranial magnetic stimulation showed that motor training induces changes in the representation of the muscles in the motor cortex as well as changes in the organization of the movement (Pascual-Leone et al., 1995; Müllbacher et al., 2001; Perez et al., 2004).

Based on these findings we believe that treadmill training can be an important tool for developing rehabilitation strategies for children with CP, and EMG-EMG coherence can be exploited as an indirect measure that can reflect improvements in the cortical drive to the muscle.

Increased representation of specific muscles during motor learning may also be related to increased attention to the task. Kristeva-Feige et al. in 2002 observed that coherence increased when individuals had to perform a motor task that required a high level of attention and a high degree of precision. More evidence showed enhanced short-term motor unit synchronization and EMG-EMG coherence during motor tasks that require high levels of attention and effort (Kilner et al., 2000; Perez et al., 2006). It is still unclear how changes in attention during a visually guided motor task influence the central common drive. However, it cannot be excluded that the change in attention modulates the central common drive and, by consequence, the change in coherence.

In order to efficiently navigate in a challenging terrain and successfully avoid obstacles, adequate processing of visual information during gait is crucial.

Being an indirect measure of corticospinal activity, increased EMG-EMG coherence in beta and gamma frequency bands during a visually guided motor task can underlie a crucial involvement of the motor cortex and the corticospinal activity in precise foot placement, which is supported by visual information during gait.

Studies showing increased corticospinal excitability during precision walking guided by visual feedback compared to normal walking (Schubert et al., 1999) and experiments in the cat showing increased firing of corticospinal neurons when careful paw placement is requested (Drew et al., 1996; Drew and Marigold, 2015) represent additional evidence that the motor cortex and the corticospinal pathway play a critical role in visually guided modifications of the gait pattern.

Furthermore, our results support previous research that showed that visuomotor training can also improve human motor performance (Roche and O'Mara, 2003) and that cerebral palsy patients' ankle function improves following a training technique involving the use of visual feedback (Willerslev-Olsen et al., 2014b, 2015).

The correlation analysis between changes in coherence and improvement in performance performed in our study showed that there is indeed a correlation between the increase in coherence and the performance improvement, meaning a reduced error rate between the first and the last trial of the visuomotor gait task. This finding appears to be consistent with the research stated above, confirming that visuomotor training can improve motor performance and motor functions in both TD and CP individuals.

8. Conclusion

The results from the experiment we conducted show that intramuscular coherence recorded during static contraction of the Tibialis Anterior muscle in the beta and low gamma frequency bands changes following visuomotor gait training in both typically developed individuals and individuals with cerebral palsy, even though the magnitude of the increase is significantly different between the two groups. The learning process of the motor task helps the individual to successfully perform the motor act significantly reducing the number of errors. This performance improvement is also correlated with the increase in intramuscular coherence in the beta and gamma frequencies.

We propose that even short-lasting gait training produces plastic changes that result in increased intramuscular coherence, which represents an indirect measure of changes in the central drive to motoneurons.

These results support the idea that coherence analysis, specifically intramuscular coherence analysis, can be exploited as a measure of corticospinal drive in gait training therapy in individuals with cerebral palsy.

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